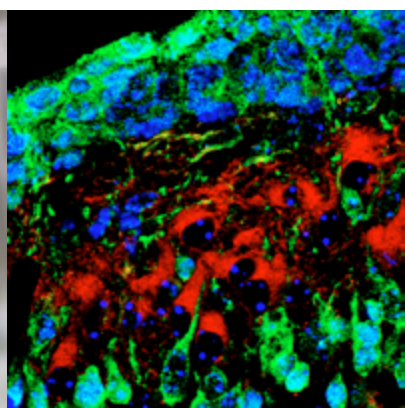
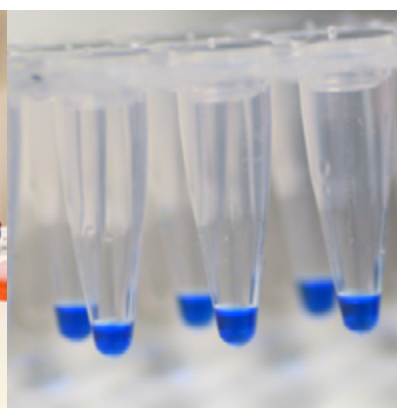
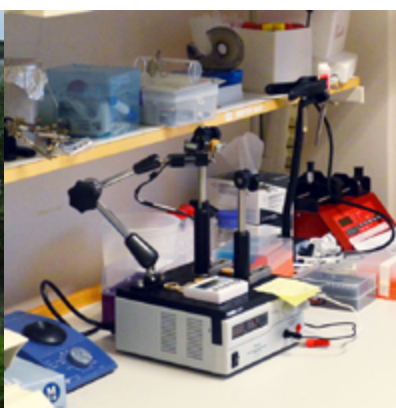




UPPSALA
UNIVERSITET

Department of Immunology, Genetics and Pathology

Annual Report 2014



Annual Report

2014

Department of Immunology,
Genetics and Pathology

Uppsala University

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Introduction

The Department of Immunology, Genetics and Pathology (IGP) hereby presents its activities during 2014. This has been another successful year for the department. Several new recruitments of excellent scientists were finalized, continuing a very important strategic development for IGP. We are proud that Professor Elisabetta Dejana was awarded a grant for International Recruitment of Leading Researchers from the Swedish Research Council. Prof. Dejana will build a new research group at IGP focused on cerebral vascular malformations while retaining fifty per cent research activity at Milano University. During 2014, Linda Holmfeldt, who was selected as KAW Academy Fellow in 2013, established her new research group focused on hematological malignancies, at IGP. We warmly welcome them both to IGP.

Several of the principal investigators at IGP were successful in attracting prestigious grants and awards. Taija Mäkinen received a Research Grant for Junior Researchers from the Swedish Research Council, and Fredrik Swartling obtained a prestigious ERC starting grant. Warm congratulations to both! Other achievements include the Athena prize to Alex Karlsson-Parra, the grants to Peetra Magnusson and Sara Mangsbo from Bio-X/Uppsala BIO for project commercialization. Sara Mangsbo also received the Göran Gustafsson prize for younger scientists. Moreover, IGP's researchers did very well in the competition for funding from the Swedish Research Council, the Swedish Cancer Society and the Swedish Childhood Cancer Foundation, EU funding and other granting agencies. Here we can only mention a selection of the grants, but each one is important, and will be put in the best of use for research at IGP. We thank everyone for their efforts in applying for external grants, which is absolutely critical for the continued growth and prosperity of the Department.

Another very important development for the Department during 2014 was the decision by the Disciplinary Domain of Medicine and Pharmacy that the Oncology, Biomedical radiation sciences and Medical radiation physics units from the ROS department, would fuse with IGP. The process was initiated during the late fall of 2014 and resulted in a restructuring of the Research programs that will continue during 2015. We know from our previous experience that fusion of units means fusion of cultures, which needs to be given time. Moreover, even though some of these changes are difficult, the positive effects will dominate with time. We wish the new units and their personnel very welcome to IGP. The fusion will be described in more detail in the yearly report for 2015.

In 2014, the Swedish Higher Education Authority evaluated master programs in biomedicine, on a national basis. IGP runs two master programs, in Molecular Medicine and Forensic Science, which were given the excellent grade "Very high quality". We thank Lena Åslund, in charge of the Molecular Medicine program, Marie Allen, in charge of the Forensic Science program, and Nils-Erik Heldin, Head of Education at IGP, for their efforts with the report and successful evaluation, and for their continuous hard work to ensure that education at IGP remains of the highest quality.

In 2014, an almost complete version (85%) of the Human Protein Atlas, an atlas covering all human proteins, was launched. The atlas includes 13 million images that show in which tissue or cell the respective proteins are present. Approximately 25 people at IGP participate in the project, with a focus on determining where and when proteins are present in tissues and cells.

2014 marks the end of the first 5-year period of the Strategic research programs (SFOs). IGP hosts or co-hosts several of the SFOs, including U-CAN, EXODIAB, StemTherapy and

SciLifeLab. IGP researchers are heavily involved with these programs and several are in charge of technical platforms at SciLifeLab. Therefore many of the Department's co-workers have spent time and energy on reporting on the research activities to show that the investment into the SFO programs has been successful. We hope that the efforts will bear fruit and that the programs will continue.

Finally, during 2014, the reconstruction of the Rudbeck Laboratory was initiated and will continue for all of 2015 and part of 2016. It is exciting but also challenging to continue the research and educational activity during the reconstruction phase. We very much look forward to seeing the new lab and office space completed.

The overriding common goal of IGP's research activities is to improve prevention, diagnostics and treatment of diseases, such as cancer and diabetes. Heads of Department, we have strived to support this endeavor to the best of our capacity. We gratefully acknowledge all who assist in these efforts: the IGP administrative staff, project coordinators at the Disciplinary Domain of Medicine and Pharmacy, the Grants Office, the Legal Affairs Division, UU Innovation, and the Central University Administration.



Lena Claesson-Welsh
Head of Department until October 31st, 2014



Karin Forsberg-Nilsson
Head of Department from November 1st, 2014

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Employees during 2014

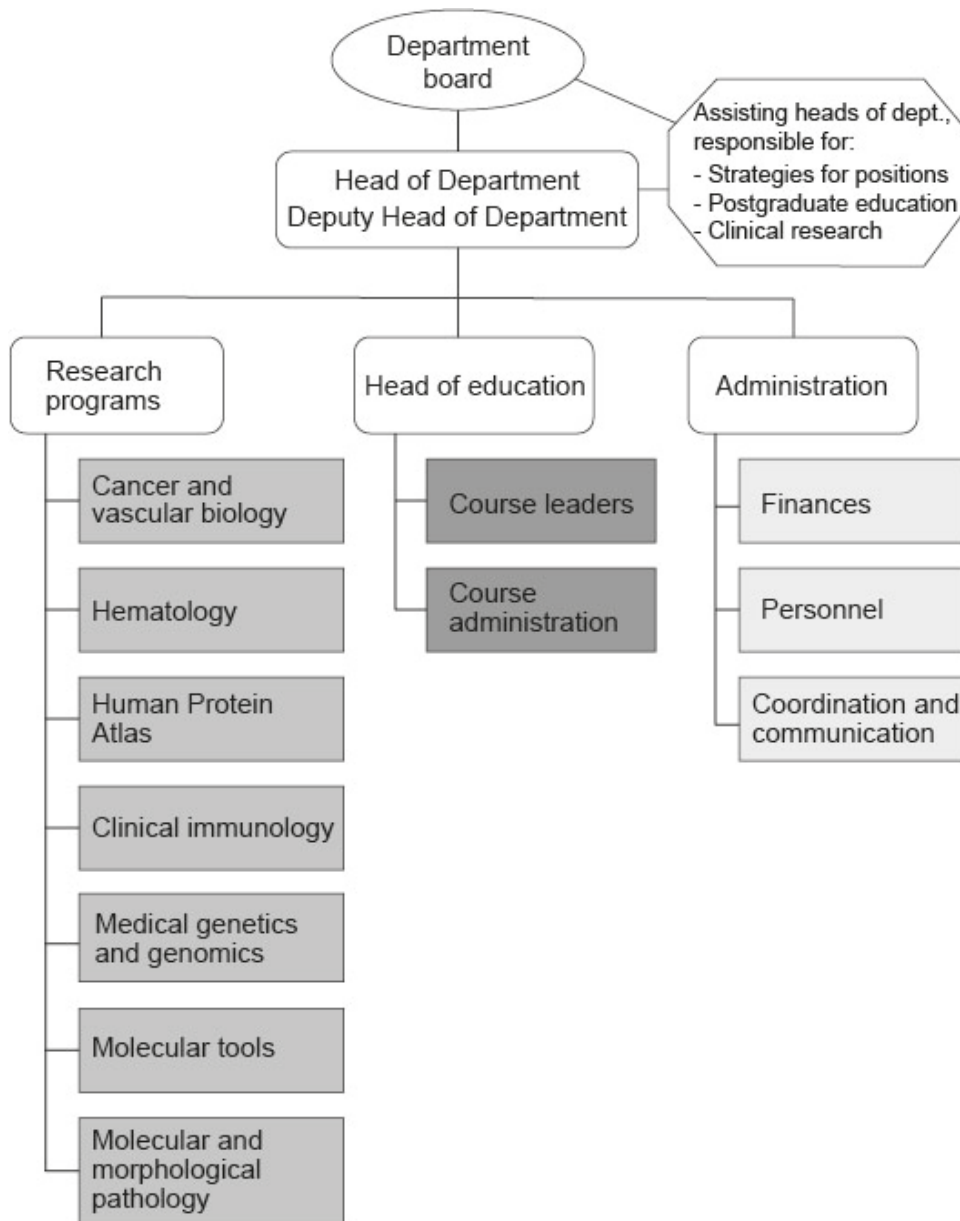
Abramsson Malin	Chmielniakova Jana	Gyllensten Ulf
Adler Jeremy	Chugunova Elena	Gängel Konstantin
Adlerteg Tom	Chytraeus Lisa	Hallqvist Osterman Erik
Agarwal Prasoon	Claesson-Welsh Lena	Halvardson Jonatan
Ahlander Anders	Classon Christina	Hamad Osama
Ahlgren Viktoria	Clausson Carl-Magnus	Hansson Tony
Ahlstav Suzanne	Cortese Diego	Hartman Karin
Alafuzoff Irina Ingeborg	Cui Tao	He Liqun
Al-Amin Abdullah	Dahl Niklas	Hede Sanna-Maria
Alemayehu Groom	Danielsson Louise	Hedlund Lindberg Julia
Ali Muhammad Akhtar	Davies Hanna	Hedlund Marie
Allen Marie	Davoodpour Padideh	Heldin Nils-Erik
Al-Walai Somar	Dimberg Anna	Hellström Ann-Charlotte
Alzrigat Mohammad	Djureinovic Dijana	Hellström Mats
Ameur Adam	Dohlmar Ulf	Henriksson Kerstin
Anagandula Mahesh Kumar	Dumanski Jan	Hermansson Annika
Anand Manivel Vivek	Dührkop-Sisewitsch Claudia	Herö Johanna
Andaloussi Mäe Maarja	Ebai Tonge Brunhilda	Hikmet Noraddin Feria
Andersson Linda	Edqvist Per-Henrik	Hillerdal Viktoria
Andersson Magdalena	Edvinsson Åsa	Holmfeldt Linda
Andersson Sandra	Ek Weronica	Hong Jaan
Andrae Johanna	Ekberg Elin	Huang Hua
Aronsson Maria	Ekvall Sara	Humieniecki Lukasz
Asif Sana	Elfineh Lioudmila	Häggqvist Susana
Asp Michaela	Elmén Karin	Höijer Ida
Asplund Anna	Elshafie Amir	Ilbäck Carolina
Backeryd Lindström Anna	Emanuelsson Hanna	Israelsson Katarina
Banski Piotr	Enroth Stefan	Jeansson Marie
Barbu Andreea	Essand Magnus	Jernberg Wiklund Helena
Baskaran Sathishkumar	Falk Sörqvist Elin	Jin Chuan
Berggrund Malin	Ferletta Maria	Johansson Patrik
Bergman Julia	Feuk Lars	Johansson Sebastian
Bergström Tobias	Flamourakis Georgios	Johansson Swartling Fredrik
Betsholtz Christer	Fletcher Erika	Johansson Åsa
Bhoi Sujata	Fonnaland Karin	Jonasson Inger
Bjerke Mia	Forsberg Lars	Juko-Pecirep Ivana
Björkesten Johan	Forsberg Nilsson Karin	Kalushkova Antonia
Blokzjil Andries	Fransson Moa	Kamali-Moghaddam Masood
Bodare Sofia	Frisk Gun	Kampf Caroline
Bolin Sara	Fristedt Duvefelt Charlotte	Karlsson Hannah
Bondeson Marie-Louise	Fromell Karin	Karlsson Marie
Boox Pirkko	Gallant Caroline	Karlström Therese
Borgenvik Anna	Galli Joakim	Karoutsou Maria
Broström Ulrika	Georganaki Maria	Kastemar Marianne
Brännström Johan	Grannas Karin	Katona Borbala
Bunikis Ignas	Grånemo Joakim	Keller Annika
Bus Magdalena	Gu Jijuan	Kermani Shila
Cahill Nicola	Gubanova Evgenia	Kesti Dennis
Cancer Matko	Gupta Rajesh	Kiflemariam Sara
Cane Gaëlle	Gustafsson Birgitta	Klaesson Axel
Cavalli Marco	Gustafsson Karin	Klar Joakim
Chen Dan	Gustafsson Victor	Koos Björn
Chen Lei	Gustavsson Inger	Korsgren Olle

Kozarcanin Huda
Krona Cecilia
Kuhnemund Malte
Kundu Snehangshu
Kundu Soumi
Kunze Elene
Källström Lillemor
La Fleur Linnea
Ladenvall Claes
Landegren Ulf
Langenkamp Elise
Larsson Chatarina
Larsson Erik
Lavina Siemsen Barbara
Leja-Jarblad Justyna
Li Xiujuan
Liljenfeldt Lina
Lindahl Erik
Lindau Cecilia
Lindblom Susanne
Lindholm Carlström Eva
Lindskog Bergström Cecilia
Lindström Anne-Christine
Ljungström Viktor
Ljungström Viktor
Loskog Angelica
Lundberg Marcus
Lundin Erika
Löf Liza
Lönn Peter
Löf Johan
Magnusson Christina
Magnusson Kristina
Magnusson Petra
Mangsbo Sara
Mansouri Larry
Marinescu Voichita
Marques Souza de Oliveira
Martinez-Corral Ines
Martinsson Pernilla
Mathot Lucy
Mattsson Johanna
Melin Malin
Mickelsen Jansson Annlouise
Milennova Yoanna
Molnar Matyas
Morin Eric
Mulder Sara
Mäkinen Taija
Nelander Sven
Niaudet Colin
Niklasson Mia
Nilsson Berith
Nilsson Bo

Nilsson Camilla
Nilsson Ekdahl Kristina
Nilsson Mats
Nitzsche Anja
Nong Rachel
Nord Helena
Nordling Sofia
Nordling Torbjörn
Nordqvist Marcus
Oelrich Johan
Ohlin Elisabet
Olerud Johan
Olsson Cecilia
Olsson Ingmarie
Ortsäter Henrik
Oskarsson Linda
Oskolkov Nikolay
Pacholsky Dirk
Pan Gang
Pandzic Tatjana
Papadaki Evangelia
Paul-Wetterberg Gabriella
Persson Skare Tor
Peterson Pia
Petersson Sara
Petri Anna
Pettersson Ulf
Pontén Fredrik
Pristovsek Nusa
Pääbo Svante
Quarfordt Pernilla
Ramachandran Mohanraj
Rasi Chiara
Raykova Doroteya
Reddy Bysani Madhusudhan
Rendo Verónica
Roche Francis
Rosén Gabriela
Rosenquist Brandell Richard
Roy Ananya
Rung Johan
Russell Camilla
Rönnelid Johan
Sandberg Charlotta
Savov Vasil
Schenström Maria
Schmidt Linnéa
Schuster Jens
Simu Tuulikki
Singh Umashankar
Sjöblom Tobias
Sjöstedt Evelina
Sjösten Anna
Skog Oskar

Smith Ross
Sobol Maria
Sohrabian Azita
Stamatopoulos Konstantino
Stanczuk Lukasz
Steffen Henrik
Steimer Ulla
Stenbeck Funke Lillemor
Stoimenov Ivaylo
Strand Ann-Sofi
Stratmann Svea
Strid Stina
Sun Ren
Sutton Lesley Ann
Svensson Emma
Söderberg Ola
Tan E-Jean
Taussig Mike
Tellgren-Roth Christian
Testini Chiara
Tibbling Gunilla Birgitta
Tötterman Thomas
Uhrbom Lene
Ullbors Anna-Maria
Ullerås Erik
Ulvmar Maria
Wadelius Claes
van Hooren Luuk
Weishaupt Holger
Wester Kenneth
Westermark Ann
Westermark Bengt
Weström Simone
Wicher Grzegorz
Wik Lotta
Wikner Charlotte
Wilbe Maria
Williams Nina
Vinnere Pettersson Olga
Wu Di
Xiong Anqi
Yaka Cane
Yan Junhong
Young Emma
Yuan Xie
Zaghlool Ammar
Zhang Lei
Zhao Hongxing
Zhao Jin
Zieba Agata
Åslund Lena
Öberg Fredrik

Organization of the Department of Immunology, Genetics and Pathology



Head of Department

Lena Claesson-Welsh, Karin Forsberg Nilsson

Vice Head of Department

Ulf Landegren

Assistant Heads of Department

Anna Dimberg, postgraduate education

Claes Wadelius, postgraduate education

Karin Forsberg Nilsson, strategies for positions and recruitments

Bo Nilsson, clinical research

Department Board

Members during 2014

Lena Claesson-Welsh, Head of Department
Karin Forsberg Nilsson, Head of Department/teacher representative
Christer Betsholtz, teacher representative
Sara Bolin, graduate student representative
Niklas Dahl, teacher representative
Anna Dimberg, teacher representative
Elin Ekberg, representative for technical/administrative staff
Emelie Ekstrand, undergraduate student representative
Lars Feuk, teacher representative
Sarah Galien, undergraduate student representative
Birgitta Gustafsson, representative for technical/administrative staff
Marie Hedlund, representative for technical/administrative staff, deputy
Ellen Hertz, undergraduate student representative
Feria Hikmet Noradin, undergraduate student representative
Masood Kamali-Moghaddam, teacher representative, deputy
Inger Jonasson, representative for technical/administrative staff, deputy
Ulf Landegren, teacher representative, deputy
Viktor Ljungström, graduate student representative
Johan Lööf, undergraduate student representative, deputy
Sven Nelander, teacher representative, deputy
Sara Nordling, graduate student representative, deputy
Fredrik Pontén, teacher representative
Fredrik Swartling, teacher representative, deputy
Ola Söderberg, teacher representative
Lene Uhrbom, teacher representative, deputy
Sawin Yousef, undergraduate student representative

Teaching organization

Nils-Erik Heldin, head of education
Sofia Bodare, course administrator
Lisa Chytraeus, course administrator
Viktoria Ahlgren, course administrator
Suzanne Ahlstav Hernandez, course administrator
Gunilla Tibbling, course administrator

Administration

Anna Backeryd Lindström, accounting	Shila Kermani, accounting
Pirkko Boox, senior advisor	Barbro Nelson, accounting
Jenny Djerf, accounting	Camilla Nilsson, personnel coordinator
Ulf Dohlmars, accounting	Klara Nilsson, personnel
Birgitta Gustafsson, financial coordinator	Christina Magnusson, administrator of postgraduate education
Holger Henningsson, personnel	Sara Mulder, personnel
Kerstin Henriksson, communication	Tuulikki Simu, accounting
Katarina Israelsson, accounting	Ulla Steimer, accounting
Therese Karlström, personnel	

Core Facilities

BioVis

In 2010, the former Cell Analysis Core Facility was reshaped to create BioVis, a national resource for advanced visualization and analysis of biological material. The BioVis Facility is part of Science for Life Lab since 2010.

BioVis provides services and instrumentation for Electron and Light microscopy, flow Cytometry, cell sorting and Image Flow Cytometry. Researchers from Academia as well as non-Academia users are welcome to analyze their own samples on the instruments available in the lab. We provide hands-on training and advice for handling and we offer advice on the use of appropriate methods and experimental setups. For users who wish to have this service provided we can, time permitting, also perform sorting and analysis experiments.

Instrumentation at BioVis includes a FACS Aria III flow sorter and a BD LSR II SORP multilaser flow cytometer as well as a Merck/AMNIS Flowsight Imaging Flow Cytometer. (first in Scandinavia). A ZEISS LSM 700 confocal microscope, a ZEISS 710 NLO multiphoton microscope and ZEISS 710 Superresolution SIM and a ZEISS AxioImager brightfield and fluorescence microscope with an apotome (for optical sectioning) are also installed. BioVis is proud to be first in Scandinavia having a ZEISS Lightsheet Z.1 microscope installed. In addition, users have access to workstations for image analysis and documentation including IMARIS and AMIRA software. BioVis is collaborating with the group of C. Wählby from Centrum for Image Analysis (CBA) for in-depth image analysis.

An FEI Tecnai Biotwin transmission electron microscope has also been added to the facility. This instrument has increased our capacity to provide the services requested by various research groups. A laboratory and staff to prepare samples for imaging on the electron microscope is available.

In 2014 the services offered by the BioVis were regularly used by 94 academic and 7 non-academic research groups, covering 179 projects, coming from the medical and pharmaceutical faculties, from other Uppsala University faculties, as well as from other universities and research establishments.

The service level is high with a lab manager and experts for microscopy, flow cytometry, electron microscopy and image analysis available to instruct and advise users, to ensure instrument performance, to perform experiments and to administrate instrument service and reservations.

BioVis was proud to host the 5th Swedish Bioimaging National meeting, Uppsala, May 2014. In May 2014, BioVis arranged the first Image J workshop in Uppsala (and probably Sweden), again highly appreciated by the participants coming from all over Sweden, and the first Flowsight Seminar. Other workshops included Image Analysis software Huygens (June) and Imaris (December). BioVis staff members were giving lectures on different courses arranged by the Rudbeck Laboratory and SLU Uppsala.

A highly appreciated graduate student course on cell analysis techniques is given annually at the facility. The course contents include fluorescence theory, basic and advanced confocal microscopy and flow cytometry, as well as a substantial amount of hands-on instrument time. BioVis is organizing on a regular basis workshops and symposia covering the techniques available at the Facility.

To meet its standards BioVis started to form a BioVis Advisory Board, which was to be installed in 2015.

Staff

Dirk Pacholsky, facility manager (management, flow cytometry, cell sorting, microscopy)
Anders Ahlander, research engineer (electron microscopy)

Matyas Molnar, research engineer (light, confocal and multiphoton microscopy)
Sara Peterson, research engineer
Kenneth Wester, researcher

Clinical Sequencing Facility

In 2013, Uppsala University, Uppsala University Hospital and Science for Life Laboratory together formed a new facility at the department of Immunology, Genetics and Pathology: the Clinical Sequencing Facility. The mission of the facility is to provide services for high throughput genomics in real clinical applications, from new genetic tests in routine diagnostics to translational research projects based on next-generation sequencing (NGS). The facility is one of three facilities constituting the national SciLifeLab platform for Clinical Diagnostics.

In the first year of operation, the facility purchased its first Illumina HiSeq 2500 and MiSeq instruments and formed administrative routines for operations, and agreements between the different partners of the facility. Two molecular biologists and three bioinformaticians were recruited to the facility. Development projects and services are divided into three separate work-packages, i.e. solid tumors, hematological malignancies, and inherited diseases. Two additional work packages are dedicated to the development of bioinformatic analysis pipelines and data management, and policies and practises for ethics and reporting. In 2014, we developed new diagnostic gene panel tests for inherited cardiac and connective tissue diseases that are now used in routine practice at Clinical Genetics, Uppsala University Hospital. These tests are also accredited with ISO 15189. We also developed diagnostic gene panel tests for cancer; as a prime example, a panel of 32 genes aimed at several cancer types is now in production at Uppsala University Hospital, Molecular Pathology. This panel is designed for formalin-fixed paraffin-embedded (FFPE) tumor material.

We collaborate actively with the facilities within National Genomics Infrastructure (NGI) on NGS-based tests for clinical diagnostics that use technology other than what is installed in our own facility. In particular, whole-exome sequencing with HiSeq instruments, and long-read sequencing with PacBio, are important parts of this. We also collaborate with UPPMAX, the Uppsala facility for high performance computing, which is part of SNIC, the Swedish National Infrastructure for Computing, and the European Bioinformatics Institute (Hinxton, UK) on sequence data management.

The facility became the Swedish country node for the UNESCO-protected international Human Variome Project in 2014.

Staff

Richard Rosenquist Brandell, platform director
Johan Rung, facility manager
Johan Botling, work-package leader, solid tumors
Lucia Cavelier, work-package leader, hematological malignancies
Rose-Marie Amini, work package leader, hematological malignancies
Marie-Louise Bondeson, work-package leader, inherited diseases
Pirkko Boox, senior legal advisor
Lotte Moens, molecular geneticist
Nicola Cahill, molecular geneticist
Britt-Inger Jonsson, BMA
Elin Falk Sörqvist, bioinformatician
Malin Melin, bioinformatician

Claes Ladenvall, bioinformatician
Viktor Ljungström, bioinformatician

NGI-Uppsala / Uppsala Genome Center

NGI-Uppsala / Uppsala Genome center (UGC) is one of three nodes in the National Genomics Infrastructure (NGI) hosted by Science for Life Laboratory. The facility moved to new localities at the Biomedical Center during January 2014.

The facility is open to academic research groups in Sweden on a non-profit basis and provides a facility for Massively Parallel Sequencing (MPS) and qualified bioinformatics support. The facility can also provide service for companies and customers outside Sweden if labour and instrument capacity is sufficient.

UGC offers a broad range of services for genetic analysis such as

- Massively Parallel Sequencing (MPS) on Ion Torrent (PGM™), Ion Proton™ systems from Life Technologies and on RSII from Pacific Biosciences.
- Sanger Sequencing Service
- Genotyping with STR-markers
- Service for separation of custom prepared samples by capillary electrophoresis on AB3730XL Genetic Analyzer

During 2014 a second RSII instrument from Pacific Biosciences has been installed and taken in use for service projects. This technology is a real-time, single molecule sequencer, which generates ultra long reads up to 60 kb. This technology is a complement to the established short reads instruments at NGI.

The different MPS technologies can be used for *de novo* sequencing, whole genome re-sequencing and targeted re-sequencing of DNA. RNA sequencing can be performed either as whole transcriptome analysis, gene expression profiling, or as sequencing of small RNA molecules. With the long read technology from Pacific Biosciences UGC have the capacity to offer different technologies for different type of projects, with variation in output of sequencing data from 10 Mbp till 1000 Mbp per run and read length from 200 bp to 60000 bp.

During 2014 UGC has been working in collaboration with Clinical Genetics, Uppsala Akademiska Hospital with different projects on Pacific Biosciences to develop new protocols for detection of low frequency mutations in cancer and genetic diseases. The aim is that Pacific Biosciences will replace current methods and be used as a tool for clinical diagnosis.

UGC is a “Certified Service Provider” for exome sequencing using the Ampliseq Exome Kit from Life Technologies. This PCR-based protocol allows low input of DNA and a rapid workflow with the possibility to fast delivery of data. UGC is also taking part in different programs as “early users” to test and evaluate new chemistries and technologies from Life Technologies.

UGC performs DNA sequencing service using the Sanger method. This is done mainly by preparing the sequencing reactions or by size separation of custom prepared DNA-fragments by capillary electrophoresis. Additional services include genotyping of microsatellite markers and TaqMan® based SNP-typing.

In 2014 UGC provided service for more than 260 projects and more than 2200 samples on the MPS sequencing machinery. 150 researchers are more or less frequent users of the Sanger Sequencing Service and the other types of services that UGC offers.

Staff

Ulf Gyllensten, managing director
Inger Jonasson, facility manager
Adam Ameer, bioinformatician
Magdalena Andersson, research engineer
Ulrika Broström, research engineer
Ignas Bunikis, bioinformatician
Nicola Cahill, research engineer
Susana Häggqvist, research engineer
Ida Höijer, research engineer
Carolina Ilbäck, research engineer,
Sebastian Johansson, research engineer
Cecilia Lindau, research engineer
Anne-Christine Lindström, research engineer
Anna Petri, research engineer
Maria Schenström, research engineer
Christian Tellgren-Roth, bioinformatician
Olga Vinnere Pettersson, project coordinator
Nina Williams, research engineer

PLA Proteomics Facility

The PLA Proteomics facility that is part of the Affinity Proteomics platform of Science for Life Laboratory (SciLifeLab), was established in 2010 and provides services for the scientific community for sensitive and specific analyses of proteins and their interaction complexes using *in situ* proximity ligation assays (*in situ* PLA). Since 2012 the facility also assists users by establishing solid-phase PLA tests for sensitive and specific detection of proteins in body fluids such as plasma, cerebrospinal fluids, etc. The services further include high-performance PLA-based western blot assays.

The PLA technology was developed at the Department of Immunology, Genetics and Pathology, Division of Molecular Tools, and allows target protein molecules to be sensitively analyzed using sets of antibodies with conjugated oligonucleotides. Upon recognition of target molecules by the antibodies, the attached oligonucleotides can either be ligated to each other (for solution-phase PLA), or guide circularization of two accessory oligonucleotides (for *in situ* PLA). The reporter DNA molecules that form by ligation are amplifiable by real-time PCR for solution-phase measurements or by localized rolling circle amplification for *in situ* detection. The PLA method owes its specificity and sensitivity to the requirement for multiple recognition events and the possibility of translating the detecting signals to amplifiable DNA reporters.

During 2014 the facility offered Swedish scientists both service for fee and also participated in collaborative projects. A large and growing number of *in situ* PLA-based assays are available for analyses of proteins in cells and tissues at single cell and single molecule resolution. The facility can also establish assays for new target molecules or adapt established assay formats for new applications, by mutual agreement with users. The assistance also includes expert advice on design of experiment and for data analyses.

Staff

Ulf Landegren, facility director
Masood Kamali-Moghaddam, facility manager
Christina Classon, research engineer

Tissue Profiling Facility

The Tissue Profiling Facility was established during 2010 as part of the SciLifeLab effort in Uppsala. In 2013 it became a national facility within the SciLifeLab Affinity Proteomics platform. The expertise of the centre is focused on histopathology with special technical emphasis on tissue microarray (TMA) production, immunohistochemistry (IHC) and image digitalization of stained slides (scanning). As a technical high-throughput platform, the centre aims to provide these services to external research groups. During 2014 the facility performed service for researchers that included the construction of 59 TMAs, over 7.600 cut tissue sections, 5000 slide scannings and 2500 stained slides.

The origin of the facility builds on more than a decade of accumulated experience and know-how from being a central part of the Human Protein Atlas project. This project which is funded by the Knut and Alice Wallenberg research foundation, is set up to map the human proteome by generating and validating antibodies to be used for high throughput protein profiling of normal human tissues, different forms of cancers and multiple cell lines. As part of the Human Protein Atlas effort, the tissue profiling facility have since the start 2003 constructed over 4600 TMAs containing over 273.000 cores from tissues or cell lines, stained over 216.000 slides for immunohistochemistry, scanned over 136.000 slides and cut over 880.000 sections.

Tissue microarrays are constructed using four different systems; a fully automated TMA production system (TMA GrandMaster), an automated system (Beecher ATA-27), a semi-automated system (Pathology Devices) and a manual arrayer (Beecher MTA-1) depending on tissue used and amount of tissue available. Sections are cut using a waterfall microtome (Microm HM355S).

Immunohistochemistry is performed in an automated slide staining system (Lab Vision Autostainer 480) on formalin fixed paraffin embedded material, using a polymer based detection system. Slides are deparaffinized and dehydrated in an automated slide staining system (Leica Autostainer XL) and mounted in an automated glass coverslipper system (Leica CV5030).

By using bright field digital scanners based on line scanning technology (Aperio Scanscope XT and AT), stained glass slides are transformed to digital images. Images are subsequently exported and up-loaded to a server for viewing. Slides are scanned using 20x or 40x magnification. The high-resolution images can be viewed using a freely available software (ImageScope) from Aperio.

Staff

Fredrik Pontén, Platform director
Per-Henrik Edqvist, Head of Facility
Ing-Marie Olsson, Technician
Dennis Kesti, Technician
Erik Lindahl, Technician

Associated staff

Dijana Cerjan, Technician
Sofie Gustafsson, Technician
Lillemor Källström, Technician
Ann-Sofie Strand, Technician

Prizes and awards

Bengt Westermark was awarded **H.M. The King's Medal of the 8th size with the ribbon of the Order of the Seraphim** for his valuable contributions in and for cancer research.

Lena Claesson-Welsh received **the Olof Rudbeck Prize for 2014** for her important achievements in basic science that have had significant clinical impact.

Carl-Magnus Clausson received the **Hwasser Prize** for best preclinical PhD thesis.

Alex Karlsson-Parra was awarded **the Athena Prize** for his work on therapeutic cancer vaccines.

Undergraduate Education at IGP

The Department participates in the education programs in Medicine, Biomedicine, Biomedical Laboratory Science and Physiotherapy. The master programs in Forensic Science and Molecular Medicine are organized by IGP.

Medicine

We participated in the courses "Growth and Degeneration" in the second semester, "Attack and Defense" in the fourth semester, and in the integration periods in the fourth, fifth, sixth, seventh and ninth semester of the medicine program. Students on the sixth semester also had a clinical rotation "Clinical Pathology". A two-week course in Cancer Genetics and Tumor Biology was given on the seventh semester. Approximately hundred students attended the different courses, which are given twice a year.

Biomedicine

In this program we gave a 7.5 credit course in Medical Genetics. This course is given during the fourth semester of the Biomedicine program. About thirty-five students participated in the course. We also teach general pathology in the course "Diseases - Clinical Survey" on the sixth semester of the program.

Biomedical Laboratory Science

During the fall we gave a course in Pathology and Clinical Genetics within the Biomedical laboratory science program. This course is for 11 credits and is given to students in the third semester of their education. We also headed two other courses at the program during the spring semester "Immunology and Transfusion Medicine 12 credits" (fourth semester on the program) as well as "Advanced Course I 7.5 credits" (sixth semester). Approximately fifty students attended each course.

Physiotherapy

A course in Pathology, within the Physiotherapy program, was given twice during 2014. The course was for 1.5 credits and around forty students were enrolled on each occasion.

Single Subject Courses

We offer a web-based course in Basic Medical Genetics. The course is for 4.5 credits and was given twice during 2014. It was completely web-based, with lectures, study questions and exam available via the PING-PONG platform. Students from all over Sweden, as well as abroad, enroll in this course.

Three courses, "Medical Genetics 7.5 credits", "Immune, Gene and Cell Therapy 7.5 credits" and "Molecular Mechanisms in Cancer 7.5 credits", were given during the fall.

Master Programs

The Department is heading two international master programs. The master program in Forensic Science is based on knowledge from leading international research and is closely linked to research in the field. Courses in Medical Genetics, Forensic Science and Criminalistics, Forensic Genetics and Medicine, Criminology, Forensic Chemistry and Analytical Toxicology are included. In addition to IGP, other departments at Uppsala University and Stockholm University are arranging courses within the program. Approximately twenty students are enrolled on each occasion.

The international master program in Molecular Medicine has twenty-five student positions. The program is focused on molecular mechanisms causing diseases and new technologies in genomics, epigenetics and proteomics. The courses in Medical Genetics and Cancer, and Advanced Techniques in Molecular Medicine, are given on the first semester of the program. Courses in Epigenomics, Biomarkers, Bioimaging and Regenerative Medicine are included in the program on the second and third semester.

In 2014 both master programmes organised by IGP received the highest credentials “very high quality” in an evaluation performed by the Swedish Higher Education Authority.

Postgraduate Education at IGP

In 2014 the Department had 80 students registered for a postgraduate education. Ten PhD students defended their PhD theses and four students obtained licentiate degrees.

Postgraduate education at IGP is performed as main scientific work in research groups under the guidance of at least two supervisors. Postgraduate studies also require participation at postgraduate courses. The Department encourages postgraduate students to attend international courses and has allocated funds from which students can apply for funding to participate in such courses, or to visit research laboratories to learn techniques required for their research projects. In 2014 two students received this type of funding.

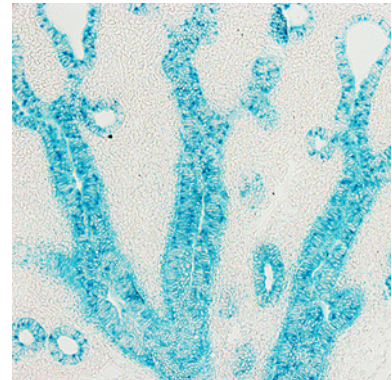
At the Rudbeck Laboratory, the BioVis facility organises the postgraduate student course “Methods in Cell Analysis”. The course contents include fluorescence theory, basic and advanced confocal microscopy and flow cytometry. The students also have the opportunity to try out suitable methods in their own projects. Another postgraduate course given at the Rudbeck Laboratory is Advanced Molecular Technology and Instrumentation for Proteome Analyses, which is organized by the proteomics platforms at Science for Life Laboratory in Uppsala. Some students also follow “NatiOn” which is a national school for graduate students in clinical cancer research held at Karolinska Institutet in collaboration with Uppsala University, including teachers from IGP.

The Rudbeck Seminar Series, organized by IGP, was given as a course for PhD students in both the spring and fall semesters. PhD students who regularly attend the seminars can account for three credit points per semester in their PhD education. The seminars are held by invited speakers from other Swedish universities as well as from abroad, on topics relevant for the PhD students at the Department. In 2014, 33 seminars were given in the series. Some students working in IGP laboratories at the Biomedial Centre (BMC) also attended the SciLifeLab/The Svedberg seminar series held there. All postgraduate students attend a pedagogical course given at Uppsala University.

The department organized the IGP Day on 16 June 2014, where many students presented their work on posters.

Cancer and Vascular Biology

A common characteristic of the many different types of cancer is disruption of the normal growth pattern, leading to uncontrolled cell division and growth, invasiveness and distant spread. Several different forms of cancer are studied at the department, with a particular focus on brain tumors and colorectal carcinoma. For example, we study glioma and medulloblastoma, where currently efficient therapies are lacking, resulting in poor prognosis. Our research aims to increase the knowledge about molecular mechanisms in tumor development with the ultimate goal to contribute to better treatment. We work closely together with clinicians to set up novel patient-derived cell models, and are engaged in several large efforts to find new therapeutic targets.



The formation of new blood and lymphatic vessels (angiogenesis and lymphangiogenesis respectively), is normally a strictly controlled process, which is critical for embryonic development as well as for growth and repair of tissues after birth. Moreover, several diseases, such as cancer, involve exaggerated (lymph)angiogenesis, leading to disorganized and dysfunctional vasculature that helps to propagate the disease. Our research focuses on how (lymph)angiogenesis is regulated in health and disease. We study the role of stimulating growth factors and negatively regulating proteins that control (lymph)angiogenesis during development, inflammation, eye disease and cancer.

Developmental Genetics

Christer Betsholtz

Our group studies cellular and molecular mechanisms of angiogenesis, vascular permeability and other vascular functions (vessel tone, molecule transport, cell transmigration across the vessel wall), in embryonic development, adult homeostasis and disease.

A particular focus is placed on the microvascular pericyte. Pericytes are obligatory components of all blood capillaries, yet their functions in health and disease are still poorly understood. Our on-going research addresses pericyte functions in different situations in organs using *in vivo* and *in vitro* techniques.

Other areas of focus concern the mechanisms of angiogenic sprouting, and the specific role of G-protein coupled receptors in this process as well as in other microvascular functions. A large project relates to the blood-brain barrier (BBB), a complex and specific feature of the neurovascular unit, and the role of pericytes in this structure.

Some of our questions go beyond vascular biology. In a broad sense we address the roles of platelet-derived growth factors (PDGFs) and other growth factors and their intracellular signal transducers during embryonic and postnatal development, as well as in pathological processes in the adult organism, such as cancer and brain calcification and neurodegeneration.

The Angiopoietin/Tek system in fibrosis

Marie Jeansson

Angiopoietins are proteins that bind the tyrosin kinase receptor Tek (also called Tie2), expressed on the endothelium of blood vessels. Angiopoietin-1 is an agonist and results in stabilization and quiescence of the vessel whereas Angiopoietin-2 is an antagonist and inhibits the protective Angiopoietin-1/Tek signaling.

Several clinical conditions, including cardiovascular disease, malaria, and sepsis, increase the serum level of Angiopoietin-2, and the increased ratio between Angiopoietin-2/Angiopoietin-1 has been shown to predict adverse outcomes. One of our objectives is to define the role of the Angiopoietin/Tek system in fibrotic diseases.

Mechanisms of angiogenesis and vascular permeability: the role of G-protein coupled receptors.

Konstantin Gaengel, Colin Niaudet, Barbara Lavina-Siensen, Marco Castro

We previously identified a core set of 58 gene transcripts expressed specifically (and quite universally) in endothelial cells. This set of genes included some 20 well-established endothelial markers, many of which are known to play critical roles in vasculogenesis and angiogenesis.

Interestingly, however, approximately half of the 58 gene transcripts had not been previously implicated in vascular biology. Many of them are highly interesting as candidate novel regulators of angiogenesis since they 1) are highly endothelial-specific in their expression, and 2) encode proteins predicted to play a role in cell signaling, such as GPCRs. In our current research program, we are investigating cellular and molecular mechanisms involved in angiogenesis, with focus on new regulators and regulatory processes involved in vascular morphogenesis, stabilization and barrier formation.

Analyses of PDGF signaling during organ development

Johanna Andrae, Radosa Gallini, Leonor Gouveia

The overall aim for this project is to analyze and describe developmental processes where members of the platelet-derived growth factor (PDGF) family play important roles.

We are focusing on processes that are dependent on proper signaling through the tyrosine-kinase receptor PDGFR α . Generally viewed PDGFR α is expressed by mesenchymal and glial cells, whereas adjacent epithelial, muscle or neuronal cells express the ligands PDGF-A and/or PDGF-C. This is true for example in brain, lung, intestine, palate and hair follicles.

Our goal is a detailed understanding of how correct PDGF signaling contributes to different developmental processes. What are the characteristics of the cells that express PDGFR α ? Where are they located in relation to the ligand expressing cells? What happens to those cells in the absence of PDGF, or if they are over-stimulated? It is important to know how different cells contribute to a specific tissue organization.

All cell types use specific molecular signals to communicate with each other, and knowing the normal signaling pathways may be crucial for understanding a pathological behaviour.

Pericyte biology and markers

Elisabeth Raschperger, Bongnam Jung, Michael Vanlandewijck

Pericytes are essential for development and stabilization of the vascular networks. These cells also regulate capillary blood flow, and are a component of the neurovascular unit that controls blood-brain permeability. In addition, immune, phagocytic and contractile functions are assigned to pericytes. Genetic mutation and cell-based studies have demonstrated pericyte engagement in physiological functions and in diseases, including vascular/ organ

development, wound healing, scarring, fibrosis and tissue remodeling. For example, PDGF-B or PDGFRb- deficient mice die perinatally exhibiting vascular dysfunction due to a lack of pericyte investment around blood vessels, suggesting the critical role of PDGFB/R signaling in vascular maturation.

Although the biological significance of pericytes is appreciated, a lack of pericyte-specific markers have hampered in-depth study on their origin, presence and function during physiological and pathological processes. To date, existing pericyte markers, such as PDGFR β , NG2, desmin and CD13, cannot distinguish pericytes from vascular smooth muscle cells (vSMCs) or other mesenchymal cells. The expression patterns of these markers also vary between species, developmental stages and tissues. Therefore, 1) pioneering a reliable, pericyte-specific marker and 2) characterizing known marker expression in a timely- and organ- specific manner are necessary for proper analysis of pericyte biology in health and disease.

We take advantage of the double fluorescent transgenic mouse model, PDGFR β -EGFP/NG2-dsRed, to study pericyte expression in embryonic and adult mouse tissues by immunofluorescence staining and imaging. Further, we use these mice to FACS pericytes for deep sequencing-based transcriptional profiling to investigate not only novel and specific pericyte markers but also transcriptional differences in pericytes from various organs. Utilizing the PDGF knock out mice crossed to the NG2-dsRed mouse, we hope to address the precise mechanism of PDGFs in regulation of pericyte function, and differential behavior of pericytes throughout development and in adulthood.

Zebrafish models

Lwaki Ebarasi

We use the zebrafish as a model organism for the study of angiogenesis, pericyte and mesangial cell biology, glomerular development and function in the context of the developing zebrafish embryo. We exploit the experimental advantages of rapid development, transparency, *ex utero* development, and a rapidly expanding arsenal of genetic tools to explore the cellular interplay and molecular regulators of these cells and processes.

Organogenesis and patterning are complete in the first two days of the developing zebrafish embryo's life. Endothelial, podocyte, erythrocyte, tubular, and astrocyte cell-specific reporter lines are some of the tools we apply in our research. The mechanisms, cell types and molecular regulation of angiogenesis and glomerular development and function in the zebrafish are the same ones at play in the higher vertebrates. We apply both forward and reverse genetic approaches to elucidate the molecular mechanisms important to endothelial cell and glomerular development, homeostasis, and function as well as high-resolution live imaging to study cellular behavior and interactions.

Group members during 2014

Christer Betsholtz, professor, group leader

Maarja Andaloussi Mäe, researcher

Johanna Andrae, researcher

Marco Castro, PhD student

Jana Chmielniakova, technician

Lwaki Ebarasi, post doc

Radosa Gallini, PhD student

Maria Leonor Segurado Gouveia, PhD student

Rajesh Gupta, researcher

Konstantin Gängel, research fellow

Liqun He, researcher
Jennifer Hofmann, post doc
Marie Jeansson, researcher
Bongnam Jung, post doc
Annika Keller, researcher
Barbara Lavina Siemsen, researcher
Thibaud Lebouvier, post doc
Helene Leksell, biomedical analyst
Krishnapriya Loganathan, PhD student
Colin Niaudet, researcher
Marta Oliveira, student
Cecilia Olsson, technician
Pia Peterson, technician
Elisabeth Raschperger, research fellow
Ebtisam Salem Said, student
Alexandros Sountoulidis, researcher
Michael Vanlandewijck, post doc

Funding during 2014

European Research Council (advanced grant), 4 500 kSEK
Knut and Alice Wallenberg Foundation (KAW Scholar), 3 000 kSEK
Swedish Cancer Society, 2 000 kSEK
Leducq Foundation, 1 200 kSEK
Swedish Research Council, 1 350 kSEK (C Betsholtz), 750 kSEK (M Jeansson)
Åke Wiberg foundation, 250 kSEK (M Jeansson)

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Regulation of Blood Vessel Formation

Lena Claesson-Welsh

Vascular endothelial growth factors (VEGF) are essential regulators of blood vessel formation, angiogenesis, and survival of existing blood vessels. VEGF was originally denoted VPF, vascular permeability factor (VPF), reflecting another essential function of VEGF. Angiogenesis is initiated by binding of VEGF to receptor tyrosine kinases, VEGFR1 and VEGFR2, on endothelial cells. VEGFR2 is the most important receptor for VEGF; activation of VEGFR2 by VEGF is essential for establishment of the vasculature during embryogenesis and for regulation of angiogenesis in physiological and pathological processes.

We employ *in vivo* models to study VEGF signal transduction in healthy organs, in tumors and in other disease models such as retinopathy and myocardial infarction. Our particular interest is to identify signal transduction pathways regulating i) vascular morphogenesis to create functional vessels that lead blood, and ii) vascular permeability, the process where fluid, molecules and cells leave the blood and extravasate into the surrounding tissue. We furthermore study the biology of the heparin-binding plasma protein histidine-rich glycoprotein (HRG), which acts on inflammatory cells and indirectly, on blood vessels. Treatment with HRG normalizes tumor vessels, and decreases metastatic dissemination. One important goal of our research is to exploit our findings for therapeutic applications.

Regulation of inflammation and angiogenesis by histidine-rich glycoprotein (HRG)

Hiroshi Kaito, Oriol Noguer, Frank Roche, Miguel Sainz Jaspeado, Ross Smith

The heparin-binding plasma protein HRG was originally identified as a regulator of tumor angiogenesis (Olsson et al., *Can Res* 2004, Dixelius et al., *Blood* 2006). We have shown in a number of models that expression of HRG in tumors results in reduced primary tumor growth and reduced metastatic spread. These effects of HRG depends on polarization of macrophages from an M2 to an M1 phenotype (Rolny et al., *Cancer Cell* 2011), accompanied by reduced production of angiogenic growth factors and promotion of an anti-tumor immune response. In accordance, peritoneal macrophages in the *hrg*^{-/-} mouse are arrested in M2, with 10-fold upregulated M2 markers, and the knockout mice grow larger tumors with increased metastatic spread (Tugues et al., *Can Res* 2012). Iodinated HRG binds specifically to mononuclear phagocytes and depletion of these cells using neutralizing CSF1R antibodies leads to accumulation of HRG in blood (Tugues et al., *PlosOne* 2014). Current aims include to identify the HRG-binding molecule, the HRG receptor, on mononuclear phagocytes, and to explore the potential therapeutic benefit of HRG in combinatorial cancer immunotherapy.

Regulation of angiogenesis and vascular permeability

Daisuke Fukuhara, Emma Gordon, Marie Hedlund, Naoki Honkura, Xiujuan Li, Eric Morin, Elisabet Ohlin, Narendra Padhan, Chiara Testini, Charlotte Wikner

Dysregulation of VEGF and its receptor VEGFR2 in tumors leads to exaggerated formation of leaky and dysfunctional vessels, which in turn promotes tumor invasiveness and metastatic spread. We have identified the *in vivo* signal transduction pathway regulating vascular permeability in response to VEGF. The pathway involves a particular phosphotyrosine, Y949 in VEGFR2, which binds the Src Homology 2 (SH2) domain-containing adaptor molecule TSAd (T cell specific adaptor) that in turn couples to the cytoplasmic kinase c-Src. Gene targeting to eliminate expression of TSAd results in a block in VEGF-induced vascular permeability. We study the effects of Tsad targeting and of Y949F VEGFR2 mutation on

vascular permeability and angiogenesis during development. We also study the role of the VEGFR2/TSAd pathway in a number of disease models such as cancer (melanoma, glioblastoma, insulinoma) and myocardial infarction (in collaboration with Prof. Jan Borén, Sahlgrenska Academy). In related project, we examine the biology of other VEGFR2 phosphotyrosine sites such as Y1212. We also work with tamoxifen-regulated floxed in vivo models in order to identify the expression patterns molecules regulating vascular permeability, and to eliminate expression of genes of interest, only in endothelial cells.

We furthermore address the role of VEGF coreceptors (heparan sulfate and neuropilin) in presentation of VEGF to VEGFR2, their ability to regulate VEGFR2 internalization and the subsequent biological response. We have shown that neuropilin-1 expressed on tumor cells can present VEGF to VEGFR2 expressed on endothelial cells (denoted “trans” presentation), leading to retention of VEGFR2 on the cell surface, reduced signaling and vascular quiescence (Koch et al., Dev Cell 2014).

Cancer signaling signatures

Narendra Padhan

By robotized nanofluidic isoelectric focusing in a ProteinSimple Nanopro 1000 equipment, we determine protein activity using antibody-mediated identification of posttranslationally modified (phosphorylated) signal transducers with unprecedented sensitivity and resolution. The strategy allows the identification of all posttranslationally modified versions of a particular signal transducer with one reagent, which saves time and resources. By combining patterns of signal transduction pathway events into algorithms, we can now predict normal tissue from cancer. The goal is identify human cancer stages with this technique.

Group members during 2014

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Funding during 2014

Swedish Cancer Foundation, 2 000 kSEK
Swedish Research Foundtion, 1 800 kSEK
AICR/Worldwide Cancer Research, 650 kSEK

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Tumor Vascular Biology

Anna Dimberg

Blood vessel formation and inflammation are closely linked processes that affect the clinical outcome of several pathological conditions, including cancer. Endothelial cells, lining the inside of vessels, are central players in both these processes. They initiate the formation of new vessels after growth factor stimulation and regulate extravasation of inflammatory cells from the blood stream into the tissue.

Tumor vessels are morphologically and functionally distinct from normal vessels, at least partially as a consequence of ongoing angiogenesis and extensive growth factor stimulation. Proteins specifically expressed in endothelial cells during tumor angiogenesis may constitute new targets for cancer treatment. Importantly, heterogeneous protein expression in tumor endothelium may affect leukocyte recruitment, permeability and establishment of a vascular niche. The focus of our research is to understand how the vasculature affects cancer progression through regulation of the tumor microenvironment.

Molecular regulation of vascular abnormalization in glioblastoma

Lei Zhang, Elise Langenkamp, Hua Huang, Liisi Laaniste, Xavier Catena Parrado, Johan Lööf, and Anna Dimberg

Glioblastoma, the most aggressive type of glioma, are characterized by high mitotic activity, nuclear atypia, microvascular proliferation, hemorrhage and necrosis. The median survival of adult glioblastoma patients is only twelve months. Extensive angiogenesis and markedly abnormal vessels are a hallmark of glioblastoma, leading to enhanced permeability and brain oedema. However, the molecular mechanisms that underlie the extensive morphological and functional changes observed in glioblastoma vasculature are largely unknown.

To identify changes in gene expression in abnormal GBM vessels, we have employed laser capture microdissection to isolate vessels from human GBM, low grade gliomas and normal brain and analyzed gene expression by microarray analysis. We identified 95 genes that are differentially expressed in glioblastoma vessels and found that many of these genes are induced in response to VEGF and/or TGF- β , growth factors highly expressed in the tumor microenvironment. We have selected some of the proteins that we found to be highly expressed in glioblastoma vessels and are currently investigating how these contribute to aberrant vascular function and tumor progression in glioblastoma.

Pleiotrophin is a small heparin-binding growth factor that is frequently expressed in human glioblastoma and low grade glioma, but not detectable in normal adult brain tissue. It is considered to be a pro-angiogenic growth factor, but its net effect appears to be context dependent as it can also oppose angiogenesis in some systems. In glioma, pleiotrophin has been shown to affect migration and proliferation of tumor cells that express its receptors. We are currently evaluating the role of pleiotrophin in regulation of tumor angiogenesis in glioblastoma.

Cross-talk between pro-angiogenic and pro-inflammatory signalling pathways in the tumor microenvironment and its impact on immunotherapy

Hua Huang, Maria Georganaki, Luuk van Hooren, Elise Langenkamp and Anna Dimberg

Tumor growth is significantly affected by recruitment of inflammatory cells. This process is regulated by *endothelial activation*, endothelial up-regulation of adhesion molecules that capture leukocytes and enable slow rolling, firm adhesion and transmigration into the tissue. Pro-angiogenic signalling in the tumor microenvironment affects endothelial activation

through negative crosstalk with pro-inflammatory signalling pathways. Also, the aberrant architecture and blood flow in combination with changes in endothelial gene expression may limit effector lymphocyte recruitment into the tumor.

The success of cancer immunotherapy relies on efficient recruitment of immune cells into the tumor mass. Despite recent breakthroughs, the tumor vasculature still presents a hurdle for infiltrating leukocytes that limits the efficacy of cancer immunotherapy in solid tumors. We are currently investigating how anti-angiogenic therapy affects endothelial gene expression and function, and the possible benefits and pitfalls of combining anti-angiogenic therapy with immunotherapy approaches. The goal is to find new combinatorial therapies for cancer.

Group members during 2014

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Maria Georganaki, PhD student
Hua Huang, PhD student
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Funding during 2014

Swedish Research Council, 1 000 kSEK
Swedish Cancer Society, 400 kSEK
Swedish Childhood Cancer Society, 300 kSEK
EU FP7 MC ITN TIMCC, 1 616 kSEK

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Neural Stem Cells and Brain Tumors

Karin Forsberg Nilsson

The overall goal of our research is an improved treatment of malignant brain tumors, in particular glioblastoma and medulloblastoma. In our projects we incorporate experience of neural stem cells with glioma biology, leveraging the close relationship between these two fields. We also investigate the neuro-inflammatory responses to traumatic brain injury and brain tumors. The main aims are:

- To establish how interactions between tumor cells and the extracellular matrix influence glioma development and stem cell differentiation
- Modeling glioma *in vitro* and exploring novel regulators of tumor formation

Some projects in the group focus on the role of mast cells in brain tumors.

Extracellular matrix interactions of importance for brain tumor formation and neural development

Soumi Kundu, Anqi Xiong, Grzegorz Wichor, Annika Hermansson, Argyris Spyrou and Lulu Rama Haseeb, Yuyuan Xiong, Misbah Riaz

The focus of this project is the “brain tumor niche” that allows tumor cells to detach from the original site, remodel the extracellular matrix (ECM) and migrate to seed new tumors that ultimately leads to death of the patient. Based on our increased understanding of the biochemical and molecular determinants of brain tumor invasion, new drug targets in the glioma microenvironment could be identified.

Heparan sulfate (HS) proteoglycans are main components of the ECM where they interact with a large number of physiologically important macromolecules, thereby influencing biological processes. HS modulate growth factor activities, and we have shown a vital role for HS in formation of the neural lineage (Forsberg et al., 2012). The major enzymatic activity degrading HS is heparanase. In this project we address HS proteoglycan biosynthesis and degradation in clinical brain tumor samples, human glioma and medulloblastoma cell culture as well as mouse and human models of glioma and medulloblastoma. We found that high heparanase expression is correlated to an enhanced tumor growth, both in teratoma and brain tumors. We aim to clarify the mechanism of heparanase activation in the enhancement of tumor growth. Furthermore, we examine the role for heparanase in neural differentiation.

Human glioma cell cultures as a new experimental platform

Grzegorz Wichor, Annika Hermansson, Argyris Spyrou, Lulu Rama Haseeb

Basic cancer research, including preclinical tumor models and testing of candidate drugs needs optimized *in vitro* models that better reflect the patient’s disease. There are major challenges in generating model systems at the scale necessary to demonstrate patient tumor heterogeneity. The availability of “tumor stem cell” culture techniques has opened the possibility to create well-characterized human tumor cell cultures. However, to establish these experimental tools requires simultaneous access to the technical know-how of culturing and analyzing cancer cells, and a systematic biobanking pipeline of patient tissue combined with clinical data acquisition. All these parameters are now in place at the Rudbeck Laboratory through a collaborative effort between K. Forsberg Nilsson, L. Uhrbom, B. Westermark, and S. Nelander, clinical collaborators G. Hesselager and I. Alafuzoff, Uppsala University Hospital and the U-CAN project (www.u-can.uu.se). In our recently published study (Kitambi et al, 2014) these cell lines were screened to identify cellular processes amendable for

development of targeted treatments. This is the first example of how the new platform can be used successfully towards novel therapeutic opportunities.

Investigating regulators for brain tumors and neural stem cells

Anqi Xiong

We previously reported that malignant brain tumors and neural stem cells share a common transcriptional signature (Demoulin et al, 2006) and selected the pseudokinase nuclear receptor binding protein 2 (NRBP2), for further study because of the high level of regulation (Larsson et al, 2008). Pseudokinases have high sequence similarity to mechanistically validated enzymes, but are devoid of the catalytic activity (NRBP2 lacks 7 out of 15 residues of the kinase domain) and are now increasingly viewed as components of signaling pathways. We are now working to identify the function of NRBP2 and its role in brain tumor development.

The role of IL-33 in development, brain injury and brain tumors

Grzegorz Wicher, Anastasia Magoulopoulou,

IL-33 has important functions in inflammatory and autoimmune diseases (Enoksson et al, 2013). Little is known, however, about IL-33 in brain development, injury and brain tumors. Our data suggest that IL-33 expression is under tight regulation in the normal brain but can be triggered by injury. Its detection during the first three weeks of postnatal life coincides with important parts of the CNS developmental programs, and opens the possibility of IL-33 involvement in normal developmental processes (Wicher et al, 2013). De novo expression of IL-33 after injury suggests involvement of this alarmin in the neuro inflammatory response. A high level of expression in glioma samples implies a role in tumor development and progression.

The role of mast cells in gliomagenesis

Elena Chugunova, Ananya Roy, Anna Sjösten

Human cancers maintain a complex inflammatory program triggering rapid recruitment of inflammatory cells, including mast cells (MCs), to the tumor site. MCs are crucial players in various inflammatory conditions, including cancer. The potential contribution of MCs in glioma has not been addressed previously.

Just recently we have expanded our understanding of the role of inflammation in gliomas by showing, for the first time, that MCs infiltrate mouse and human glioma, and that the extent of MC infiltration, both in mouse and human gliomas, shows a strong positive correlation with the malignancy grade of the tumor.

Considering novel data it becomes increasingly important to thoroughly elucidate new trends in interactions between MCs and glioma. i) The revealing of pro- or antitumorigenic role of MCs upon glioma development and presumably opposing MC functions depending on glioma grade. ii) The determination of conditions at which glioma cells cause the potential functional switch in MCs and iii) To what extent the parallels can be made between the well-defined mouse model and poorly understood human condition.

Glioma Derived Plasminogen Activator Inhibitor-1 (PAI-1) Regulates the Recruitment of LRP1 Positive Mast Cells

Elena Chugunova, Ananya Roy, Antoine Coum

Here we investigated the role of plasminogen activator inhibitor 1 (PAI-1), previously identified by us as a glioma secreted candidate, to MC recruitment.

PAI-1 is a primary regulator in plasminogen-plasmin or fibrinolytic system. It is capable of forming complex with fibrinolytic system proteins together with low-density lipoprotein receptor-related protein 1 (LRP1). Our study demonstrated the expression of LRP1 in LAD2 cells as well as revealed the presence of LRP1 in MCs in human high-grade glioma tissue. The activation of potential PAI-1/LRP1 axis with purified PAI-1 promoted increased phosphorylation of STAT3 and subsequently exocytosis in MCs.

In conclusion, these findings indicate the important influence of PAI-1/LRP1 axis on the recruitment of MCs in gliomas, suggesting that this information could be used to improve patient stratification in future therapeutic trials.

Mast cell contribution to brain metastasis

Elena Chugunova, Ananya Roy, Ida Gustavsson

Brain metastases are becoming an important problem because of the progressive neurological disability and the lack of effective treatment due to the unique structure of the blood-brain-barrier (BBB). Recent studies in this field point towards a link between the immune system and metastases pathogenesis but many aspects still need to be investigated. In order to clarify the role of MCs and other immune cells in brain metastasis we aim to understand the mechanisms underlying the MC-brain metastatic cell interactions and identify key factors regulating these interactions.

Our preliminary data, for the first time, demonstrated the abundant accumulation of MCs in human brain metastases originated from different primary tumors (lung, prostate, kidney, ovarian and rectum). We expect MCs to contribute to the expansion of angiogenesis within brain metastases with specific addressing the role for MC proteases in this process. We plan to investigate MC-brain metastases cell interaction (*in vitro* studies), early stages of brain metastasis development (*in vivo* studies), as well as gather clinical data by exploring patient brain metastases samples and corresponding primary tumors. Our final goal is to reveal the correlation in these studies and support it with mechanistic findings.

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Swedish Research Council, 700 kSEK

Swedish Cancer Society, 500 kSEK

Swedish Childhood Cancer Foundation, 400 kSEK

Elena Chugunova

Swedish Research Council, 1 195 kSEK (incl. funding for E Chugunova's position)

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Characterization of Novel Regulators of Blood Vessel Formation

Mats Hellström

In recent years scientists have clarified how important the formation of new blood vessels is in various diseases. Despite this fact, there is still a lack of knowledge about the signalling pathways that regulate blood vessel formation and only a few blood vessel-specific drugs have been developed.

Our research focuses on finding signalling components that are specific for endothelial cells, the cells that line the inner walls of blood vessels. We have identified several possible candidates and one of these, called paladin, we have analysed in more detail. We have shown that mice that lack paladin have altered blood vessels in the retina, and we are studying the role of paladin in tumour development. Paladin belongs to a group of proteins that are commonly involved in cell signalling. We hope that our results will contribute to an increased understanding of signalling during the formation of new blood vessels in tumours.

Characterization of Novel Regulators of Blood Vessel Formation

Isabel Egana, Anja Nitzsche, Chiara Testini

Although the importance of angiogenesis in pathological conditions is well established few blood vessel-specific drug targets have been identified and information is still limited about endothelial-specific molecular pathways. Hence, there is a great need to better characterize the process in order to provide new ideas for improved and novel therapies.

In the search for endothelial-specific regulators we have used several approaches, including expression profiling of mouse vasculature and other mouse tissues, zebrafish gene knock down, and screening of drug-like compounds in human cellular assays. This led to the identification of several new regulators of angiogenesis, including kiaa1274/x99384/Pald1 (or Paladin), a putative cytoplasmic S/T/Y phosphatase. Paladin is one of the first examples of a cytoplasmic, potential phosphatase with an endothelial-specific expression.

We have generated a mouse knock-out for *Paladin* with a functional β -galactosidase reporter, verifying endothelial specificity in many mouse tissues. We have characterized the expression pattern of mouse and human Paladin during development and in cancer tissue. Paladin is preferentially expressed in the vasculature and shows a dynamic expression pattern changing from expression in capillaries and veins during development, to vascular smooth muscle cells in arteries in the adult organs. The knock-out mice are viable and fertile. Our preliminary data show that Paladin knock-outs display increased vascular density in the postnatal retina. We plan to further study vascularization of normal tissues as well as tumors. We will also perform comprehensive biochemical and signal transduction analyses *in vitro*, including over-expression and siRNA knock down of *Pald1*.

Kinases belong to an important drug target class in oncology, which strongly suggests that our studies on *Pald1* will contribute to the understanding of kinase/phosphatase signaling in general and angiogenesis/tumor angiogenesis in particular.

Group members during 2014

Mats Hellström, researcher, group leader

Isabel Egaña, post doc

Anja Nitzsche, PhD student

Chiara Testini, PhD student

Funding during 2014

Swedish Cancer Society, 800 kSEK

Publications 2012-2014

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Regulation of lymphatic vasculature

Taija Mäkinen

Lymphatic vasculature constitutes a network of vessels critical for the maintenance of the body's fluid balance. Failure of the lymphatic vessels can lead to a disabling disease called lymphoedema for which there is no cure or effective treatment. Recent studies have revealed important new roles of lymphatic vasculature in inflammation, immunity, lipid metabolism, blood pressure regulation and cancer metastasis. Understanding mechanisms of lymphangiogenesis may thus enable development of new therapies for common diseases that affect a large number of people worldwide.

Our laboratory aims to understand, at the molecular level but in the context of a living organism, the regulation of lymphatic vascular morphogenesis. We utilise and develop advanced mouse genetic tools to spatially and temporally control expression of genes in specific cell types of interest. By identifying and functionally characterising genes causative of hereditary lymphoedema we additionally aim to uncover mechanisms of vascular development that are directly relevant to human pathology.

Organ- and vessel-type –specific mechanisms of lymphatic development

Lukas Stanczuk, Ines Martinez-Corral, Maria Ulvmar, Yang Zhang, Henrik Ortsäter

The lymphatic system is composed of a hierarchy of vessels with specific features serving their unique functions: the blind-ended lymphatic capillaries that absorb the interstitial fluid and the collecting lymphatic vessels that transport the lymph to the cardiovascular system. Failure of the lymphatic vessels, caused by a genetic defect (primary) or damage following surgery or radiation therapy (secondary) can lead to lymphoedema, which is a progressive and lifelong condition characterised by gross swelling of the affected tissue. Notably, several primary lymphoedemas are characterised by defects that affect specifically either the collecting vessels or the capillaries. In addition, specific area(s) of the body are affected in different types of lymphoedemas. What underlies tissue-specific vessel failure is not understood yet this knowledge is instrumental in designing therapeutic strategies for lymphoedema and other lymphatic disorders that are currently lacking. In this project we will identify genes and mechanisms required for organ-specific lymphatic vascular development by characterising the features of specific lymphatic vascular beds, and by identifying and functionally characterising genes regulating lymphatic development in an organ- and/or vessel-type specific manner using genetic mouse models.

Functional characterisation of causative genes for human primary lymphoedema

Ines Martinez-Corral, Andrea Taddei (London), Maike Frye

Recently gained insights into mechanisms of lymphangiogenesis have been driven by the characterisation of animal models with specific lymphatic defects, and identification of genes causative of human primary lymphoedemas. In collaboration with Steve Jeffery, Peter Mortimer and their teams at St George's Hospital in London, we have recently identified *GATA2* and *KIF11* as two novel causative genes for primary lymphoedema by whole-exome sequencing. We are currently investigating the biological function of *GATA2* and *KIF11* in lymphatic development by combining state-of-the-art mouse genetics with in vitro studies on primary lymphatic endothelial cells. The results from this project are expected to increase our understanding of normal lymphatic development and pathophysiological mechanisms involved in lymphoedema and other lymphatic disorders.

Group members during 2014

Taija Mäkinen, senior lecturer, group leader

Maike Frye, post doc

Ines Martinez-Corral, researcher

Henrik Ortsäter, research engineer

Lukas Stanczuk, researcher

Maria Ulvmar, researcher

Andrea Taddei (post doc, Cancer Research UK London Research Institute)

Yang Zhang, PhD student

Funding during 2014

Swedish Research Council, 1 000 kSEK

Swedish Cancer Society, 1 250 kSEK

Beijer Foundation, 1 000 kSEK

Medical Faculty, 500 kSEK

Publications 2012-2014

(The group came to IGP in 2013 and some papers have therefore not been published with IGP as affiliation)

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New strategies to understand and target brain cancer

Sven Nelander

The group develops new, systems biology based strategies to chart the molecular networks that cause cancer. The goals are to understand the importance of regulatory changes in cancer cells and to guide the development of new anticancer therapies, tailored to target specific vulnerabilities in the individual patient.

We mainly focus on the brain tumor glioblastoma, which affects more than 10,000 Europeans every year. We are developing a unique platform to study the cancer stem cell (CSC) population of these tumors. CSCs represent a small fraction of cancer cells that play a crucial role in disease progression and recurrence.

Using a Swedish collection of more than 100 patient-specific CSC cultures, we are applying high resolution molecular profiling, computer modeling and cell experiments using microfluidic technology, which enables us to track complex cellular responses to drugs.

We also develop new computational tools and resources for cancer research, such as EPoC and the new resource Cancerlandscapes.

High resolution molecular mapping and functional biology of brain tumor stem cell lines

Linnéa Schmidt, Sathishkumar Baskaran, Santhanam Kulasekara, Voichita Marinescu, Ludmila Elfineh

In this project, we develop systems biology strategies for the targeting of cancer stem cells (CSCs) in individual patients suffering from glioblastoma. CSCs are crucial for the maintenance and progression of these cancers, but the systems-scale characterization of CSCs has so far been limited by the lack of relevant model systems for large-scale functional studies. Our project takes advantage of the Human Glioma Cell Culture—HGCC biobank, a world-unique clinical material that comprises an extensive collection of early-passage glioblastoma CSC cultures derived from more than a hundred consecutive patient cases at Uppsala University Hospital during 2012-2014.

Unlike the classical cell lines used in the field today, the HGCC lines (i) have the defining characteristics of CSCs (expression of neural stem cell markers, sphere formation and tumor initiating capacity), (ii) are collected in a consistent manner in the same institute, and linked to the excellent Swedish clinical registries; and, (iii) are cultured in defined, serum free medium for a short period of time. Our effort involves several coordinated activities:

- Systematic testing of HGCC line vulnerabilities at the SciLifeLab facilities
- Development of computational models to predict the vulnerability of each cell line
- Using state of the art mouse models to evaluate therapeutic strategies

The work is highly inter-disciplinary and involves collaborations with the IGP neurooncology groups (Urbom, Forsberg-Nilsson, Westermark), SciLifeLab screening and imaging platforms (Thomas Helleday, Bo Lundgren, Carolina Wählby), as well as international partners (Stella Carro, Freiburg).

Computational strategies to understand the molecular diversity of human cancers

Patrik Johansson, Teresia Kling, José Sanchez, Rebecka Jörnsten

A major challenge in current cancer research is to gain biological insight from large scale molecular data from patient samples. In this project, we invent new mathematical methods to construct regulatory maps of individual cancer diagnoses. Our system uses data from both

public sources and from IGP/SciLifeLab and is implemented on national computing infrastructure (UPPMAX and C3SE). The results are made available on a new web resource, Cancerlandscapes, which will open soon. A unique feature of Cancerlandscapes is that very complex data become available in an intuitive form, which lab biologists can use to design experiments.

Group members during 2014

Sven Nelander, Associate Professor, group leader

Satishkumar Baskaran, PhD student

Ludmila Elfineh, research engineer

Evgenia Gubanova, post doc

Lukasz Huminiecki, researcher

Patrik Johansson, PhD student

Marianne Kastemar, technician

Cecilia Krona, researcher

Ingrid Lönnstedt, researcher

Torbjörn Nordling, research assistant

Linnea Schmidt, researcher

Part of the group is working in Gothenburg

Teresia Kling, PhD student

Funding during 2014

Swedish Cancer Society, 3 000 kSEK

Swedish Research Council, 4 500 kSEK

Swedish Childhood Cancer Foundation, 1 350 kSEK

Publications 2012-2014

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Development of Childhood Brain Tumors and Targeting of MYC Proteins

Fredrik Swartling

MYC proteins (like MYC or MYCN) are transcription factors and potent mitogens with essential roles in normal brain development. Misexpression of MYC proteins occurs frequently in medulloblastoma, the most common malignant childhood brain tumor of the hindbrain. *MYC* or *MYCN* amplifications in medulloblastoma are strongly correlated with poor prognosis suggesting MYC proteins are clinically relevant targets for brain tumor therapy. MYC proteins are also overexpressed in glioma, adult malignant brain tumors of the forebrain.

Our research group is exploring how MYC proteins are stabilized in malignant brain tumors with a focus on identifying cells of tumor origin as well as understanding critical signaling pathways and new treatments for MYC/MYCN-driven brain tumors. We have generated clinically relevant models for MYCN-driven brain tumors and we also study a large number of primary cell lines obtained from childhood brain tumor patients.

In search for the cellular origin of MYCN-driven medulloblastoma

Sara Bolin, Holger Weishaupt and Fredrik Swartling

We recently showed that MYCN could generate tumors from a glutamate transporter (GLT1) promoter in a transgenic inducible model (GTML) of medulloblastoma (Swartling et al. *Genes & Dev.*, 2010). By mapping cellular fate we found that GLT1-positive neural stem cells (NSCs) represent putative cells of brain tumor origin. GTML mice generate aggressive medulloblastoma after about 3-6 months. Before tumor onset we found significantly more proliferating cells in thalamic forebrain cells and of cerebellar Bergmann glia as compared to controls. Currently we study cellular fate using the promoter for the glial cell marker BLBP as well as retroviral tagging with the RCAS/tv-a system to understand how tumors develop. We are also isolating putative cells of tumor origin using laser-capture microdissection. Detailed bioinformatic analysis of expression profiles of laser-captured cells or distinct brain regions are now being compared in order to reveal the cellular origin for these malignancies.

FBW7 regulates MYCN protein stabilization during brain tumor formation

Sanna-Maria Hede, Vasil Savov and Fredrik Swartling

Medulloblastoma can be divided into four distinct molecular subtypes (WNT, SHH, Group 3 and Group 4). Group 3 and 4 medulloblastoma often show amplifications of MYC and MYCN, respectively, and correlates with the worst prognosis and poor patient survival. MYC proteins are unstable oncoproteins with short half-lives. We recently found that protein stabilization of MYCN is essential for brain tumor initiation (Swartling et al. *Cancer Cell*, 2012). MYCN stability is regulated by the ubiquitin ligase FBW7, which normally targets it for proteasomal degradation. FBW7 is a tumor suppressor gene mutated in various types of cancer including medulloblastoma and we study loss of function of FBW7 in our animal models of medulloblastoma. We have crossed FBW7 knock-out mice to GTML mice and currently study how FBW7 loss alters brain tumor formation. Molecular profiles/subtypes of these novel brain tumors will be analyzed using advanced cross-species comparisons.

A new model for childhood brain tumor recurrence

Vasil Savov, Gabriela Rosén, Holger Weishaupt and Fredrik Swartling

Tumor recurrence is the main cause of death in children with medulloblastoma. In this project we are studying how MYCN interacts with SOX9, a transcription factor involved in glial fate

determination in the brain. Few scattered SOX9-positive cells are found in GTML tumors that are similar to Group 3 or Group 4 human MB. By using a combination of Tet-ON and Tet-OFF inducible systems we managed to target this rare population of SOX9-positive GTML tumor cells *in vivo* to show how they were capable of initiating recurrence after primary tumor removal. The relapsed medulloblastoma has similar characteristics as the initial one but develops at a distant site in the brain, in line with recent human tumor data. We further showed that isolated metastases in Group 3/4 patients had consistently higher SOX9 levels as compared to corresponding primary tumors. Further characterization of SOX9-positive cell types will help us understand the mechanisms behind metastatic medulloblastoma recurrence.

Primary cilia loss in malignant brain tumors driven by stabilized MYCN

Sanna-Maria Hede, Anna Borgenvik and Fredrik Swartling

Primary cilia are tiny organelles that could be described as the cells antenna as they protrude on the surface of cells. This structure is essential for SHH signaling (described above). We are studying primary cilia loss during brain tumor formation and have found a strong correlation with lack of cilia and the most aggressive types of MYCN-driven brain tumors, especially as MYCN is stabilized by certain mutations (at residue T58 of the protein). We are presently investigating how oncogenes downregulate cilia from the surfaces of cultured NSCs and tumor cells. Cilia length and structure are analysed by confocal and electron microscopy. We are also studying how loss of cilia affects brain tumor formation *in vivo*, by crossing our brain tumor models to mice lacking KIF3A, a protein essential for cilia formation. In this project we also study primary cilia loss in primary cultures from patient samples from both medulloblastoma and from adult brain tumors, glioma. Our goal is to understand the role of the primary cilia in tumor initiation and study how these antenna-like structures are lost during brain tumor progression and if we can use this information to prevent disease.

Targeting MYCN through Bromodomains and by using CDK2 inhibitors

Sara Bolin, Holger Weishaupt and Fredrik Swartling

We recently showed that MYCN levels and early proliferation of brain tumors could be reduced by specific inhibition of the bromodomain inhibitor JQ1, which targets MYC proteins epigenetically (Bandopadhyay et al. Clin Can Res., 2014). We also found good efficacy controlling MYCN phosphorylation and stabilization by using a CDK2 inhibitor called Milciclib. Both drugs induced tumor cell senescence or apoptosis in our brain tumor models and also in primary human brain tumor cells. As compared to either drug alone, when combining the two drugs we further reduced MYCN levels and completely abolished brain tumor growth after long-term treatment *in vitro*. We are currently evaluating these treatment effects in our models *in vivo*. Our goal is to understand the underlying mechanisms of this MYCN inhibition and further evaluate the potential of using these promising drugs in the clinic.

New models using human hindbrain cells to study medulloblastoma development

Matko Čančer, Anna Borgenvik, Sara Bolin, Holger Weishaupt and Fredrik Swartling

In this project we are transforming human hindbrain neural stem cells in order to model the different subgroups of medulloblastoma using lentiviruses carrying clinically relevant cancer driver genes for the distinct tumor subgroups. We will evaluate the ease and relevance in using well-defined human hindbrain stem cells to generate childhood brain tumors and we will compare these to subtype-specific cells similarly cultured from medulloblastoma patients. We hope we will understand what actually drives the initiation of various molecular

subgroups of medulloblastoma and if the various subgroups match certain human hindbrain cell types. Finally, we will use genetic and epigenetic analyses to predict how these cells could be specifically treated or if they would be resistant to targeted therapies.

Using a forward genetics screen to identify novel cancer-causing genes in brain tumors

Holger Weishaupt, Matko Čančer, Gabriela Rosén, Sara Bolin and Fredrik Swartling

We use a tumor model to study human glioma development from cell-type specific and retrovirus-driven Platelet-Derived Growth Factor (PDGF)-B overexpression. Virus integration into the host genome presents a risk for insertional mutagenesis, which can alter proximate genes, giving a particular tumor cell an advantage over other cells during tumorigenesis.

We have used whole genome sequencing (WGS) to identify genes that, together with clinically relevant cancer drivers like PDGF-B, contribute to tumor development. For this purpose we have developed a streamlined analysis pipeline for integration detection, followed by integration site annotation against functional genes and enhancers from reference genome assemblies. We hope to identify several common integration sites that harbor novel important genes that promote glioma in collaboration with the PDGF pathway.

Group members during 2014

Fredrik Swartling, researcher, group leader

Sara Bolin, PhD student

Anna Borgenvik, PhD student

Matko Čančer, PhD student

Sanna Hede, researcher

Gabriela Rosen, lab technician

Vasil Savov, PhD student

Holger Weishaupt, post doc

Funding during 2014

Ragnar Söderbergs Stiftelse, 1 200 kSEK

Åke Wibergs Stiftelse, 1 000 kSEK

Vetenskapsrådet, 950 kSEK

AICR/WCR, 800 kSEK

Cancerfonden, 500 kSEK

Barncancerfonden, 400 kSEK

Svenska Läkaresällskapet, 260 kSEK

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A cell of origin-based strategy to decipher glioma biology

Lene Uhrbom

Glioma is a large and heterogenous group of primary CNS tumors comprising astrocytoma, oligodendroglioma and ependymoma of all malignancy grades (I-IV). Glioma can strike at any age but the majority of patients are adult at diagnosis. Only grade I tumors are benign while grade II-IV tumors are malignant. Glioblastoma is a grade IV glioma and the most common form of all primary malignant brain tumors with dismal prognosis and essentially no cure. *In my group we study all types of malignant glioma with a particular interest in glioblastoma.*

Recent large-scale efforts to map the genetic and epigenetic landscape of glioblastoma has led to a comprehensive molecular characterization of a great number of tumors which has provided highly valuable information on the molecular basis of glioblastoma. Notwithstanding, the cell of origin for glioma (including glioblastoma) remains unknown. It is generally presumed to be a neural stem cell or glial progenitor cell but this has not been formally proven. For a complete understanding of glioma biology we believe that it is essential to know from where it originates and how that affects the phenotype of the tumor.

My research is focused on understanding how various glioma-relevant genetic alterations affect tumor development, progression and response to treatment depending on which cell type the tumor originated from. The goal is to uncover glioma-specific mechanisms to which directed therapies will be tested in our pre-clinical models.

To perform our studies we have built up an experimental platform consisting of several transgenic mouse models in which different and defined CNS cell types can be targeted to develop glioma (RCAS/tv-a) and many different types and grades of life-like glioma can be modelled. In addition, we have a large biobank of new human glioma cell cultures (HGCC) maintained under stem cell conditions to enrich for glioma stem cells. The HGCC project is performed in collaboration with Karin Forsberg-Nilsson, Sven Nelander and Bengt Westermark. In all, our mouse and human glioma models provide a unique and relevant platform for our basic and pre-clinical glioma research.

Projects

- **Establishment of the HGCC biobank of cultured glioblastoma cells.**
Yuan Xie, Yiwen Jiang, Smitha Sreedharan, E-Jean Tan, Marianne Kastemar, in collaboration with Karin Forsberg-Nilsson, Bengt Westermark and Sven Nelander
- **Cell of origin for glioblastoma as a basis for stratification, target identification and drug screening.**
Yiwen Jiang and Yuan Xie, in collaboration with Voichita Marinescu, Sven Nelander, Rolf Larsson, Mårten Fryknäs, Malin Jarvius and Caroline Haglund
- **The interplay between cell of origin, oncogenic activation and developmental age in glioma development.**
Smitha Sreedharan and Yuan Xie
- **Role of LGR5 in glioma stem cells.**
Yuan Xie and Yiwen Jiang, in collaboration with Voichita Marinescu, Sven Nelander, Andries Blokzijl and Ulf Landegren
- **Oligodendrocyte precursor cells as origin of both astrocytoma and oligodendroglioma.**
Yiwen Jiang, Yuan Xie and Marianne Kastemar, in collaboration with Nanna Lindberg and Eric Holland, FHCRC, Seattle

- **Investigations of human glioblastoma cell cultures of the mesenchymal subtype.**
E-Jean Tan and Yuan Xie

Group members 2014

Lene Uhrbom, researcher, group leader
Ann-Charlotte Hellström, research technician
Yiwen Jiang, post doc
Marianne Kastemar, research technician
Smitha Sreedharan, post doc
E-Jean Tan, post doc
Yuan Xie, PhD student

Funding during 2014

Swedish Research Council, 700 kSEK
Swedish Cancer Society, 800 kSEK (project grant), 1 060 kSEK (Senior Investigator Award)

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Forsberg-Nilsson K, Westermark B, Uhrbom L, Linnarsson S, Nelander S, Andäng M. Selective calcium sensitivity in immature glioma cancer stem cells. *PLoS One*. 2014, 9(12):e115698.

9. Etomidate, propofol and diazepam potentiate GABA-evoked GABAA currents in a cell line. Babateen O, Jin Z, Bhandage A, Korol SV, Westermark B, Forsberg Nilsson K, Uhrbom L, Smits A, Birnir B. derived from human glioblastoma. *Eur J Pharmacol*. 2015 Feb 5;748:101-7. Epub 2014 Dec 12.

Human Malignant Glioma – from Oncogenic Mechanisms to Treatment

Bengt Westermark

Our research is focused on glioblastoma, the most common form of malignant brain tumors in adults. Our main goal is to understand the molecular mechanisms of glioblastoma development. This knowledge may increase the possibilities of developing novel treatment modalities.

Human glioblastoma cell lines are established from fresh tumor surgical specimens taken in connection with brain tumor surgery. The aim here is to identify novel lead substances that inhibit tumor cell growth.

CGGBP1 in cell cycle checkpoint regulation and telomere protection

Umashankar Singh, Bengt Westermark

Using a genetic screen in mice, we have identified a number of glioblastoma candidate genes. One of these, NFIX, was used as a bait to find binding partners in a yeast-two-hybrid screen. One of the binding partners, the transcriptional regulator CGGBP1 is involved in DNA damage response, localizes to midbodies, regulates abscission and prevents tetraploidy. A novel role of CGGBP1 as a protector of telomeres was studied in human diploid fibroblasts. Expression of a mutated form of CGGBP1, in which an ATR phosphorylation site (Serine-164) has been mutated, leads to telomere shortening, DNA damage response at telomeres, telomere fusions resulting in chromatin bridges between dividing cells, and cell cycle arrest. The finer mechanistic details of CGGBP1 as a protector of telomeres are being analyzed. Further, we have found by chromatin immunoprecipitation sequencing that CGGBP1 binds to repetitive DNA sequences of the LINE1 and Alu families and regulates Alu expression.

Search for candidate drugs for the treatment of malignant glioma

Anna Segerman, Bo Segerman, Mia Niklasson, Tobias Bergström, Erika Lundin, Bengt Westermark

We aim to identify novel targets and lead substances with the ultimate goal to improve glioblastoma therapy. While taking tumor heterogeneity into account, we will characterize the subgroup of tumor cells with relapse potential (glioma initiating cells, GICs).

Glioma cell lines are continuously established from fresh biopsies and characterized with regard to genotype (structural alterations in known oncogenes and suppressor genes), phenotype (e.g. expression of stem cells and differentiation markers and tumorigenicity in immunocompromized mice) and treatment response using the standard glioma regimen (radiation and temozolomide). Selected cell lines and clonal derivatives are subjected to transcriptome and proteome analysis to define biomarker signatures.

Using growth inhibition as endpoint, we analyze the response of individual glioma cell lines to BMP4. Using CRISP-Cas9 knock out technology, we aim to define BMP4 signaling in growth inhibition. Further, chemical libraries of a total of >20,000 compounds will be used for high throughput screening. After substantial in vitro testing, the efficacy of the identified substances and combinations will be analyzed in vivo (orthotopic xenotransplantation in mice).

The role of Sox21 as a suppressor gene during glioma progression

Maria Ferletta, Erika Lundin, Bengt Westermark

The transcription factor Sox2 is required for maintaining the pluripotency of embryonal stem cells. Sox2 is expressed in neuronal stem cells and down regulation of Sox2 is accompanied

by neuronal differentiation. We have shown that both Sox2 and Sox21 are expressed in adult and pediatric brain tumors and that the expressions of the transcription factors are correlated. Our *in vitro* studies indicate that Sox21 can down regulate Sox2 in glioma cells and the *in vivo* studies show that an up regulation of Sox21 decreases the tumor growth significantly as well as prolong the survival extensively. Sox21 appears to decrease the stem-like cell properties of the tumor cells and induce abnormal differentiation and apoptosis as well as reduce cell proliferation in glioma cell *in vivo*. Further, tumor cells with increased expression of Sox21 demonstrated an improved formation of Sox2:Sox21 complexes. Our studies indicate that Sox21 function as a tumor suppressor during gliomagenesis mediated by a shift in the complex formation of Sox2:Sox21. These results imply that the Sox2/Sox21 axis could be a potential therapeutic component.

So far very little is known about which signaling pathways Sox21 take part in, so to investigate that we have performed cDNA arrays to identify signaling pathways and components important for mediating the suppressor effect of Sox21 in glioma cells. We are at the moment focusing on the TGF- β /BMP4 signaling pathways and the JAK/STAT-signaling pathway. In addition we will study if these signaling pathways or if the Sox2/Sox21 axis can be inhibited by low molecular weight inhibitors to prevent brain tumor progression.

Molecular studies of growth and carcinogenesis in the thyroid gland

Nils-Erik Heldin

Undifferentiated (anaplastic) tumors are highly malignant, rapidly growing and invasive, and constitute a major clinical problem. This project focuses on anaplastic thyroid carcinoma (ATC) and our aim is to elucidate the genetic events involved in generating the tumor.

Our laboratory has established several cell lines from human anaplastic thyroid cancer biopsies. Analyses of their karyotypes showed an abundance of double minute chromosomes (DMs) in two of the cell lines. DMs are known to harbour amplified gene sequences. With this in mind, we are currently using “next generation” sequencing technology to identify the amplified sequences.

Group members during 2014

Bengt Westermark, professor, group leader
Tobias Bergström, post doc
Maria Ferletta, researcher
Nils-Erik Heldin, associate professor
Marianne Kastemar, lab technician
Erika Lundin, research engineer
Mia Niklasson, researcher
Anna Segerman, researcher
Bo Segerman, associate professor
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Jacob Wall, student
Ann Westermark, teaching assistant

Funding during 2014

Swedish Cancer Society, 1 500 kSEK
Knut and Alice Wallenberg Foundation, 6 000 kSEK

Publications 2012-2014

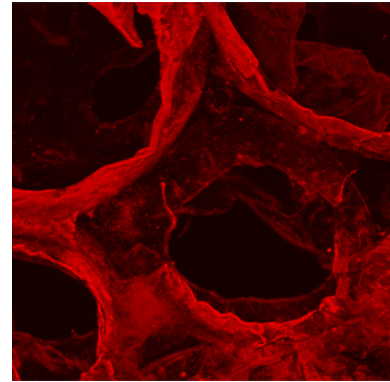
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Clinical Immunology

The Clinical Immunology research groups have a strong translational focus. The research projects aim to increase the understanding about immunological mechanisms in patients with cancer or autoimmune disease (diabetes, rheumatoid arthritis, systemic lupus erythematosus or multiple sclerosis) and to explain the immune reactions that occur when immune cells or components come in contact with biomaterial or cells or viruses used for therapy.

Within this research area we are developing novel immune, gene and cell therapies and diagnostic/prognostic markers, which are tested in clinical trials in collaboration with the Uppsala University Hospital, other national and international universities, the immune diagnostic industry, EU networks and the Nordic Network for Langerhans Cell Transplantation.



Gene, Cell and Immunotherapy of Cancer

Magnus Essand

Immunotherapy is currently being established as cancer treatment. Immune checkpoint blockade antibodies that fine-tune T cell activity to kill tumor cells can cure 20-25% of patients with refractory melanoma, and adoptive transfer of patient-derived T cells that are engineered *ex vivo* to express a chimeric antigen receptor (CAR) results in complete remission in a majority of patient with refractory B cell acute lymphoblastic leukemia. Oncolytic vaccines, which are replication-competent viruses engineered to selectively kill tumor cells and deceive the immune system to believe that the tumor is a foreign entity that needs to be eradicated are emerging as the next breakthrough in cancer immunotherapy.

Although successful in part, immunotherapy has so far not delivered for most forms of cancer. It is apparent that the technologies need to be improved and refined further to optimize treatments. We are focusing on translational cancer immunotherapy research specializing on virus, dendritic cell (DC) and T cell modifications to develop new potent anti-cancer agents. Some virus and T cells developed in our laboratory are already in clinical phase I trials and others are about to enter clinical trials.

Oncolytic Virus Therapy

Viruses are genetically engineered to selectively kill tumor cells and induce a potent and adequate anti-tumor immune response. Virus infectivity is altered through genetic modification of the virus capsid to favor infection of tumors cells. Virus replication is altered by introduction of regulatory elements, such as promoter and/or microRNA target sequences into the virus genome to specifically control viral gene expression to tumor cells. Progeny virus, produced upon virus replication in tumor cells, can infect neighboring tumor cells, thus amplifying the initial inoculum. The lytic cell death induced by virus is not dependent on the ability of the tumor cell to go into apoptosis, thus also drug-resistant cancer stem cells can be killed. Furthermore, the presence of immunogenic virus in the tumor microenvironment can alter the otherwise immunosuppressive milieu in favor of an anti-tumor immune response. To further boost adequate anti-tumor immune responses the virus encodes a transgene, such as

Helicobacter pylori neutrophil-activating protein (HP-NAP), for a Th1-type immune activation.

In order to target metastatic sites we are evaluating various cell types as carriers to deliver oncolytic virus at the tumor site. In particular, we are evaluating macrophages as virus carriers, in collaboration with Claire Lewis at Sheffield University, as macrophages are attracted to inflammatory cancer environments. We focus our efforts on prostate cancer, neuroendocrine cancer and neuroblastoma and we are primarily working with oncolytic adenovirus but also Semliki Forest virus and Vaccinia virus.

T Cell Therapy

T cells are genetically engineered to express novel T cell receptors (TCR) or chimeric antigen receptors (CAR) that recognize antigens that are expressed and presented by u cells. This way the engineered T cells specifically target and kill tumor cells. We have recently cloned a TCR against a prostate tumor-associated antigen called TARP and shown that genetically engineered T cells expressing this TCR can selectively kill prostate and breast cancer cells. We are also developing CAR T cells targeting PSMA on prostate cancer cells, GD2 on neuroblastoma or CD19 on B cell malignancies.

Besides developing new CAR transgenes for T cell therapy we are also developing new viral vectors (lentivirus and adenovirus) for efficient transfer of CAR transgenes to T lymphocytes and other hematologic cells. In addition, we are developing optimized protocols to expand the engineered T cells to make them resistant to oxidative stress and immunosuppressive factor.

Allogeneic DC Vaccination

Patient-derived DCs modified with u-associated antigens have been evaluated as therapeutic cancer vaccines with some success. It has however become clear that *ex vivo*-modified DCs are short-lived when re-injected and do not migrate to draining lymph nodes. The therapeutic effect obtained from administration of *ex vivo*-modified DCs, with respect to functionality and maturation characteristics, appears to come from resident tissue (bystander) DCs that take up material from dying injected DCs and bring it to lymph nodes for antigen presentation to naïve T and B cells.

We therefore investigate if allogeneic DCs (DCs from a different individual) or a DC cell line can be used instead of patient-derived DCs. The logistic would be simplified and costs would be significantly reduced. Importantly, the HLA mismatch will most likely act as a strong adjuvant both for NK and T cells. We perform both efficacy studies and mechanistic studies in mice to evaluate which cell types are attracted and activated in response to allogeneic DCs. We use real-time intravital confocal microscopy imaging, in collaboration with Mia Phillipson, Uppsala University to study these events. We also investigate whether the therapeutic effect can be improved if the allogeneic DCs are transduced with an adenoviral vector secreting HP-NAP, IL-1b and other immune modulators.

Group members during 2014

Magnus Essand, professor, group leader
Grammatiki Fotaki, PhD student
Victoria Hillerdal, postdoc
Alex Karlsson-Parra, adjunct professor, chief physician
Chuan Jin, PhD student
Jing Ma, scholarship student
Justyna Leja-Jarblad, researcher

Berith Nilsson, project leader
Mohanraj Ramachandran, PhD student
Di Yu, post doc

Dissertations during 2014

Victoria Hillerdal, The Multiple Faces of Genetically-Modified T Cells: Potential Applications in Therapy, November 15, 2014.

Funding during 2014

Swedish Cancer Society, 800 kSEK
Swedish Childhood Cancer Foundation, 500 kEK
Swedish Research Council, 600 kSEK
Immunicum, 600, SEK
Donations for clinical trial, 1 500 kSEK

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Diabetes Research

Olle Korsgren

Our research focuses on the cause of diabetes and on possibilities to prevent and cure the disease. The research has a broad multidisciplinary translational approach, which integrates genetics, bioinformatics, physiology, cell biology, clinical immunology, diabetology and transplantation research.

Diabetes mellitus is a lifelong, incapacitating disease affecting multiple organs. Estimates of worldwide prevalence suggest that 250 million patients have diabetes today and that this number by 2025 will increase by fifty percent. In Sweden, at least 500,000 persons suffer from diabetes today. Diabetes and its complications impose an immense burden on the quality of life of patients and account for more than ten percent of health care costs in Sweden.

Although type 2 diabetes accounts for most of the diabetes epidemic, type 1 diabetes (T1D) is in Sweden the most common chronic disorder in children. More than two children per day are diagnosed with T1D, reaching more than 800 patients per year. In Finland one child out of 123 will be diagnosed with T1D before the age of 15 years. The figures are frightening and for unknown reasons the incidence of T1D has doubled during the past twenty years and continues to increase by four to six percent per year.

The aim of our research is to clarify the etiology of T1D and to pave the way for development of new strategies for prevention and cure of T1D.

The work is organised in five projects with the following objectives:

- a) Unravel the etiology of T1D.
- b) Halt or prevent T1D in newly diagnosed patients by transplantation of autologous mesenchymal stem cells.
- c) Islet Imaging: Antibody-based proteomics for discovery and exploration of proteins expressed in pancreatic islets
- d) Transplantation of isolated islets to cure patients with the most severe T1D, experimental and clinical studies.
- e) Induction of immunological tolerance: Regulatory T cells for treatment of transplantation induced immune reactions

Group members during 2014

Olle Korsgren, professor, group leader
Mahesh Kumar Anagandula, PhD student
David Berglund, researcher
Marcus Bergström, PhD student
Torsten Eich, PhD student
Karin Fonnaland, research engineer
Maria Hårdstedt, researcher
Sofie Ingvast, research engineer
Marie Karlsson, research engineer
Enida Kuric, research engineer
Marcus Lundberg, PhD student
Johan Olerud, researcher
Oskar Skog, research engineer
Per-Anton Stenwall, PhD student
Magnus Ståhle, PhD student
Anna-Maria Ullbors, adm. assistant

Anna Wiberg, PhD student

Dissertations during 2014

David Berglund, Preparatory Studies to Introduce Regulatory T Cells in Clinical Transplantation, May 10, 2014.

Maria Hårdstedt, Studies of Innate and Adaptive Immunity in Islet Transplantation, November 7, 2014.

Publications 2012-2014

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Immunotherapy for Cancer and Autoimmune Diseases

Angelica Loskog

Our research group develops new immunotherapies for cancer and autoimmune diseases. The immune system has an important role both in the development and control of these diseases and our research is based on the potential to affect the disease by modifying the immune response.

Tumor cells differ from normal cells both in appearance and growth pattern. They are therefore often recognized and killed by cells of the immune system. However, some tumor cells avoid recognition, for instance by producing immunosuppressive substances. These cells will continue to grow in an uncontrolled way, eventually causing cancer. We use gene technology to enhance anti-tumor immune reactions. For example, we are evaluating gene engineered T cells for the treatment of lymphoma and leukemia and immunostimulating gene therapy for the treatment of solid cancer such as melanoma and pancreatic cancer.

Multiple sclerosis (MS) is an autoimmune disease where the immune system attacks cells in the nervous system. We are investigating the immunological mechanisms of hematopoietic stem cell transplantation for MS patients.

Immunostimulatory gene therapy for cancer

Hannah Karlsson, Emma Svensson, Stina Söderlund, Ioanna Milenova, Jessica Wenthe, Victor Gustafson, Lina Liljenfeldt, Berith Nilsson, Ann-Charlotte Hellström, Angelica Loskog

The immune system has the capacity to destroy tumor cells by the same mechanisms that it clears viral infections. However, tumor cells require skills to turn off, or even kill, immune cells. We are investigating the role of different immune escape mechanisms and how they are affected by conventional or experimental treatment. By genetic engineering it is possible to shift the immunosuppressive milieu and/or to shield the effector immune cells from tumor-induced escape mechanisms. In this project the overall goal is to develop novel biological therapies for cancer focused on gene engineering cells and tumor tissues.

CD40 ligand (CD40L) is an immunostimulatory molecule that can be transferred to the tumor site by adenoviral vectors. CD40L production in the tumor area will enhance immune activation against the tumor resulting in tumor cell destruction, reduce the level of immunosuppressive molecules in the tumor area and drive Th1-mediated cytokine production. Moreover, stimulation of CD40 present on certain tumors such as those of epithelial origin will lead to tumor cell apoptosis which not only lead to decreased tumor mass but as well to increased uptake by antigen-presenting cells. We are evaluating the effects of AdCD40L gene therapy on tumors in both experimental models and in collaboration with other researchers at IGP as well as with Lokon Pharma AB and the Dept of Oncology at Uppsala University Hospital we are performing clinical Phase I/II trials on solid tumors currently with a focus on melanoma and pancreatic cancer.

T-cells are immune effector cells with high capacity to target and kill tumor cells. Adoptive transfer of *ex vivo*-cultured and expanded tumor-reactive T-cells has been investigated extensively. Due to the sensitivity of these cells to tumor-induced immunosuppression novel means are needed to enhance their survival and to restore their killing capacity. Lately, T-cells have been strengthened by gene technology prior to infusion into patients and multiple clinical trials are ongoing worldwide to test their safety and efficacy. In collaboration with Baylor College of Medicine, Houston, TX, Vecura at Karolinska University Hospital and Dept of Oncology at Uppsala University Hospital we have just initiated a clinical trial using CD19-targeting chimeric antigen receptor (CAR) T

cells for lymphoma and leukemia and we are also developing novel improved gene technology vectors that are currently evaluated in preclinical models.

Development of novel therapies for multiple sclerosis (MS)

Joachim Burman (contact person: joachim.burman@neuro.uu.se), Pooja Vijay Ghopal, Angelica Loskog

MS is an autoimmune disease of the central nervous system (CNS) in which the immune system attacks myelin-producing cells. The immune attack results in the destruction of the myelin sheath that covers nerves which leads to deteriorated function and may, in severe forms, cause paralysis. Most patients exhibit relapsing-remitting MS (RRMS) and these patients have shown possible to treat with autologous hematopoietic stem cell transfer (HSCT). Within this project we investigate the role of the immune system during different phases of the disease (relapse and remission) to determine how and why the immune cells are activated against myelin and why the normal tolerance mechanisms fail to prevent immune attacks during relapses. Patients subjected to HSCT stops to relapse and can even recover from previous symptoms to some extent. The major part of our current work is related to these patients and how HSCT has affected the immune system. This project is done in collaboration with the Dept of Neurology at Uppsala University Hospital. In experimental models we have investigated CNS-targeting immunosuppressive cells developed in our lab by genetic engineering. These cells target the CNS and locally suppress unwanted immune reactions without hampering peripheral control of infectious disease.

Group members during 2014

Angelica Loskog, professor (adj), group leader
Joachim Burman, post doc, specialist in neurology
Victor Gustafsson, research assistant
Hannah Karlsson, post doc
Lina Liljenfeldt, PhD student
Yoanna Milenova, research assistant
Berith Nilsson, project coordinator (part time)
Emma Svensson, PhD student
Stina Söderlund, PhD student, resident in hematology
Ann-Charlotte Hellström, technical assistant
Jessica Wenthe, student

Dissertations during 2014

Lina Liljenfeldt, CD40L gene therapy of solid tumors, June 3, 2014.
Joachim Burman, Curing multiple sclerosis – how to do it and how to prove it, June 13, 2014.
Hannah Karlsson, CD19-targeting CAR T Cells for Treatment of B Cell Malignancies: From Bench to Bedside, November 21, 2014.

Funding during 2014

AFA Försäkring AB, 1 000 kSEK
Cancerfonden, 500 kSEK
Barncancerfonden 350 kSEK
Lokon Pharma AB 1 500 kSEK (commissioned research)

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Blood vessel function after transplantation

Peetra Magnusson

Disturbances in vascular function contribute to the development of several diseases and as a further consequence to human mortality. Diseases such as diabetes, heart failure and ischemia reperfusion injury share many of the same risk factors and consequential damage of the endothelium.

In replacement or regeneration of human cells, tissues or organs there is a need of functional revascularization. To improve recruitment of recipient's vessels to the new tissue utilization of biomaterials or supportive cells is an attractive strategy.

Our research focuses on the endothelium in health and disease. By using cutting edge techniques and our signature methods for endothelial cell interactions with the blood compartment, stem cells and heparin compounds we are investigating different possibilities to protect the endothelium in disease and to improve islet transplantation.

Ischemia reperfusion injury

A majority of kidney complications in diabetic patients lead to end-stage renal disease causing a need of kidney replacement. A challenge in organ transplantation is the great risk of ischemia-reperfusion injury occurring when the organ is connected to the vasculature of the recipient that may cause endothelial cell activation, triggering events leading to microvascular thrombosis and severe risk of graft failure.

Strategies to protect the vasculature upon transplantation are crucial. To be able to investigate the effects upon activated endothelial cells on a cellular and molecular level we are using our described blood endothelial cell chamber model where therapies with complement regulators/inhibitors will be investigated.

The strategy to protect the vasculature in transplantation is part of an EU FP7 supported project, DIREKT. The DIREKT project is coordinated from Uppsala by Prof. Bo Nilsson and the consortium has partners in Sweden, Norway, Denmark, The Netherlands, Greece, USA, Germany and Australia.

Heparin conjugate for vascular protection

In collaboration with Tomas Lorant (UU) and Corline Systems AB, Uppsala.

During a 2-year period the project "Heparin conjugate for vascular protection" will through funding from BIO-X/Vinnova develop the Corline Heparin Conjugate (CHC) into an *ex vivo* tool for repairing ischemia reperfusion injury (IRI) in kidneys prior to transplantation.

The aim of the project is to show that CHC will significantly reduce the vascular reperfusion injury for donated kidneys. The clinical therapy will use CHC to counteract the devastating effects of thrombosis that occurs upon reperfusion of the kidneys. Attenuating reperfusion injury will possibly improve function of the donated kidneys and reduce delayed graft function (DGF) after transplantation. Tasks related to market analysis, market plans and regulatory/toxicology will be addressed during the project period.

The project will fill an important gap by providing proof of concept (POC) data needed for submitting a clinical trial application to the Swedish Medical Product Agency (MPA).

Tissue bioengineering utilizing mesenchymal stromal cells

In collaboration with Katarina LeBlanc (Karolinska Institute), Olle Korsgren, Joey Lau, (UU) and the Science for Life laboratories

Mesenchymal stromal stem cells (MSC) are a heterogeneous population of stem cells that originates from the bone marrow and other tissues. Bone marrow derived MSC are currently

used in the clinic in patients with graft vs host disease (GvHD) with promising results and are at present subjects for clinical trials for a variety of diseases.

We have via collaboration with the Karolinska Institute access to human MSC from healthy donors and are currently investigating their role in health and disease. It is well known that MSC migrate to inflamed tissues and we have observed that MSC are communicating with neighboring cells via organelle transfer. By using cutting edge techniques such as CyTOF, Seahorse, FlowSight, 2-photon microscopy, LightSheet and SPIM we are able to define the organelle transfer and the effect it has on the recipient cell. We have established a co-culture protocol of MSC and endothelial cells that allow investigations of cellular mechanisms and function.

In the process of revascularization upon transplantation MSC can support endothelial cells by the production of growth factors and matrix proteins. MSC also produce proteases enabling vessels to migrate into the surrounding tissue during angiogenesis. We have a model system of combining MSC with islets of Langerhans investigating their cellular contributions to the graft. Furthermore, we are investigating their potential in supporting a vascularized site pre transplantation by utilizing biomaterials and surface treatments.

Signature methods: Blood endothelial chamber

In collaboration with Carolina Wählby and Petter Ranefall (UU)

In many infectious and inflammatory diseases the cells of the endothelium are affected, leading to secondary complications such as nephropathy, retinopathy and coronary artery disease due to endothelial dysfunction. To be able to investigate the interaction between vascular cells and blood cells, we have a system of blood chambers combined with cultured primary cells, a blood endothelial chamber.

The model will symbolize the scenario of patients with inflammatory disease or ischemia reperfusion injury during organ transplantation. This makes it possible to study blood interactions in combination with hypoxia or cytokine stimulated human primary endothelial cells, symbolizing an activated and inflammatory state of disease.

The model will be expanded towards a possibility to perform high throughput screening. The aim is to automate the processing of staining, imaging and image analysis to enable efficient analyses of candidate compounds, especially developed within the EU DIREKT consortium. This model supports the European Parliament Directive 2010/63/EU to reduce and replace the use of research animals.

Group members during 2014

Peetra Magnusson, researcher, group leader

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Funding during 2014

Uppsala BIO/Vinnova, 2 000 kSEK
Barndiabetesfonden, 117 kSEK
SLS, 100 kSEK

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Thromboinflammation in Therapeutic Medicine

Bo Nilsson

The cascade systems of the blood consist of the complement, the contact, the coagulation and the fibrinolysis systems. In particular the complement system, but also the other systems, are part of the innate immune system. The primary function of the **complement system** is to act as a purging system of the body to remove foreign substances including microorganisms, apoptotic cell debris, immune complexes and foreign bodies/materials. The primary function of **the fibrinolysis, the coagulation and the contact systems** is in hemostasis. However, all three systems are also engaged in inflammation.

Physiologically, thromboinflammation is an initiator of the healing and repair process of the body and is triggered by the humoral innate immune system, which primarily consists of the cascade systems of the blood. These subsequently activate leukocytes, platelets and endothelial cells, finally resulting in thrombotic and inflammatory reactions.

Thromboinflammation is also an important pathophysiological process involved in several clinical conditions and treatments:

1. Cell and cell cluster transplantation and therapies.
2. Whole organ transplantation
3. Thrombotic events such as cardiac infarction, stroke and other cardiovascular conditions
4. Rheumatic conditions (scleroderma, SLE, antiphospholipid syndrome).
5. pharmacological delivery systems e.g. lipid micelles, polymers, virus vectors etc.
6. Treatments with biomaterials implants (joint replacements, scaffolds for tissue engineering etc), extracorporeal treatments (hemodialysis, cardiopulmonary bypass)

Cross-talk between the cascade systems and activated platelets

Osama Hamad Huda Kozarcanin, Kristina Nilsson Ekdahl, Bo Nilsson

Platelet activation during thrombotic events is closely associated with complement and contact system activation, which in turn leads to inflammation. Chondroitin sulfate A (CS-A), released from alpha granules during platelet activation, is a potent mediator of cross-talk between platelets and the complement system. Under physiological conditions, no complement activation seems to occur on the activated platelet surface, but C3 in the form of C3(H₂O) is bound to the surfaces of activated platelets. C3(H₂O) is a non-proteolytically cleaved but activated form of C3, with C3b-like properties. Platelet-bound C3(H₂O) acts as a ligand for leukocyte CD35 and CD11b/CD18, enabling platelet-leukocyte interactions.

Furthermore, we have shown that activated platelets and fibrin elicit activation of the lectin pathway enzymes, MASP-1 and -2 without complement activation. The MASP proteases thereby represent a crossover between the complement and coagulation. Thus, in addition to their traditional role as initiators of secondary hemostasis, platelets also act as mediator and regulator of inflammation in thrombotic events.

Disarming the intravascular innate immune response to improve treatment modalities for chronic kidney disease

Sana Asif, Karin Fromell, Yuji Teramura, Andreea Barbu, Kristina Nilsson Ekdahl, Bo Nilsson

Chronic kidney disease is world wide a major cause of end-stage renal disease (ESRD). 800.000 patients in Europe and in the US, respectively, require long-term treatment initially with peritoneal dialysis, followed by hemodialysis and kidney transplantation. Each ESRD patient on hemodialysis costs ≈€40000 to €80000 per year, has extremely poor quality of life

and an average life expectancy of only 4 years. Kidney transplantation totally changes life for ESRD patients who can then return to normal life, but this treatment is hampered by the low number of available kidney grafts. All these treatments are, however, associated with adverse reactions that cause damaging thromboinflammation, triggered by the intravascular innate immune system, which may lead to poor results and non-function.

The overall aim of this project is to clarify the innate immune mechanisms that cause thromboinflammation and identify nature's own specific control points of regulation in these adverse reactions. By applying these concepts of regulation in hemodialysis and kidney transplantation, we intend to significantly improve the quality of hemodialysis devices and kidney grafts. We envisage to 1) convey a novel soluble complement inhibitor to the clinical stage via phase 1/2a clinical studies, 2) create of nano-profiled surfaces with low activating properties and 3) generate easy-to-apply one step-coatings for treatment of biomaterials (hemodialysis) and endothelial cell surfaces (kidney grafts) that will significantly improve the treatment modalities of ESRD. We expect that these advances will result in extended periods during which hemodialysis can be applied to patients and that the quality of life will improve. In kidney transplantation attenuation of innate immune reactions is anticipated to protect the grafts against damage thereby making a larger number of kidneys accessible for transplantation. The novel techniques are also likely to be applicable on other types of implantations, extracorporeal treatments and transplantations and in the future to be used in xenotransplantation and stem cell therapies. This project is part of the FP7 grant DIREKT coordinated by our group.

Thromboinflammation induced by nanoparticles

Padideh Davoodpour, Jaan Hong, Bo Nilsson, Kristina Ekdahl

Nanoparticles (NP) and nanostructured materials are used in a growing number of applications and their use is expected to increase dramatically in the future. We have found that NP of different origin induce thromboinflammation, and our aim is to apply the technology that we developed for elucidating the biocompatibility of biomaterials in contact with blood, to characterize the biological responses and toxicity of NP in contact with tissue fluid / blood plasma / whole blood. We have applied this technology to investigate TiO₂ NPs. These particles are widely used and applied in a number of applications e.g. sun protection, white paint and toothpaste etc. Our investigations have revealed that they are highly thrombogenic, despite that they have been considered to be mostly inert. The project will help to clarify the mechanisms of toxicity of NPs, and help to develop techniques for evaluating the toxicity of present and future NP materials that are disseminated in the environment. This project is supported by AFA.

Coatings of liposomes in order to avoid innate immune recognition

Claudia Dührkop, Bo Nilsson, Kristina Ekdahl

Drug delivery by liposomes is a technique to contain and neutralize toxic drugs, e.g. various chemotherapies, in order to avoid release of the drug to off-target cells. Liposomes injected into the blood are, however, recognized by the innate immune system, leading to accelerated removal of the particles and to adverse reactions. Attempt to conceal the surface with polyethyleneglycol (PEG) have been partially successful, but also this coat has been shown to be recognized by the innate immune system. The so-called accelerated blood clearance (ABC) phenomenon has been suggested to be triggered by natural IgM antibodies. In this project, which is supported by the FP7 project DECENT AID, we attempt to find alternative coatings to avoid innate immune recognition and ABC.

Coatings of liposomes in order to avoid innate immune recognition

Jaan Hong, Bo Nilsson, Kristina Ekdahl

Development of biomaterials intended for applications in tissue engineering is time-consuming and costly, due to design of the material and repeated testing in animal models. Therefore, there is a need to find screening techniques that at an early stage can be used to predict the biocompatibility of the material. One of the major properties of a biomaterial that determines the fate of the implant is the recognition by the innate immune system. We have developed two different tentative screening techniques that are applicable for this purpose. The first one employs the adsorbed protein profile after exposing the material to blood plasma. We have demonstrated that the proportion of complement and coagulation protein profiles, are closely correlated with the biological response. The second technique is a migration assay that allows blood cells to migrate through a membrane in response activation products generated by the biomaterial in contact with blood plasma. Both assays are at present under evaluation in animal models. This project is supported by FP7 Project BIODESIGN.

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Swedish Research Council, 1 000 kSEK
AFA, 1 300 kSEK
EU FP7 DIREKT, 2 400 kSEK
EU FP7 Biodesign, 600 kSEK
EU FP7 Decent Aid, 900 kSEK
STINT/Science Council of Japan, 700 kSEK

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Immune Complexes in Rheumatic Diseases

Johan Rönnelid

Our research focuses on the functional and prognostic impact of immune complexes and immune complex-associated autoantibodies in rheumatic diseases and chronic infections. We study immune complex (IC)-mediated mechanisms in chronic rheumatic diseases, primarily rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and chronic infections like *Leishmania donovani*. We also study how IC and IC-associated autoantibodies act as prognostic markers for future disease development.

Our research aims to make IC more central in the etiologic perception of chronic diseases from a modernized functional approach. This can lead to definition of new IC-dependent disease phenotypes, as has been the case in RA, as well as to phenotype-based therapies in autoimmune diseases with IC-driven pathology.

Characterization of immune complexes

Vivek Anand Manivel, Azita Sohrabian, Amir Elshafie, Linda Mathsson, Mohammed Mullazehi, Sahwa Elbagir, Johan Rönnelid

The project relies on close collaboration between basic immunological research and clinical research, mostly within rheumatology. The basic research concerns characterization of IC-induced immune/inflammatory reactions and development of new techniques to measure effects of IC and IC-associated autoantibodies. The measurement outcomes are then related to the clinical situation for the individual patients at the time of sampling (pathogenetic issues) or later (prognostic issues).

The role of IC in disease

Vivek Anand Manivel, Azita Sohrabian, Amir Elshafie, Linda Mathsson, Mohammed Mullazehi, Johan Rönnelid

At the clinical level we investigate the importance of IC-triggered mechanisms for the development and maintenance of disease activity in RA, SLE and chronic infections. One of our main interests is currently to describe in detail the group of RA patients with high levels of circulating autoantibodies reacting with collagen type II in joint cartilage. We have shown that these antibodies, which show the highest levels very early (at the time of RA diagnosis) are found in patients which also have maximum inflammation and joint destruction at this early time point.

With two *in vitro* models reflecting anti-collagen containing IC in the joints, we have shown that these IC induce the production of inflammation-promoting and joint-degrading substances. Thereby we have explained the link between the early appearance of anti-collagen antibodies and the simultaneously appearing inflammation and joint destruction in anti-collagen antibody positive RA patients.

We purify IC from blood or inflamed joints, whereupon these IC are used to stimulate cells *in vitro*. In other *in vitro* systems we create artificial IC with human components, and use these IC to stimulate different cell types. In these experiments we aim to mimic immune reactions that take place in specific target organs in patients, e.g. RA cartilage or in the soft tissues in close vicinity to bone/cartilage erosion in RA joints. This work is done in close collaboration with researchers from many rheumatology centers in Sweden, Holland, United Kingdom, USA, Iceland and Sudan, as well as tropical medicine specialists in Sudan.

We believe that a greater functional understanding of IC-mediated mechanisms can lead to new principles of treatment in IC-associated diseases like RA, SLE and chronic infections.

Such knowledge will also lead to better understanding and distinguishing of pathogenetically separate subgroups of patients in traditional criterion-based diseases like RA and SLE. Thereby it will be possible to treat each phenotypical patient subgroup in an individually and biologically adequate way.

Comparative studies of rheumatic diseases in Sweden and Sudan

Amir Elshafie, Sahwa Elbagir, Johan Rönnelid

Little is today known about the natural history of rheumatoid arthritis in third world countries, and nothing has been published from Sudan. In the first part of the project we investigate Sudanese RA patient and preliminary data show very high disease activity and severe joint destructions.

The world's highest rates of stillbirths are found in sub-Saharan Africa. The anti-phospholipid syndrome (APS) is characterized by thromboses and severe pregnancy complications. APS is associated with anti-phospholipid antibodies, and often related to systemic lupus erythematosus (SLE) a disease with a very strong female preponderance and increased pregnancy risk. The second part of the project will investigate Sudanese pregnancies and SLE patients in an APS context. Sahwa Elbagir is a PhD student within this project. Laboratory analyses of standard APS test, APS tests that have shown bigger impact in Africa than elsewhere and new recently described phospholipid antibodies will be investigated. As control groups we use Swedish SLE and APS patients from Karolinska Hospital and Sudanese women with uncomplicated pregnancies.

Group members during 2014

Johan Rönnelid, professor, senior consultant in clinical immunology, group leader
Vivek Anand Manivel, PhD student
Sahwa Elbagir, PhD student
Azita Sohrabian, research engineer
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Linda Mathsson, affiliated researcher

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Swedish Research Council, 700 kSEK
ALF, 444 kSEK
Agnes and Mac Rudberg fund, 250 kSEK
Swedish Rheumatism Association, 175 kSEK
King Gustav Vth 80-year fund, 100 kSEK

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Immunotherapy of Cancer

Thomas Tötterman, Sara Mangsbo

Growing tumors have the capacity to counteract the attack and control of the immune system by creating an immunosuppressive milieu. This is the result of recruitment of several types of immunosuppressive cells and their cytokines.

We have initiated a series of novel locally applied immunotherapies in which we aim to revert this negative milieu. Therapies include the use of Adenovectors expressing CD40L, monoclonal antibodies and Toll-like receptor agonists. We have pioneered local AdCD40L immunotherapy of bladder cancer and melanoma in man and melanoma in the dog. Our adoptive T cell therapy trial in human melanoma was one of the first. We currently develop novel protein-based agonists/blockers in collaboration with the Biotech industry (See auto-commentary in *Oncoimmunology* 2014). In addition we are currently developing a novel therapeutic long peptide vaccine for the treatment of prostate cancer. The project is financed by BIO-X/Vinnova and is a collaboration effort between Uppsala University, Leiden University Medical Center and Immuneed AB.

Immune stimulating gene therapy

Tötterman/Mangsbo et al.

Over the past several years, we have developed and characterized Adenoviral vectors expressing immunostimulatory genes in several tumor models, with special focus on bladder cancer. Injection of AdCD40L directly into the tumor area effects tumor regression and specific immunity. A first-in-man clinical phase I/IIa study utilizing this vector in aggressive bladder cancer has been published. Several patients experienced tumor regression or disappearance, with minimal side effects. We have in collaboration with SLU (Swedish Agricultural University) treated 19 dogs with aggressive malignant melanoma, again with very encouraging results. We are currently pursuing a clinical trial with our AdCD40L therapy on melanoma patients. The therapy is given with or without low dose cyclophosphamide and the latter is applied as means to inhibit the function T regulatory cells thereby aiding anti-tumor responses. We have also, together with professor Magnus Essand, validated a second generation vector for gene therapy that can target a wider range of cells allowing us to modulate the whole tumor micro environment including antigen-presenting cells (Liljenfeldt et al *JIT* 2014).

Adjuvant therapies in combination a block of inhibitory receptors to target tumors

Mangsbo et al.

Immune activation can be hampered by two major immune checkpoint regulators (CTLA-4 and PD-1). In order to ensure proper and sustained T cell activation one can use antibodies that block these two receptors. We have combined the synthetic DNA sequences; CpG ODNs (described in the previous project) with CTLA-4 or PD-1 blockade to examine if the combination strategy could improve therapy.

Single and combination strategies were assessed in an experimental bladder cancer model. CTLA-4 blockade alone prolonged survival of mice. When anti-CTLA-4 or anti-PD-1 antibodies were combined with CpG, survival was enhanced and elevated levels of activated T cells were found in treated mice. We believe that this strategy can be used to further improve on immunotherapy for patients with aggressive bladder cancer or other solid tumors

and we are now investigating novel therapies that can be used in a clinical setting together with our industrial partners.

Cancer Vaccines

For the last years we have pursued a track of research aiming to improve T cell priming/activation by facilitating the delivery of synthetic long peptides (SLPs) into DCs via Fc receptors (FcR). The SLPs are overlapping ~20-30 long amino acid sequences spanning tumor or pathogen related antigens (Ags) and can be used to trigger T cell responses in conjunction with adjuvants. SLPs have the advantage, over short single peptides, to span a whole tumor associated protein. They include a plethora of CD4 and CD8 T cell epitopes for various HLA alleles. Importantly, they require processing by antigen-presenting cells (APCs) and will therefore not directly interact with MHC class I on non APCs, thus lowering the risk of anergy induction.

SLPs have successfully been assessed by our collaborators in Leiden in a clinical trial for high-grade vulvar intraepithelial neoplasia using long peptides spanning the E6 and E7 oncoproteins but the work demonstrate that improvements are needed to cure larger lesions. Our work to improve SLP vaccination has led to the discovery that a B cell epitope (a hapten/Ag), when coupled to SLPs, can facilitate Ag-SLP uptake. The idea is that circulating antibodies (Abs) will bind the hapten and immune complexes will form that can subsequently interact with Fc receptors which will lead to Ag-SLP uptake, processing and presentation to T cells. The subsequent T cell response will be improved as the DCs are loaded with significantly more Ag-SLP due to immune complex mediated uptake. Additionally DCs are activated by the FcR interaction, enabling upregulation of CD80/CD86 as well as cytokines, crucially important for optimal T cell activation (Schematic illustration in Figure 1).

We are currently investigating this novel vaccine in a human blood loop system to establish how the immune complexes behave in the presence of intact human blood components.

Via funding from Bio-X (Vinnova) we are now preparing a clinical grade batch of a prostate cancer vaccine based on long peptides with the aim to progress to a clinical trial 2017.

Myeloid cells in the tumor micro environment

We are collaborating with both industrial and academic partners in the TIMCC network (EU Marie Curie ITN grant to Dr Mangsbo and associate professor Dimberg). Our previous data in this area demonstrate that we can affect myeloid cells by our well know AdCD40L therapy. Herein we are further exploring how this recruitment and modulation of myeloid cells occur in response to immunotherapy and how the vasculature can affect this. This is an exciting new project that will continue until the end of 2016.

Group members during 2014

Thomas Tötterman, professor, group leader
Sara Mangsbo, researcher, assisting group leader
Erika Fletcher, PhD student
Luuk van Hooren, PhD student
Wictor Gustafsson, research assistant
Lina Liljenfeldt, PhD student
Gabriella Paul-Wetterberg, engineer
Ann-Charlotte Hellström, technician
Joachim Burman, PhD student and MD

Dissertations during 2014

Lina Liljenfeldt, CD40L Gene Therapy for Solid Tumors, June 3, 2014.

Joachim Burman, Curing Multiple Sclerosis: How to do it and how to prove it. June 13, 2014

Funding during 2014

Sara Mangsbo

Swedish Research Council, 3R, 700 kSEK

Göran Gustafsson stiftelse, 500 kSEK

EU, Marie Curie TIMCC (PI Anna Dimberg), 800 kSEK

Thomas Tötterman

Swedish Cancer Society, 800 kSEK

ALF, 135 kSEK

Alligator Bio, 1 400 kSEK (commissioned research)

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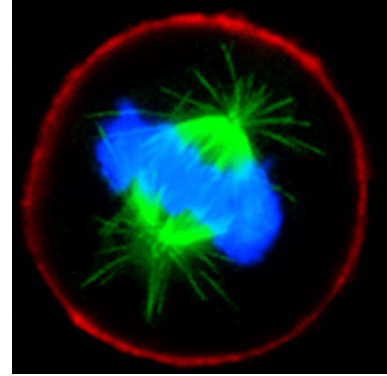
Hematology

In addition to exhibiting an abnormal growth pattern, tumour cells are also often less sensitive, or even resistant, to signals that regulate cell death, apoptosis. A common objective in our research on hematological malignancies is to understand the mechanisms behind the defective control of growth, differentiation and apoptosis of the tumour cells.

Another common theme is to investigate the role of genetic and epigenetic alterations in disease development, to study how the presence or absence of disease-related mutations and epigenetic patterns can contribute to tumour growth, and how these influence patient outcome.

Ultimately, we aim to identify new prognostic and predictive markers, and to reveal new treatment strategies for hematological malignancies.

The research projects are translational with a close collaboration between our research groups and the clinical departments in Uppsala, as well as with our extensive network of national and international collaborators.



Molecular Characterization of Acute Leukemia

Linda Holmfeldt

Acute leukemia is a blood disorder that is diagnosed in about 400 Swedes annually. Despite the best possible treatment with the drugs available today, a large fraction of the patients do not respond to the treatment or experience a relapse after an initial response. It is not possible to increase the survival by intensifying the treatment, since the cancer medicines available today are toxic themselves. Thus, to find new, more efficient treatment alternatives with fewer side effects, more knowledge about the origin and growth of the cancer cell is needed.

Our research aims to increase the understanding of why many patients do not respond to treatment or suffer from a relapse of the disease. We also want to identify changes in the tumor cells that can be used to develop more efficient treatment alternatives for high-risk leukemia, that today is associated with a very poor outcome.

What are the underlying causes of initiation and progression of acute myeloid leukemia?

Svea Stratmann, Henrik Steffen

To identify which alterations that favour primary treatment failure and/or the outgrowth of resistant relapse clones, we perform unbiased multilevel analyses comparing newly diagnosed and relapse AML specimens.

Complementary high-resolution techniques are used to identify any alterations that may explain treatment failure or the onset of relapse. Techniques included are, amongst others, whole genome and/or exome sequencing, RNA sequencing and various studies of the epigenome. We also study the proteome of the leukemic cells, comparing these to corresponding normal cells. By employing systems biology approaches, data generated from the above mentioned analyses are integrated to generate hypotheses that could explain tumour progression.

To complement the exploratory studies, we functionally evaluate the hypotheses generated using a combination of cellular studies and *in vivo* modelling. Finally, evaluation of novel therapeutic alternatives for AML is performed.

What are the downstream consequences of aberrant epigenetic regulators in leukemia?

Ren Sun, Henrik Steffen

My previous studies of pediatric high-risk and relapsed acute lymphoblastic leukemia identified a high frequency of alterations of epigenetic modifiers. Among these, alterations in the histone H3K27 methyltransferase Polycomb repressive complex 2 (PRC2) stand out, especially the catalytic subunit EZH2, which has been shown to act as both an oncoprotein and tumor suppressor in different types of malignancies. This suggests that perturbation in epigenetic regulation facilitates a reduced response to therapy and/or the onset of relapse.

We want to answer the question whether specific alterations in epigenetic regulators cause i) stochastic changes at the epigenetic level, or ii) specific and recurrently found epigenetic changes of genes that favour tumorigenesis.

One of the aims in our lab is thus to interrogate the downstream consequences of aberrant PRC2 on leukemogenesis. The approach we take includes everything from biochemical enzymatic assays utilizing purified protein complexes, through analyses at the cellular level to *in vivo* modelling, followed by epigenomic and transcriptomic analyses of manipulated cells.

Group members during 2014

Linda Holmfeldt, researcher, group leader
Henrik Steffen, research engineer
Svea Stratmann, PhD student
Ren Sun, post doc

Funding during 2014

Knut & Alice Wallenberg's Foundation, Wallenberg Academy Fellow, 1 820 kSEK
Swedish Research Council, 1 300 kSEK
Swedish Cancer Society, 500 kSEK
Swedish Childhood Cancer Foundation, 500 kSEK and funding for L. Holmfeldt's position
Kjell and Märta Beijer Foundation, "Beijerforsknare", 1 000 kSEK
Jeansson's Foundations, 300 kSEK

Publications 2012-2014

(The group came to IGP in 2014 and the papers have therefore not been published with IGP as affiliation)

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The Control of Survival and Apoptosis in Human Multiple Myeloma

Helena Jernberg Wiklund

Our research focuses on multiple myeloma (MM). MM is a genetically heterogeneous plasma cell malignancy characterized by the accumulation of well-differentiated tumor cells of B cell origin in the bone marrow. Recent treatment improvements have proven successful in selected subfractions of MM patients. However, due to large intratumoral heterogeneity the identification of common causative alterations for MM has been hampered. Further understanding of the biology of MM is of primary importance in order to be able to tackle the disease complexity. The long-term goal of this research is to identify targets essential for tumor cell survival and explore in models of MM whether their function can be blocked in parallel survival pathways.

To study the molecular mechanisms and therapeutical use of target proteins in survival pathways of MM, a prerequisite has been to select and implement relevant models *in vitro* and *in vivo*. Immunocompetent syngeneic murine models of MM *in vivo* and *in vitro* models, a large well characterized authenticated panel of cell lines representing all common genetic subtypes of MM, primary patient cells and normal age-matched normal counterpart cells now constitutes our highly clinically relevant model of human MM.

Previously accomplished results in our highly relevant model of MM has generated proof-of-principle that the IGF-1R is an attractive target for intervention, and that survival circuits act via gene silencing by epigenetic mechanisms. The hypothesis that tumor stemness may lie within a novel tumor-associated epigenetically silenced gene signature by the Polycomb complex (PcG) is an attractive idea since it may be reverted and genes reactivated by pharmacological intervention.

Novel and combinatorial experimental targeted therapy *in vitro* and *in vivo*

Charlotte Fristedt, Prasoon Agarwal, Pernilla Martinsson

We focus predominantly on pairing promising novel anti-tumor agents with agents conventionally used in MM management and demonstrated sensitization to cytotoxic drugs.

Taking into account the predicted development of drug resistance using highly selective targeted drugs, we have selected downstream candidates of the IGF-1R pathway potentially amenable to future combinatorial intervention, one of these agents targeting epigenetic modulators in clinical trials for MM (Kharaziha et al 2012) (Lemaire et al 2012) (Bieghs et al 2014) (Maes, manuscript 2015).

In parallel, we are studying downstream molecules (cIAP2) for drug resistance using lentivirally introduced gene expression. In our present research program a panel of MM cell lines subgrouped to different usage of constitutive NF- κ B activation pathways by genetical aberrations have been generated to express potential target genes of importance for altered drug resistance. We are now evaluating novel mechanisms underlying resistance to proteasome inhibitors and are currently performing gene expression signatures following drug exposure. In parallel, clinically relevant drugs to cIAP2 are studied in single and combinatorial regimens in our MM model *in vitro* and *in vivo* (Fristedt, in press 2015).

A novel epigenetically regulated gene signature in MM

Antonia Kalushkova, Mohammad Alzrigat, Alba Atienza, Prasoon Agarwal, Charlotta Sandberg

In our overall aim to dissect the disease-specific global epigenetic pattern of MM, and to evaluate possible links between certain exogenous survival factors/genetic alterations and the epigenome of MM, we have recently taken an integrative genomics approach on dissecting the differences in

gene expression between non-malignant and malignant plasma cells. This novel approach resulted in our seminal findings of a novel common silenced gene profile present in a large cohort of >200 MM patients (Kalushkova et al 2010). The novel signature revealed a significant overlap to the histone methylated and silenced genes previously known to be involved in self-renewal of embryonic stem cells. We could also find the ICSBP/IRF8 gene, the expression of which we have previously reported to be silenced in patient cells and MM cell lines (Tshuikina et al 2008).

The fact that an epigenetic mark maintaining self-renewal is found in well differentiated tumor cells, supports our hypothesis that MM tumor cells by this signature harbor a proliferative potential that may be maintained by additional genetic lesions or environmental factors e.g. IGF-1 and may be unleashed by physiological and pharmacological inhibition (Jernberg Wiklund et al 2012). This is especially important in MM, displaying extensive intraclonal genetic heterogeneity, and where whole-genome sequencing has not substantially contributed to our understanding on the origin of the tumor, or lead to the development of novel therapeutic strategies. Our identification of the Polycomb repressive (PRC2) as a mediator of gene silencing in MM strongly suggests that this profile may be a common feature of myeloma tumor cells, rather than representing features of a specific stemcell subpopulation. Therefore, this gene signature in MM populations is evaluated in relation to clonogenic growth and tumor initiating capacity (Van Valckenborgh et al 2012).

The silenced gene expression in MM may thus be a possible requirement for tumor initiation, progression and survival of the MM tumour initiating clone within the bone marrow niche *in vivo*. Functional validation including mapping of the global epigenome in MM patients, studies of the tumor initiating capacity in potential tumor stem cell populations of MM, and consequences of gene reactivation by direct and indirect biological and pharmacological inhibition of the Polycomb complex are the current focus of our investigations. Currently, newly developed clinically relevant drugs targeting the silencing complex are evaluated for potential use and therapeutical implications in MM models *in vivo* and *in vitro*.

Molecular Networks for Transcriptional Regulation and Epigenetic Control of Differentiation

Fredrik Öberg, Antonia Kalushkova, Mohammad Alzrigat, Prasoon Agarwal

The project is focused on how epigenetic mechanisms regulate molecular networks with implications for two major disease processes; the pathogenesis of hematopoietic tumors and chronic inflammation. We are specifically interested in the epigenetics of transcription factors and have investigated the possibilities of reprogramming the epigenetic status of the transcription factor network in leukemic cells. The data generated by the project will increase the basic knowledge of how epigenetic mechanisms play a role in disease, and discover new target molecules/pathways, amenable to future therapeutic intervention.

Mechanisms of epigenetic control are often disturbed in cancer, and aberrant DNA-methylation or histone modifications of specific transcription factor genes, with key functions in the differentiation process, are likely to be important for the pathogenesis of leukemia. Although less well understood, epigenetic changes are also observed in chronic inflammation and influences disease activity.

The current aims of the project are (1) To investigate the molecular mechanisms for nuclear receptor mediated epigenetic reprogramming involved in the control of acute myeloid leukemia (AML) cell differentiation, (2) To identify genes required to maintain silencing of tumor suppressor genes, and to discover new compounds with the capacity to relieve epigenetic silencing and reprogram gene expression in leukemia cells, (3) To investigate the epigenetic influence on the gene-regulatory network operating in monocytes during chronic inflammation associated with mood disorders.

The long-term goal is to achieve a better understanding of the role malignancy-associated epigenetic changes play in perturbing differentiation and activation. Thereby we hope to identify

signals or drugs that can re-initiate the blocked differentiation process in leukemia or modulate disease-causing inflammatory activation of monocytes.

Group members during 2014

Helena Jernberg Wiklund, professor, group leader
Prasoon Agarwal, PhD student
Mohammad Alzrigat, PhD student
Alba Atienza Párraga, PhD student
Charlotte Fristedt, PhD student
Antonia Kalushkova, PhD student
Pernilla Martinsson, research engineer
Charlotta Sandberg, research engineer
Kenneth Nilsson, professor emeritus
Fredrik Öberg, adj associate professor
Mårten Fryknäs, associated bioinformatician

Dissertations during 2014

Prasoon Agarwal, Regulation of Gene Expression in Multiple Myeloma Cells and Normal Fibroblasts: Integrative Bioinformatic and Experimental Approaches, November 13, 2014.

Funding during 2014

Von Kantzow Foundation, 684 kSEK
Swedish Research Council, 1 250 kSEK
Swedish Cancer Society, 800 kSEK

Publications 2012-2014

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Molecular Hematology - Chronic Lymphocytic Leukemia

Richard Rosenquist Brandell

The main goals with our translational research program on chronic lymphocytic leukemia (CLL) are to increase our understanding of mechanisms behind disease development, to improve and optimize the diagnostic and prognostic information as well as to reveal new strategies for therapy.

CLL, the most common adult leukemia in Western countries, is a biologically and clinically heterogeneous malignancy with varying disease course. Many patients survive for years or decades even without treatment, whereas others succumb rapidly to the disease despite therapy. Men are more commonly affected than women with a median age at diagnosis of 72 years. At present two staging systems are used in clinical practice (Rai and Binet), however both have a limited ability to predict the clinical course at an early stage. The disease has remained incurable although new treatment strategies, including antibody-based therapy and small molecular inhibitors, appear promising.

In recent years, molecular genetic studies have revealed new prognostic markers, which have significantly improved the subdivision of the disease. Two of the most important molecular predictors are the mutation status of the immunoglobulin heavy variable (*IGHV*) genes and certain recurrent genomic aberrations, which divides CLL into prognostic subgroups.

Stereotyped B-cell receptors in CLL

Lesley-Ann Sutton, Panagiotis Baliakas, Anastasia Hazidimitriou, Diego Cortese, Sujata Bhoi, Emma Young, Larry Mansouri, Richard Rosenquist

An interesting theory that has emerged is the potential role of antigens in the development of CLL. Many reports, including ours, indicate a very biased *IGHV* gene repertoire in CLL, and virtually identical B-cell receptors (BcRs) have been identified in multiple subsets of CLL. In a large collaborative work, we analyzed the complementarity determining region 3 (CDR3) sequences, the main determinant of antigen specificity, in more than 7400 CLL patients, where up to 30% of CLL patients could be assigned to stereotyped subsets. In this study, we proposed a novel molecular classification of CLL based on BcR stereotypy, since patients expressing certain stereotyped BcR appear to have high intra-subset homogeneity both regarding clinical outcome as well as biological features. As an example of the latter point, we demonstrated that subset #2 (*IGHV3-21/IGVL3-21*) patients exhibit a remarkable 44% frequency of mutations in the *SF3B1* gene, encoding a core component of the spliceosome, whereas other aggressive subsets had frequencies in the range of 0-10%. This finding alludes to subset-biased acquisition of genomic aberrations, perhaps consistent with particular antigen/antibody interactions.

To further investigate the clinical relevance of this new molecular classification based on stereotypy, we performed a multi-center study comprising 8593 CLL patients where individual stereotyped subsets showed profound differences in e.g. demographics, clinical presentation and presence of cytogenetic aberrations. Importantly, members of the same subset followed a similar clinical course, e.g. subsets #1 (*IGHV1/5/7/IGKV1-39*) and #2 had very short time to treatment and poor overall survival, similar to patients with *TP53* dysfunction, while subset #4 patients (*IGHV4-34/IGKV2-30*) followed an indolent disease course and were rarely in need of treatment.

Novel prognostic markers in CLL

Larry Mansouri, Lesley-Ann Sutton, Anastasia Hazidimitriou, Diego Cortese, Emma Young, Sujata Bhoi, Viktor Ljungström, Mattias Mattsson, Panagiotis Baliakas, Richard Rosenquist

Next-generation sequencing (NGS) studies have revealed a number of novel recurrent mutations in e.g. the *NOTCH1*, *SF3B1* and *BIRC3* genes in CLL with higher frequencies in patients with a more aggressive disease. We investigated the presence of these mutations in a large Scandinavian population-based cohort and found a considerably lower frequency of these mutations than in the pivotal studies, probably reflecting the unselected nature of our material. Importantly, *SF3B1* and *NOTCH1* mutations were shown to confer particularly poor prognosis similar to patients with *TP53* aberrations.

In a multi-institutional collaborative effort, coordinated under the auspices of the European Research Initiative on CLL (ERIC), we investigated the presence of mutations within *SF3B1*, *NOTCH1*, *TP53*, *BIRC3* and *MYD88*, in the largest cohort ever studied (based on 3490 cases from ten European institutions). We provide strong evidence that different recurrent mutations are associated with distinct clinico-biological profiles and outcomes. The prime example is the finding of *SF3B1* mutations as an adverse indicator among early stage CLL cases, independently of other factors including *TP53* aberrations. We believe that this type of study will be very relevant for the design of future novel prognostic schemes integrating cytogenetic and molecular findings in CLL.

Next-generation sequencing of CLL subgroups

Lesley-Ann Sutton, Diego Cortese, Sujata Bhoi, Emma Young, Viktor Ljungström, Larry Mansouri, Richard Rosenquist

Due to recent technical advancements, it is now possible to investigate many genes, including genes with a large number of exons, as well as many patient samples simultaneously, by taking advantage of HaloPlex probes in conjunction with NGS technology. These developments have not only open up the possibility to investigate large genes, such as the *ATM* gene (62 exons), which has been virtually impossible to investigate in routine practice, but also result in a substantially higher sequencing depth compared to Sanger sequencing technology. Recently, we utilized HaloPlex technology and designed a gene panel including nine prognostic genes: *ATM*, *BIRC3*, *MYD88*, *NOTCH1*, *SF3B1*, *TP53*, *KLHL6*, *POT1* and *XPO1*, and investigated 188 CLL patients with poor-prognostic features. Sanger validation confirmed 93% (144/155) of mutations; notably, all 11 discordant variants had a variant allele frequency between 11-27%, hence at the detection limit of Sanger sequencing. Technical precision was assessed by repeating the procedure for 63 patients; concordance was found for 94% mutations. Considering the increasing number of prognostic genes in CLL, we foresee that this new approach will be applied to include such genes for genetic screening in CLL, eventually as a stand-alone test without the need for confirmation by Sanger sequencing.

Group members during 2014

Richard Rosenquist Brandell, professor, group leader
Panagiotis Baliakas, PhD student
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Funding during 2014

Swedish Cancer Society, 1 2500 kSEK
Swedish Research Council, 950 kSEK
Part of EU Grant “DiaTools” (PI: Ulf Landegren), € 50 000.
Part of EU Grant “AEGLE” (PI: Exodus S.A), € 85 000
UU/SciLifeLab Grant, 2 500 kSEK
SciLifeLab National Project Grant, 50% deduction for 360 whole-genomes

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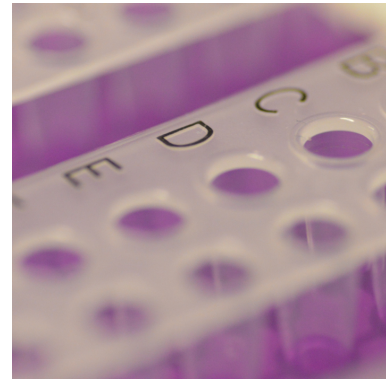
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Medical Genetics and Genomics

The research groups in this program are addressing basic mechanisms in genetics, epigenetics and genomics as well as more applied questions in clinical genetics, genetic epidemiology, cancer genetics and forensic medicine.

We use methods that can identify differences in single genes as well as in our genome as a whole. The aim is to understand the function of our genome and to identify causes of diseases such as cancer, mental retardation and congenital malformations. Studies on the genetic variability of the human genome will also increase our knowledge of our evolutionary origin. New methods for forensic DNA testing are developed to increase sensitivity and allow smaller amounts of DNA to be analysed.



Improved Forensic DNA Analysis

Marie Allen

The general objective of our research is to develop highly sensitive and discriminating assays for forensic DNA analysis of challenging samples. Evidence samples at a crime scene have often been subjected to harsh environments and have therefore commonly degraded DNA that may also be present in very small amounts. Our research involves development of quantification assays and typing systems for analysis of mitochondrial DNA (mtDNA) as well as autosomal markers. In addition, a Y-chromosome analysis can allow resolution of mixed DNA samples (common for instance in sex offence cases). The use of mtDNA markers will allow a highly sensitive analysis due to a high copy number of mtDNA molecules per cell, while the autosomal markers in very short fragments will give a high discrimination power.

Several new assays based on pyrosequencing, microarrays, real-time quantification or Sanger-sequencing have been developed and used successfully in analysis of challenging evidence material in forensic cases. In a new study, a combination of traditional methods and next generation sequencing (NGS) technologies will be evaluated for DNA analysis of degraded, limited and damaged samples. A target selection and enrichment is performed using Agilent's HaloPlex system for customized panels of a large number of targets that is based on a capture technology with high sensitivity. The MiSeq sequencer will be used for the final sequence analysis. This strategy will allow high throughput analysis of multiple markers in the genome and will allow improved relationship analysis, prediction of visible characteristics and individual identification. In general, new identification assays allow smaller amounts and also degraded DNA to be analysed. As an ultimate test for success with challenging samples, the novel techniques may be used in genetic investigations of historical samples.

Saint Birgitta (Saint Bridget of Sweden) lived between 1303 and 1373 and was appointed one of Europe's six patron saints by the Pope in 1999. According to legend, the skulls of St. Birgitta and her daughter Katarina are maintained in a relic shrine in Vadstena abbey in mid Sweden. The authenticity of the two skulls was assessed first by analysis of mitochondrial DNA (mtDNA) that excluded a maternal relationship. Moreover, a radiocarbon dating suggest an age difference of at least 200 years and neither of the dating results coincides with the period St. Birgitta or her daughter Katarina lived. Similarly, we have performed DNA analyses using novel sensitive assays to identify the remains of Nicolaus Copernicus and Carin Göring.

Group members during 2014

Marie Allen, professor, group leader
Magdalena Bus, post doc
Mia Bjerke, post doc
Joakim Grånemo, research assistant
Martina Nilsson, project manager

Funding during 2014

VINNOVA, 1 000 kSEK
Swedish Crime Victim Compensation and Support Authority, 1 100 kSEK

Publications 2012-2014

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Characterisation of Syndromes Associated with Developmental Delay

Marie-Louise Bondeson

Developmental delay, with or without malformations, occurs among two to three percent of the population. For approximately half of the patients the reason for the developmental delay is still unknown, despite extensive studies. Knowledge about the genetic causes of the syndromes is important for diagnosis, prognosis, treatment and risk for recurrence. It will also increase our understanding of the molecular processes behind the disorders.

Our research projects concern Down syndrome, characterisation of novel syndromes, intra uterine fetal death (IUID) and RASopathies, including e.g. Noonan syndrome. We also have a project that focuses on genetically caused hearing loss. The research is performed in collaboration with physicians and researchers at the Uppsala University Hospital.

Down syndrome: epidemiological, clinical and molecular characterisation:

Ulrika Wester-Oxelgren, Göran Annerén,

In collaboration with Jan Gustafsson, Åsa Myrelid (UU)

Down syndrome (DS) is the most common cause for developmental delay. Patients with DS have, besides developmental delay, an increased risk of being afflicted by several other diseases. DS is a model disease for studies on the relationship between chromosome imbalance and disease. Our research includes epidemiological, clinical, as well as molecular genetic studies of the disorder.

The specific aims of this project are:

- To perform a genotype-phenotype correlation of gene-dosage effects on chromosome 21. The studies have so far been focused on aging and dementia in DS in relation to gene-dosage effects of SOD and APP and autoimmune reactions in relation to the gene-dosage effect of the *AIRE* gene and the mental retardation in relation to the *DYRK1A* gene. Studies are also in progress to study the effect of medications on ADHD
- To study the prevalence of ADHD and Autism spectrum disorders in a population of DS and to study the effects of specific treatments of the disorders.
- To study the *DYRK1A* gene on neuronal IPS cells from patients with DS and to study the effect upon those cells from treatment with Harmine.

Clinical and molecular characterization of Noonan spectrum disorders (RASopathies)

Sara Ekvall, Berivan Baskin, Cecilia Soussi Zander, Göran Annerén, Marie-Louise Bondeson

Recent advances in molecular genetic research have led to the definition of a new group of genetic syndromes, the RAS/MAPK pathway disorders or "RASopathies". They comprise Noonan syndrome and related disorders (LEOPARD, Cardio-faico-cutaneous and Costello syndromes), as well as Neurofibromatosis type 1. The aim of this study is to enable translational research into disease mechanism and therapies of the RASopathies. The RAS/MAPK pathway, which has been well studied in cancer, is an attractive target for inhibition in the treatment of various malignancies utilizing small molecule therapeutics, which specifically inhibit the pathway. Many of these are in development and several are currently undergoing clinical trials. One of the most exciting issues related to the RASopathies is the idea that causal treatment might become possible in the future to ameliorate disease progression of some manifestations.

The specific aims of the project are:

- To develop rapid, efficient and cost-effective mutation analyses of the RAS/MAPK genes in the clinical setting using next-generation sequencing
- To identify novel causative genes associated with RASopathies using whole-exome sequencing.
- To investigate the functional role of different mutations in the RAS/MAPK pathway to clarify the underlying molecular mechanisms.

The outcome of this study will have a tremendous impact on the diagnosis, treatment and management of the patients with RASopathies.

Characterisation of novel syndromes using microarray-analysis and next generation sequencing

Marie-Louise Bondeson, Sara Ekvall, Christian Wentzel, Cecilia Soussi Zander, Göran Annerén, Patrik Georgii-Hemming, Ann-Charlotte Thuresson

Intellectual and developmental disorders (IDD) are one of the main reasons for referral in paediatric, child-neurological and clinical genetic service. We are using array based technologies to screen the genomes of patients for chromosomal aberrations to identify the underlying mechanism to possibly categorise new syndromes and genes associated with IDD. In selected groups of patients, where no chromosomal aberration has been detected, next generation sequencing technologies are used to screen genomes of patients at high resolution to identify new causative genes for IDD.

Characterisation of genes associated with hearing loss in man

Sara Ekvall, Carina Frykholm, Marie-Louise Bondeson

Hearing loss is an etiologically heterogeneous trait with many known genetic and environmental causes. Genetic causes account for more than 60 percent of childhood hearing loss in developed countries. Studies are in progress to investigate familial forms of progressive hearing impairment. The investigation includes families with both X-linked non-syndromic and syndromic hearing loss. The findings of these studies may result in an increased understanding of the underlying molecular mechanisms causing hearing loss.

Group members during 2014

Marie-Louise Bondeson, professor, clinical molecular geneticist, group leader

Göran Annerén, professor, senior staff physician

Berivan Baskin, assoc. prof., clinical molecular geneticist

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Stiftelsen Säfstaholm, 350 kSEK
Regional Research Council, 500 kSEK
ALF, 465 kSEK
Borgströms foundation, 100 kSEK
Medical Faculty, 264 kSEK

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Exploring Mendelian traits using next-generation sequencing technologies and iPSC for disease modeling

Niklas Dahl

Mendelian (monogenic) disorders are extremely heterogeneous and affect approximately 5% of the population in Western societies. The approximately 8,000 Mendelian entities described to date, as well as 50% of cases with intellectual disabilities caused by single gene mutations, constitute a major socioeconomic burden worldwide. The identification of causative gene mutations is crucial for our understanding of development and organ function as well as for accurate diagnosis, appropriate follow-up and counseling to patients/families. Furthermore, identification of novel genetic mechanisms may serve as a platform for therapeutic interventions. Our goals are to identify novel genetic variants/genes causing Mendelian traits and to model these phenotypes in different biological systems. Our long-term objectives are to identify pathways and biomarkers that can be used for the development of therapies in these and similar disorders.

Next generation sequencing for the identification of gene variants associated with (novel) human phenotypes

Joakim Klar, Maria Sobol, Doroteya Raykova, Muhammad Jameel, Jens Schuster

Next generation sequencing (NGS) technologies are used in order to identify genes, gene variants and altered expression associated with unique, mainly Mendelian phenotypes. We have characterised a number of unique phenotypes/disorders and additional clinical entities are continuously identified through collaborators. Gene variants and expression profiles are analysed using whole genome sequencing, targeted sequencing, exome-sequencing and transcriptome sequencing (SciLife platforms) on selected patient samples. As an example, we have utilized the Ion Proton sequencing platforms in 50 distinct whole-exome sequencing projects. Twenty-nine of these are finalized showing a success rate of 66% (19/29). A successful outcome is defined as the identification of a disease causing mutation proven by functional analysis in biological systems or, by independent reports on similar genotype-phenotype associations. In one third of projects no candidate gene mutation was identified, possibly due to a combination of biological and technical factors. The sequencing approach as well as data handling and bioinformatic analysis are now constantly developed in order to increase the yield of successful projects.

Different imaging technologies and model systems are used in order to study the pathophysiological mechanisms caused by novel genes/gene variants, e.g. zebrafish and iPSC differentiated to neuronal lineages.

Induced pluripotent stem (iPS) cells for disease modeling: Functional analysis of disease mechanisms in neurological disorders

Maria Sobol, Jens Schuster, Doroteya Raykova, Ayda Khallifa, Laureanne Lorenzo, Feria Hikmet Noraddin

Neurodevelopmental disorders affect approximately 2% of the population. Little is known about the mechanisms leading to early neuronal defects in the central nervous system. Induced pluripotent stem cell (iPSC) technology has the capacity to recapitulate lineage specific development and pathophysiology. Human skin fibroblasts derived from patients with clinically well-defined neurodevelopmental disorders are reprogrammed to iPSC using a non-integrating vector system. The iPSC are differentiated into different neuronal lineages using established protocols.

Populations of iPSC differentiated into neuronal stem cells are analysed for e.g. growth, migration, neurite outgrowth, transcriptome- proteome- and methylome profile, and electrophysiology for the identification of disease-associated alterations. The goal is to identify biological disease markers in early steps of differentiation and to rescue these abnormalities in a screening platform available at Chemical Biology Consortium Sweden. Examples of well-defined neurodevelopmental disorders that are now being analyzed at the “stem-cell levels” are Down syndrome, Dravet disease and Mowat-Wilson syndrome.

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Funding during 2014

AstraZeneca 1 100 kSEK
Swedish Research Council, 900 kSEK
Uppsala University, 200 kSEK
Uppsala University Hospital (ALF) 700 kSEK

Publications 2012-2014

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Molecular Oncology

Jan Dumanski

Analysis of post-zygotic or somatic genetic variation (somatic mosaicism) is the overall theme of research in the group. We work with translational disease-related projects and with basic questions addressing somatic variation in normal human cells. An emphasis is on structural genetic variation, which has emerged over the past 10 years as a dominating type of human inter-individual variation.

Mosaic loss of chromosome Y (LOY) in blood cells is associated with smoking as well as shorter survival and higher risk of cancer in men

Lars A. Forsberg, Chiara Rasi, Jan Dumanski

LOY and cancer

It is well known that men have an overall shorter life expectancy compared with women. However, it is less well recognized that incidence and mortality for sex-unspecific cancers are higher in men, a fact that is largely unexplained. Age-related loss of chromosome Y (LOY) is frequent in normal hematopoietic cells and it was first described more than 50 years ago, but the phenotypic consequences of LOY have been elusive. Our latest results suggest that LOY could be a key factor to explain the higher mortality of men.

Survival analyzes performed in the Swedish ULSAM-cohort (Uppsala Longitudinal Study of Adult Men) with >1100 participants indicated that LOY in peripheral blood could be associated with risks of all-cause mortality as well as non-hematological cancer mortality. Among the elderly men in this cohort, followed clinically for up to 20 years, at least 8.2% of the subjects were affected by LOY in a significant fraction of blood cells. The median survival time in men affected with LOY was half, i.e. 5.5 years shorter, compared to the men without mosaic LOY in blood cells. The association of LOY with risk of all-cause mortality was validated in the independent PIVUS-cohort (Prospective Investigation of the Vasculature in Uppsala Seniors) in which 20.5% of men showed LOY. Our discovery of a correlation between LOY and all-cause mortality as well as non-hematological cancer mortality will be published in Nature Genetics.

These results illustrate the impact of post-zygotic mosaicism such as loss of chromosome Y (LOY) on disease risk and could explain why males have a higher mortality compared to females and are more frequently affected by cancer. They also suggests that chromosome Y is important in processes beyond sex determination and sperm production. LOY in blood could become a predictive biomarker of male carcinogenesis.

LOY and smoking

Smoking is a major preventable environmental risk factor related to human health. Smoking killed about 100 million people during the 20th century and is projected to kill one billion people during this century, assuming that the current frequency of smoking is retained. Lung cancer is the prime cause of cancer associated death in relation to smoking. However, it is less well appreciated that smoking also causes tumors outside the respiratory tract, which is predominant in men and cumulatively roughly as common as lung cancer. Moreover, it is known that males have a higher incidence and mortality from most sex-unspecific cancers, disregarding smoking status, and this fact is largely unexplained by known risk factors.

We have published a paper in *Science* showing that smoking is associated with and LOY in blood cells in three independent cohorts encompassing in total 6014 men. Our data also support a transient and dose-dependent mutagenic effect from smoking on LOY-status (Dumanski et al. 2015 Science, PMID: 25477213). Thus, smoking may induce LOY, linking

the most common acquired human mutation with a severe preventable risk factor. Our results could explain the observed sex differences and why smoking seems a greater risk factor for cancer in men than women.

Post-zygotic genetic variation: studies of human aging/longevity and age-associated aberrations

Lars A. Forsberg, Chiara Rasi, Jan Dumanski

In collaboration with: Nancy L. Pedersen (Karolinska Institutet), Dedra Buchwald, Eric Strachan, (Univ. of Washington, USA), Fred Miller (NIH, Bethesda, USA), Devin Absher (HudsonAlpha Institute, USA), and Lars Lannfelt, Martin Ingelsson, Erik Ingelsson, Lars Lind et al. (UU).

Monozygotic (MZ) twins represent an extraordinary resource in genetics; two individuals who can also be treated as a single subject genetically matched at conception and present in two copies. Therefore, it is a powerful model for analysis of *de novo* (post-zygotic or somatic) genetic variation. We have shown in 2008 that MZ twins frequently display disparate patterns of genomic copy number variation (CNV). We hypothesized that structural genetic rearrangements in human somatic cells also vary over time and these might represent a new mechanism contributing to the aging process in humans.

Using age-stratified cohorts of 318 monozygotic (MZ) twins and 296 single-born subjects, we found age-related accumulation of copy-number variation in the nuclear genomes in vivo and frequency changes for both megabase- and kilobase-range variants. Megabase-range aberrations were found in 3.4% (9 of 264) of subjects >60 years old; these subjects included 78 MZ twin pairs and 108 single-born individuals from Uppsala ULSAM-cohort. No such findings were observed in 81 MZ pairs or 180 single-born subjects who were <55 years old. Recurrent region- and gene-specific mutations, mostly deletions, were observed. Longitudinal analyses of 43 subjects whose data were collected 7–19 years apart suggest considerable variation in the rate of accumulation of clones carrying structural changes.

Furthermore, the longitudinal analysis of individuals with structural aberrations suggests that there is a natural self-removal of aberrant cell clones from peripheral blood. In three healthy subjects, we detected somatic aberrations characteristic of patients with myelodysplastic syndrome. The recurrent rearrangements uncovered here are candidates for common age-related defects in human blood cells. We anticipate that extension of these results will allow determination of the genetic age of different somatic-cell lineages and estimation of possible individual differences between genetic and chronological age. Our work might also help to explain the cause of an age-related reduction in the number of cell clones in the blood; such a reduction is one of the hallmarks of immunosenescence.

Novel bio-markers for breast cancer; disease prediction and progression

Lars A. Forsberg, Hamid Razzaghian, Chiara Rasi, Jan Dumanski

In collaboration with: Wojciech Zegariski, (Center of Oncology, Bydgoszcz, Poland), Jaroslaw Skokowski, Arkadiusz Piotrowski (Medical University of Gdansk, Poland), Janusz Rys (Jagiellonian University, Krakow, Poland), Tibor Tot (Central Hospital of Falun, Sweden), and Devin Absher (HudsonAlpha Institute, USA)

There exists a paradox in cancer research: although the high mortality from cancer is caused by metastatic spread of tumors, genetic research of metastases is underdeveloped. Contrary to the numerous transcriptome and genome analyses of primary tumors, there is a lack of comprehensive and high-resolution studies comparing genomic profiles of primary tumors and the metastases from the same patient. We have recently completed pilot breast- and ovarian-cancer projects, testing the hypothesis that, upon high-resolution analysis, there are

frequent genetic differences between matched primary tumors and lymph node metastases. We observed aberrations that can be linked to metastatic disease and many of the observed differences were previously linked to poor patient survival, based on extensive analyses of primary tumors. This provides a proof of concept that this approach towards finding new biomarkers for breast cancer progression and patient's prognosis is viable.

The second part of this project deals with search for somatic genetic events in normal breast tissue predisposing to breast cancer. Our previous discoveries of genetic differences between differentiated tissues and in monozygotic twins indicate that the somatic mosaicism for CNVs, between normal cells in the same person is underestimated. This represents a paradigm shift in somatic cell genetics, which has implications for cancer research, as cancer is predominantly a genetic disorder of somatic cells. Hence, this gives an opportunity for analysis of *de novo* somatic aberrations that may predispose normal cells to cancer development, by comparisons of CNV/CpG methylome profiles between an uninvolved margin of histopathologically normal cells surrounding a primary tumor and blood of the same patient.

We compare genomes and epigenomes (CpG methylome) of primary tumors and metastases from patients with breast cancer. We also evaluate genetic and epigenetic (CpG methylation) profiles of normal margin of tissue surrounding primary tumor and blood DNA from the same patient. The objective is to identify patterns suggesting genomic global CNV/epigenetic instability, alternatively aberrations in specific genomic loci that might be coupled to breast cancer progression and predisposition/susceptibility. Our preliminary, unpublished results support the viability of the above hypotheses.

Group members during 2014

Jan Dumanski, professor, group leader
Lars Forsberg, researcher
Hanna Davies, research engineer
Dhanu Gupta, student
Chiara Rasi, research assistant
Edyta Rychlicka, post doc

Funding during 2014

Swedish Research Council, 900 kSEK
Swedish Cancer Society, 800 kSEK
Borgström foundation, 250 kSEK (to L. Forsberg)
Vleugel Foundation and Department funding, 390 kSEK (to L. Forsberg)

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Genetic variation and gene expression in human disease

Lars Feuk

The aim of our research is to understand the importance of genetic variation in the human genome and its role in disease and evolution. We are using high throughput sequencing combined with bioinformatic analyses to characterize genetic variation and its correlation with functional data and disease outcomes. The research ranges from very basic studies of genetic variation and transcription to disease specific analysis.

Studies of human disease are mainly focused on neurodevelopmental disorders, including intellectual disability and schizophrenia. We aim to capitalize on the development of the latest sequencing technologies to identify new causative mutations. Our samples include both large pedigrees and parent-offspring trios, and we are using different analysis strategies to mine the sequencing data for potential causative mutations.

We are also interested in better understanding the process of transcription and RNA processing in human cells. Using data from RNA sequencing, we are aiming characterize splicing mechanisms and investigate the subcellular localization of different transcripts. We also develop new strategies for RNA extraction and enrichment to increase the resolution in our analyses.

Exome and whole genome sequencing of patients with neurodevelopmental disorders

Jonatan Halvardson, Eva Carlström, Lars Feuk

To sequence all the coding regions of a genome in a single experiment is a powerful tool to discover disease genes. In this project, we are mainly focusing on two groups of patients to identify causative mutations. First, in collaboration with the clinical genetics unit, we are investigating patients with severe intellectual disability and epilepsy for de novo mutations by sequencing the exomes of both parents and the patient. The second approach is to use pedigrees with multiple affected individuals to identify mutations in regions of linkage or shared homozygosity. Significant work has been invested in establishing a bioinformatics pipeline for analysis of exome and whole genome sequence data.

Functional characterization of mutations causing intellectual disability

Ammar Zaghlool, Jin Zhao, Mitra Etemadikhah, Lars Feuk

Our exome sequencing projects have led to the discovery of several mutations in genes not previously linked to disease. The most interesting genes have been selected for functional follow-up in order to clarify the role of the gene and the specific mutation in disease. Classic molecular biology (cloning, transfection, RNAi) approaches are combined with high throughput genomics such as RNA-seq and ChIP-seq to characterize the role of the genes and mutations.

Transcriptome analysis

Ammar Zaghlool, Jonatan Halvardson, Lars Feuk

Transcriptome sequencing is providing novel insights into the transcriptional landscape of cells and tissues. In this collaborative project (Lucia Cavellier and Adam Ameer at IGP), we use RNA sequencing to study transcription in human tissue samples. We are also investigating evidence for splicing in human tissues using both computational and lab based approaches. New approaches are developed to extract specific types or subcellular fractions of RNA in order to increase biological insight.

Group members during 2014

Lars Feuk, associate professor, group leader
Mitra Etemadikhah, scholarship holder
Eva Lindholm Carlström, researcher
Jonatan Halvardson, PhD student
Ammar Zaghlool, post doc
Jin Zhao, PhD student

Funding during 2014

European Research Council Starting Grant, 2 556 kSEK
Swedish Research Council, 1 500 kSEK (Medicine and Health), 800 kSEK (Natural and Engineering Sciences)

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Human Genomics and Molecular Epidemiology

Ulf Gyllensten

The research of the group is divided into two parts. The first project uses a systems biology approach to study human physiology and we will determine the biological variation in human populations at different levels. We are interested in how the genetic, epigenetic and exposure (medical history, diet, lifestyle) effects can be modelled on the proteome, glycome and lipidome?

Our second project concerns the genetics and clinical epidemiology of cervical cancer. This is the second most common cancer among women worldwide and is caused by persistent infection of oncogenic types of human papilloma virus (HPV). The research focus on the epidemiology of HPV, the identification of genetic factors contributing to the susceptibility and on the interactions between the virus and host susceptibility factors. We also have a large randomised study ongoing to compare cytology (PAP smear) and self-sampling for HPV testing in the primary screening to detect women at risk of developing cervical cancer.

Systems biology approach to human physiology

Stefan Enroth, Åsa Johansson, Ulf Gyllensten

We will determine the biological variation in human populations at the level of the genome, transcriptome, epigenome, and proteome. The variation is studied in pedigree-based population cohorts, with unique genetic backgrounds and life style, from the European Special Population Research Network (EUROSPAN). The information includes full exome sequences of selected individuals and imputed exome structure for the complete population, genome-wide analyses of epigenomic state (methylation), high-resolution studies of the plasma proteome, the glycome (glycans), the lipidome, and exposure variables such as medical history, lifestyle and diet.

These multidisciplinary data is used to model the interaction between different types of biological information and address questions that have been beyond the reach for a single discipline. What is the impact of genetic and genomic variation on the plasma proteome? How can genetic, epigenetic, medical history, diet and lifestyle effects be modelled on the proteome, and lipidome? This is the first study based on complete exome sequences for a population to address the impact of genetic factors on other levels of biological variation. It also represents the first study to integrate data from these multiple layers of biological information and model their interactions and effect on human physiology.

Identification of genetic risk factors for cervical cancer

Dan Chen, Ivana Juko, Tao Cui, Stefan Enroth, Ulf Gyllensten

In collaboration with Emma Ivansson (UU)

Cervical cancer is caused by human papillomavirus (HPV) and both genetic and environmental risk factors contribute to persistence of an HPV infection and progression to cervical carcinoma. The disease shows a strong familial clustering restricted to biological relatives, indicating that host genetic factors are important for disease development. We have established population-based affected sib-pair (ASP) and case-control cohorts, including over 2,800 cases with cervical carcinoma and 2,000 controls to be used in the identification of genetic risk factors for cervical cancer. This represent the largest set of families with cervical carcinoma identified in the world and among the largest materials for case-control studies.

We have recently performed the first genome-wide association study (GWAS) for this disease, and this has lead to the identification of pathways and individual genes associated

with susceptibility to cervical cancer. We will now perform detailed genetic and functional studies of the identified pathways and genes. This project will increase our understanding of the etiology of cervical carcinoma and provide new means for development of diagnostic and therapeutic measures.

Development of rapid and high-resolution methods for HPV typing, and their application to clinical screening of pre-stages for cervical cancer

Inger Gustavsson, Ulf Gyllensten

In collaboration with Karin Sanner, Matts Olovsson (UU)

We have developed techniques for collection of cervical smear samples (using FTA cards) and detection and quantification of HPV using real-time PCR. These methods allow for detection of individual HPV types and estimation of their titer. The method is economical, easily scalable and amendable to automation, making it suitable for use in primary and secondary screening for cervical cancer pre-stages. We are conducting studies using self-sampling and repeat-HPV typing to determine if this could be used as a strategy in the primary screening for cervical cancer as an alternative to cytology-based strategies.

Evaluation of the use of self-sampling and repeated HPV testing in primary screening for cervical cancer: a randomised study

Inger Gustavsson, Julia Hedlund-Lindberg, Pernilla Quarfordt , Ulf Gyllensten

In collaboration with Karin Sanner, Matts Olovsson, Ingrid Wikström, Erik Wilander Riina Aarnio (UU)

The organised gynaecological screening program in Sweden has reduced the incidence of cervical cancer by 50%. To further reduce the incidence of cervical cancer, the sensitivity of the diagnostic test and coverage of screening must be improved. This can be achieved by introducing human papillomavirus (HPV) typing as the primary diagnostic test and implementing a screening system where women take the samples at their own convenience (by themselves and at home) and send it in to the lab for analysis. The aim of this project is to study: A. The feasibility of using self-sampling at home for HPV testing, as an alternative to collection of samples at a mid-wife's clinic. B. The use of repeated testing for oncogenic forms of HPV as the primary screening test for early detection of cervical cancer. C. The health-economic benefits of using self-sampling and repeated HPV testing as a basis for cervical cancer screening.

Group members during 2014

Ulf Gyllensten, professor, group leader

Malin Berggrund, PhD student

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Funding during 2014

Swedish Cancer Society, 1 500 kSEK
Swedish Research Council, 2 200 kSEK
SSF, Foundation for Strategic Research, 2 600 kSEK
Borgström Foundation, 300 kSEK
Linnéstiftelsen, 200 kSEK

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Interplay between genetic, epigenetic and environmental factors in the pathogenesis of human disease

Åsa Johansson

In my research I use genome-wide approaches to study how epigenetic and genetic factors interact with the environment in the development of complex diseases. Through large-scale epidemiological and genome-wide association studies we have previously contributed to the identification of many dietary, lifestyle and genetic factors that influence our health and risk for disease.

Recently, we have also shown that various factors, such as genetic variants, chronological age and smoking, affect our genes through epigenetic alterations. Epigenetic changes are heritable from cell to cell, and can therefore persist in the body throughout a lifetime and influence the risk of disease later in life. However, to what extent it can be transmitted through the germ cells and thereby affect our next generation is still an area for debate.

The aim of my research is to determine how genetic and epigenetic factors influence human phenotypes, clinical variables and risk of disease. I also study how environmental factors introduce epigenetic alterations, with subsequent long-term health related effects.

Effects of diet and lifestyle on the epigenome

Weronica Ek, Åsa Johansson

We are investigating the effect of food items, diet and lifestyle on DNA methylation. We are using food frequency questionnaire data together with self-reported lifestyle and clinical variables, which enable us to study epigenetic alterations due to a diet high in e.g. carbohydrates, proteins, or fat, or due to lifestyle factors such as smoking, coffee and alcohol consumption.

Relative contribution of genetic and epigenetic factors in regulating gene expression of disease-related protein biomarker

Muhammad Ahsan, Allan Lind-Thomsen, Weronica Ek, Åsa Johansson

We have recently measured over 150 disease-related protein biomarkers in over 1000 participants of a population based study cohort. By performing genome-wide association study (GWAS) and epigenome-wide association study (EWAS) for each biomarker, we have identified SNPs and CpG methylation that are associated with gene regulation. Integrating GWAS and EWAS data gives a unique possibility to study their respective roles in regulating protein expression. This knowledge is important in order to better understand the role of protein biomarkers in the pathogenesis of human disease.

Infer the causal relation between epigenetic alterations, protein biomarkers and risk of disease.

Muhammad Ahsan, Allan Lind-Thomsen, Åsa Johansson

Protein and epigenetic biomarkers have been identified for many human diseases. A biomarker is increased in patients with a disease, but the direct causal effect of increased levels of most biomarkers has not been widely investigated. Mendelian randomization can be used to evaluate the causal effect of the biomarkers on disease risk and progress. In a Mendelian randomization study, a genetic variant, that increases the levels of a biomarker, is used to divide a population into genotypic subgroups, in an analogous way to how participants are divided into arms in a randomized clinical trial. The aim of this project is to

use Mendelian randomization to evaluate the causal effect of biomarkers on risk of disease, and on disease progress.

Genetic determinants of complex phenotypes

Anna-Maria Denes, Åsa Johansson

In later years we have been involved in the identification of hundreds of genetic variants influencing the risk of common diseases through GWASs. Despite this success, only a small fraction of the genetic contribution to disease has been identified, and most of the genetic factors remain unknown. This “missing heritability” might be explained by the contribution of rare and population specific single nucleotide polymorphisms (SNPs), which are underrepresented in GWAS. In this project we study the distribution of rare and population specific SNPs in non-cosmopolitan populations and the contribution of rare and population specific SNPs to the etiology of human traits.

Identify epigenetic changes in relation to cardiovascular diseases

David Martinsson, Muhammad Ahsan, Åsa Johansson

Cardiovascular disease (CVD) is among the leading causes of death worldwide. There are several known genetic and lifestyle risk factors, but association between epigenetics and CVD is poorly understood. We are investigating the link between DNA methylation and CVD. We have performed a genome-wide DNA methylation study in a population-based cohort. Participants were not ascertained upon disease background, but some had a history of CVD, including 48 participants with a previous myocardial infarction. The genes identified are good candidates for additional studies to further understand the pathogenesis of CVD

Group members during 2014

Åsa Johansson, researcher, group leader
Muhammad Ashan, post doc
Weronica Ek, post doc
Allan Lind-Thomsen, post doc
Anna-Maria Dénes, student
David Martisson, student
Azadeh Chizarifard, student

Funding during 2014

Swedish Research Council, 550 kSEK
Beijer Foundation, 1 000 kSEK
Vleugel foundation, 390 kSEK
Göran Gustafsson stiftelse, 500 kSEK
Borgström Foundation, 300 kSEK

Publications 2012-2014

1. Ameer, A., Enroth, S., Johansson, Å., Zaboli, G., Igl, W. et al. Genetic Adaptation of Fatty-Acid Metabolism: A Human-Specific Haplotype Increasing the Biosynthesis of Long-Chain Omega-3 and Omega-6 Fatty Acids. *American Journal of Human Genetics*. 2012, 90:809-820

2. Chasman, D., Fuchsberger, C., Pattaro, C., Teumer, A., Boeger, C. et al. Integration of genome-wide association studies with biological knowledge identifies six novel genes related to kidney function. *Human Molecular Genetics*. 2012, 21: 5329-5343.
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Mechanisms of adenovirus infection

Ulf Pettersson

Many important discoveries have been made using human adenovirus as an experimental model for control of gene expression. Adenoviruses have moreover become of great interest as gene delivery vectors in gene therapy and as oncolytic viruses in cancer treatment.

How adenoviruses take over the control of host gene expression in infected cells

Ulf Pettersson and Hongxing Zhao

The aim of our project is a detailed characterization of the transcriptome of the virus and that of the infected cell. For these studies we are using state-of-the-art cDNA sequencing technologies.

Our results demonstrate that the adenovirus transcriptome is immensely more complex than hitherto believed with many novel splice sites. An adenovirus landmark map, showing splice and polyadenylation sites, has been constructed. The cellular genes that are up- and down regulated during the course of infection have been identified. In addition, we have identified a set of micro RNAs, which are dysregulated during an adenovirus infection. Our studies of changes in expression of so called long noncoding RNAs have resulted in some unexpected finding. Gradually we are building up a map of the regulatory networks that operate during the different phases of the adenovirus infection.

Epigenetic mechanisms in the human parasite *Trypanosoma cruzi*

Lena Åslund

Some of the major human parasitic diseases are caused by trypanosomes, against which no vaccine and only a few drugs are available. The *Trypanosoma cruzi* genome project has increased our understanding of the genetic make-up of the parasite causing Chagas' disease and will reveal new drug targets, however, several fundamental cellular processes such as transcription and DNA replication are still rather unexplored in these ancient pathogens. We have recently shown that epigenetic signatures, such as acetylated histones H3/H4 and H3K4me3 are associated with transcription start sites in *T. cruzi*, demonstrating for the first time that the 'histone code' is conserved in these protozoan parasites and in polycistronic transcription. We are further investigating the histone modifications during development of the parasite, *i.e.* the replicative insect stage and the non-replicative blood stage in mammalian hosts. DNA methylation is important in several epigenetic regulations such as gene silencing, cellular differentiation and DNA replication. We have determined the genome-wide distribution of DNA methylation in the *T. cruzi* genome by deep parallel sequencing of immunoprecipitated methylated DNA (MeDIP-Seq). In addition, some of the enzymes involved in this modification are investigated. Further investigations of the function of DNA methylation in trypanosomes will reveal its possible role in the parasite. Elucidating epigenetic mechanisms in the parasite will reveal new approaches to therapies against trypanosomiasis.

Group members during 2014

Ulf Pettersson, professor, group leader

Hongxing Zhao, researcher

Lena Åslund, senior lecturer

Publications 2012-2014

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Identifying and understanding mutations causing colorectal cancers

Tobias Sjöblom

We aim at finding and understanding somatic mutations that cause common human cancers, particularly colorectal cancers (CRC) (Sjöblom *et al*, *Science* 2006). By studying these mutated genes using forward and reverse genetic approaches in human cancer cells, we want to understand their contribution to tumor development. The findings may aid in development of methods for early tumor detection, improved diagnosis, and targeted cancer chemotherapy.

Integrated data and sample collection in clinical cancer care

Tony Hansson, Lucy Mathot, Sara Kiflemariam, Maria Karoutsou, Evangelia Papadaki

Identification of mutated genes that cause cancer or resistance to cancer therapies requires systematic sample collection from cancer patients. With support from the Swedish Government, we coordinate an open access longitudinal collection of patient data, tissues, and imaging before, during, and after cancer therapy at Uppsala Academic Hospital and Umeå University Hospital (www.u-can.uu.se). At the end of 2014, more than 7.000 patients with cancers of the colorectum, brain, prostate, ovaries, neuroendocrine tissues, breast, lung, lymphoma or haematological malignancies had been included in U-CAN since 2010 (Tobias Sjöblom, Program Director; Tony Hansson, Administrative Director; and U-CAN clinical partners). Currently, ~200 tumors from patients in U-CAN are undergoing whole genome sequencing and several biomarker studies have been initiated by different research groups. U-CAN has recently received a positive review in the ongoing external evaluation of Strategic Research Areas (SFO/SRAs) and will gradually re-focus to support research based on the collected materials if continued support is granted.

Major constraints on cancer genomics include obtaining DNA from the large patient cohorts required to gain knowledge about infrequently mutated genes, and the need for improved extraction technologies in diagnostic molecular pathology. We have therefore developed, patented and automated a technology for scalable serial extraction of DNA and RNA from tissue samples (Mathot *et al*, 2011; Mathot *et al*, 2013). The spin-out company ExScale Biospecimen Solutions AB, founded in 2012, has now completed development and CE/IVD labelling of a reagent system for automated serial extraction of DNA and RNA from FFPE samples in clinical diagnostics aiming at market launch in 2015.

Mutational studies of candidate cancer genes

Tom Adlerteg, Lucy Mathot, Viktor Ljungström, Veronica Rendo

By comparing DNA sequences in cancer genomes to sequences in the constitutional genome of the same patient we can derive somatic mutations that have been acquired during tumor evolution. Such somatic mutations are the basis for modern cancer diagnostics and therapeutics development. We have determined the nucleotide sequences of 37 candidate breast cancer genes previously discovered by us, and identified novel mutations in 12 genes of which *DIP2C* is subject to further functional studies (Jiao *et al*, 2012; Larsson *et al*, manuscript). To visualize mutations in tumors, we adapted *in situ* padlock probes for use in FFPE tissues (Grundberg *et al*, 2013) and applied the technology for the first *in situ* mutational analyses of *TMPRSS-ERG* rearrangements in human prostate cancer tissues (Kiflemariam *et al*, 2014). Further, we have developed software tools for rapid and accurate mutational analysis of deep sequencing data from solid tumors with significant content of normal cells. These tools have superior indel calling capabilities, a major challenge in mutational analysis, as compared to state of the art (Adlerteg *et al*, manuscript).

Using these tools, we have completed deep mutational analyses of 676 genes in cancer pathways in 107 colorectal cancers (Mathot, Ljungström *et al*, manuscript). While the expected frequencies and types of mutations were observed in known CRC genes such as *APC*, *KRAS*, and *TP53*, we noted an enrichment of mutations in the Ephrin receptor tyrosine kinase gene family in tumors giving rise to metastasis. Ephrin receptors have previously been associated with metastatic disease development due to their role in tumor growth, invasiveness, angiogenesis and metastasis *in vivo*. However, no mutational evidence has yet been presented to explain the downregulation of Eph proteins associated with metastasis of CRCs.

The use of targeted deep sequencing in this study meant that we could uncover low frequency variants that would otherwise be overlooked (Mathot *et al*, manuscript). These findings are potentially of great clinical importance to identify patients that require close monitoring to detect recurrence and to stratify CRC patients that would benefit most from adjuvant treatments. Current efforts include whole genome sequencing of CRC cases in U-CAN where longitudinal blood samples are available.

Functional studies of novel candidate cancer genes

Muhammad Akhtar Ali, Tatjana Pandzik, Snehangshu Kundu, Chatarina Larsson, Ivaylo Stoimenov, Veronica Rendo

Gene mutation prevalence is a strong indicator of selection during tumor development, but does not suffice to prove cancer gene status - functional and phenotypic studies comparing mutant and wild-type alleles in relevant model systems are required for ultimate proof. One approach to perform such analyses is through genome editing in human cancer cells. We have developed scalable experimental and computational tools for designing rAAV gene targeting constructs to all genes in the human genome (Stoimenov, Akhtar Ali *et al*, *NAR*, 2015). This technology was used to knock out the putative breast cancer gene *DIP2C* and obtained evidence for a phenotype linked to gene inactivation, and identified more than 700 genes with altered expression, many of which affect cell proliferation (Larsson *et al*, manuscript). We have targeted the transcriptional modulator *ZBED6* in colorectal cancer cells and demonstrated effects on cell growth rate and regulation of genes in CRC pathways (Akhtar Ali *et al*, *Proc Natl Acad Sci*, in revision). We have also generated knock-ins of colorectal cancer genes (*PRDM2*, *MLL3*, and *KRAS*) that are currently being characterized by us and used by collaborators in drug discovery efforts (Larsson *et al*, Akhtar Ali *et al*, Pandzic *et al*, manuscripts).

While many low frequency cancer genes (mutated in 1-5% of patient cases) have been discovered by large scale sequencing efforts, their involvement in cancer pathways and phenotypes is often less clear. To better understand which genes belong to the Ras pathway in human CRC, we have adapted technology for forward genetics by transposon mutagenesis in human cells to map the RAS pathway in human colorectal cancers by a phenotypic screen. This resulted in assignment of 163 recurrently targeted genes to the Ras pathway. After comparing with mutational analyses of human colorectal cancer genomes and performing mutual exclusivity analysis with *KRAS/BRAF*, 15 genes were selected for further validation. Of these, 3 genes showed changes in GLUT1 expression after knock-down and differential growth in low glucose, phenotypes associated with Ras pathway activation in CRC. Two of the three genes controlled the level of pERK in CRC cells, providing independent evidence of them being components of the Ras pathway (Kundu *et al*, manuscript).

The tissue expression patterns of cancer genes may yield insights into the anatomy of cancer pathways and the expression profiles of cancer drug targets. *In situ* hybridization (ISH) offers a scalable and specific approach to mapping gene expression in tissues, and we have therefore established and automated large scale ISH on FFPE tissue arrays. We have

evaluated the expression patterns of the tyrosine kinome and the tyrosine phosphatome in ~40 normal tissues and 6 common tumor types, totalling 37 000 tissue specimens, leading to the discovery of novel tumor specific stromal and vessel biomarkers in human cancers (Kiflemariam *et al*, *Am J Pathol*, in press).

Exploiting loss of heterozygosity for a novel anti-cancer therapy

Veronica Rendo, Ivaylo Stoimenov

The success of any anti-cancer therapy is based on finding conditions resulting in selective killing of cancer cells, while the normal tissues of the patient are spared. As an alternative to the existing strategies we propose a conceptually different therapy, which exploits the genetic variation (SNPs) naturally occurring in the human population and the cancer specific phenomenon loss of heterozygosity (LOH). For example, if the patient is constitutionally heterozygous for a high efficiency allele and a low efficiency allele, and the tumour loses the high efficiency allele through LOH, it is conceivable that the tumor is sensitized to certain drugs relative to the normal tissues.

Using 1000 Genomes data, we identified human enzymes having variant amino acids in their active sites as result of SNPs and ranked the 20 putative targets according to the prevalence of SNPs and LOH in common human cancers. For the top candidate, a known drug metabolic enzyme, we estimate that >3% of patients with CRC could benefit from a tailored drug therapy, which translates to >35.000 cases worldwide per year. We therefore constructed and validated CRC cell model systems for cell based drug screens for the most promising candidate in two independent genetic backgrounds. Currently, we are screening for chemical compounds, and several promising hits that have demonstrated differential cytotoxicity after LOH are undergoing further validation.

Group members during 2014

Tobias Sjöblom, researcher, group leader
Tom Adlerteg, research engineer
Mohammad Akhtar Ali, PhD student
Tatiana Pandzik, researcher
Erik Hallqvist Osterman, teaching assistant
Tony Hansson, project coordinator
Karin Hartman, research assistant
Maria Karoutsou, research engineer
Sara Kiflemariam, researcher
Snehangshu Kundu, researcher
Chatarina Larsson, researcher
Viktor Ljungström, PhD student
Lucy Mathot, PhD student
Evangelia Papadaki, research engineer
Veronica Rendo, PhD student
Ivaylo Stoimenov, post doc

Dissertations during 2014

Lucy Mathot, From Tissue to Mutations: Genetic Profiling of Colorectal Cancer, November 7, 2014.

Muhammad Akhtar Ali, Understanding Cancer Mutations by Genome Editing, December 19, 2014.

Funding during 2014

SSF, *FFL*, 850 kSEK

SSF, *CanCure*, 350 kSEK

UU/KoF, 300 kSEK

Swedish Cancer Society, 1 137 kSEK (incl. 637 kSEK for Chatarina Larsson's position)

SciLifeLab, *Identifying acquired resistance genes in CLL/CRC* (Consortium), 1 938 kSEK

SciLifeLab, *Whole Genome Sequencing of colorectal cancers in U-CAN*, 2 600 kSEK

Support for U-CAN at Uppsala University/Akademiska Hospital

U-CAN (SRA, Uppsala part), 10 909 kSEK

UU (Regionalized sample collection in U-CAN), 2 000 kSEK

ALF (New diagnoses in U-CAN), 800 kSEK

Contract research

ExScale Biospecimen Solutions AB, 194 kSEK

Publications 2012-2014

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Genomic Analysis of Gene Regulation

Claes Wadelius

The principles for how genes are activated and inactivated are known but from a genomic perspective our knowledge is very limited. Each cell type has a unique set of active genes that are regulated by the action of a collection of the 2000 transcription factors and other nuclear proteins that bind the DNA molecule. Until recently this could only be studied *in vitro* and for parts of genes. We use chromatin immunoprecipitation (ChIP) to study this *in vivo*. For detection we have developed efficient massive parallel sequencing (ChIP-seq) techniques, which allows us to interrogate the whole genome.

The traditional view of a gene, with a single beginning and end, has been challenged and in addition to the previously known enhancers and other distant regulatory elements, multiple promoters and complex alternative splicing has been found. We therefore annotate all identified DNA-protein interactions relative to everything that is known about the genome. These studies generate massive amounts of data and in order to fully explore the information we develop new informatics strategies and collaborate with specialists in the field. The methods can be used to reveal the mechanisms for common diseases and cancer. We have started to explore this in liver cells and immune cells and have found hundreds of regulatory variants that likely explain association to common metabolic and autoimmune diseases. We have also characterized a large collection of regulatory variants that are excellent candidates to contribute to cancer.

Gene regulatory variants in metabolic and autoimmune diseases and in cancer

**Gang Pan, Marco Cavalli, Helena Nord, Madhusudhan Reddy Bysani, Emelie Wallén
Arzt**

In collaboration with Kerstin Lindblad Toh, Lars Rönnblom and their groups, (UU).

At promoters, enhancers and other gene regulatory elements, nucleosomes are replaced by transcription factors and other regulatory proteins. We map transcription factors to the bases they interact with DNA and in case the cell differs in genetic make up at one base pair, we can tell a difference between what happens at one variant and the other.

Some genetic variants predispose to common diseases and we have started a process to translate this information to molecular mechanisms of disease, primarily for metabolic and autoimmune diseases. We read chromatin signals in relevant tissues to find candidate regulatory elements and test polymorphic variants in cell-based expression systems. The regulatory elements are activated by over-expression of transcription factors that bind to them or by stimulation of primary human cells.

By layering additional large-scale in-house information we have detected thousands of SNPs that are likely to be functional. So far we have detected >100 functional SNPs that are associated to common diseases and intermediary phenotypes and in some cases the molecules that bind differentially between alleles. We have started to assay them using a newly developed high-throughput system.

In collaboration with Susanne Bornelöv, Umer Husen, Klev Diamanti, Jan Komorowski (UU)

In the cell histone molecules and 147 base pairs of DNA form nucleosomes and many of them have defined positions over genes and around gene regulatory elements. Some histones have epigenetic marks reflecting the function of the specific genomic region and we map these features at the theoretical resolution. We have found that nucleosomes are positioned over exons and have epigenetic marks that are associated to splicing. Other nucleosomes flank

gene regulatory elements and carry other epigenetic marks. We have found that nucleosomes at promoters carry specific modifications if they are located in transcribed sequences.

Cancer develops when cells acquire mutations that were not present in the person at birth. In a new project we have started to search for mutations in regulatory elements that contribute to cancer and have a large collection of candidates. The initial experimental validation has shown the expected results. This project is likely to add a new dimension to cancer etiology.

Group members during 2014

Claes Wadelius, professor, group leader

Marco Cavalli, researcher

Helena Nord, post doc

Gan Pan, post doc

Madhusudhan Reddy Bysani, postdoc

Emelie Wallén Arzt, master student

Funding during 2014

Swedish Research Council, 850 kSEK

AstraZeneca, 950 kSEK

Publications 2012-2014

1. Enroth S, Bornelöv S, Wadelius C, Komorowski J. Combinations of histone modifications mark exon inclusion levels. *PLoS One*. 2012;7(1):e29911.
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6. Enroth S, Andersson R, Bysani M, Wallerman O, Termén S, Tuch BB, De La Vega FM, Heldin CH, Moustakas A, Komorowski J, Wadelius C. Nucleosome regulatory dynamics in response to TGF β . *Nucleic Acids Res*. 2014, 42(11):6921-34.
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Molecular and Morphological Pathology

Research projects the programme Molecular and morphological pathology focus on disease related alterations in tissues. Our main objectives are to improve diagnostics and to identify potential targets that can be used for development of new therapies. We study both morphological changes and molecular alterations. e.g. in protein expression or on the DNA or RNA level. On-going projects include studies on neurodegenerative diseases and cancer, for instance tumours in the lung, brain, skin or blood.



Neuropathology

Irina Alafuzoff

Our research focuses primarily on various degenerative processes and diseases of the human brain. The material that we study is human tissue, brain or other organs, obtained post-mortem (neurodegeneration and vascular pathology) or during surgical procedure (primary brain tumours). The methods applied include among others histology, immunohistochemistry and *in situ* hybridization.

Neurodegenerative diseases

Svetlana Popova, Maria Leino, Adila Elobeid, Tuomas Rauramaa

One of the major events in neurodegeneration is misfolding of proteins that tend to accumulate in the cells or matrix. Misfolding of proteins increases with aging. Accumulation of misfolded proteins leads to functional disturbances seen as various movement disorders or cognitive impairment/dementia.

Based on current knowledge the most common form of neuronal degeneration is the hyperphosphorylation of the tau (HPTau) protein followed by alteration of beta-amyloid and alpha-synuclein.

The questions addressed by the research team during 2014 are briefly the following; initiation site of neuronal degeneration, incidence of certain neuronal degeneration, progression pattern, associated alterations such as astrogliosis, microgliosis, seeding of misfolding of proteins.

Research projects have been summarised and published (see publ list) involving PhD student Adila Elobeid, in collaboration with iNPH group in Kuopio, University of Eastern Finland (UEF); international PART consortium and in collaboration with Brain Net Europe. Manuscripts are under preparation involving Post Doc Svetlana Popova and UEF PhD student Tuomas Rauramaa.

Primary brain tumours of glial origin

Sylwia Libard

The most devastating brain tumour is glioma that can be of various grades ranging from I to IV. Currently there is no cure for these tumours and the most malignant glioma of grade IV is lethal. Treatment strategies include surgery, radiotherapy and chemotherapy. The main focus today is to identify new treatment strategies. For this approach detailed assessment of

tumours, i.e., morphology, protein expression and molecular data are required. In addition to facilitate assessment of a huge number of cases in a unified manner tissue microarray approach (TMA) is implemented.

The question addressed by the research team are briefly the following; alteration in protein expression in relation to grade, neuroanatomical region, recurrence and treatment.

Research projects have been summarised and published (see publication list) involving PhD student Sylwia Libard and in collaboration with UU/IGP research groups in the field brain tumours. Manuscripts are under preparation in collaboration with neurosurgeons at Uppsala University Hospital (Maria Zetterling), in collaboration with UU/IGP research groups in the field brain tumours and in collaboration with the national GLIOGENE group.

Vascular brain pathology

Tuomas Rauramaa, Yasmin Lundström, Patrik Lundström,

With aging the cardiac function as well as the vessels display age related changes that ultimately lead to various extent of circulatory failure. Brain tissue alterations related to insufficient circulation are common but poorly investigated. A brain infarct can be seen as a defined lesion. Assessing brain tissue with diffuse neuronal loss, loss of oligodendrocytes or activation of astrocytes or microglia that is initiated by various severity of ischemia/anoxia is more difficult.

The question addressed by the research team are briefly the following; primary protein alteration to be seen in neurons, astrocytes, oligodendrocytes or microglia at hypoxia/anoxia.

A research project has been published (see publ list) involving UEF PhD student Tuomas Rauramaa. Pilot studies have been carried out involving the Sofosko students Yasmin Lundström and Patrik Lundström.

Group members during 2014

Irina Alafuzoff, professor, group leader

Adila Elobeid, PhD student

Maria Leino, researcher

Sylwia Libard, PhD student

Patrik Lundström, student

Yasmin Lundström, student

Svetlana Popova, researcher

Tuomas Rauramaa, PhD student

Funding during 2014

ALF, 600 kSEK

Hans-Gabriel och Alice Trolle-Wachtmeisters stiftelse för medicinsk forskning, 500 kSEK

Publications 2012-2014

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Translational Tumor Pathology

Patrick Micke – Johan Botling

The Molecular Pathology of Non-small Cell Lung Cancer

Non-small cell lung cancer (NSCLC) represents a histologically mixed group of highly aggressive tumors. In subsets of patients, distinct genetic aberrations have been identified that are now successfully exploited for therapeutic intervention. However, for the vast majority of patients, treatment options are scant and overall prognosis poor. The molecular characterisation of NSCLC is challenging because of its notorious heterogeneity and its apparent genetic instability. Therefore, it is difficult to separate “driver” mutations from irrelevant genetic events in experimental model systems. Instead, correlation of molecular alterations in the tumor tissues of individual patients to actual clinical outcome is essential in order to understand the basic tumor biology, and necessary for the development of diagnostic biomarkers and new treatment strategies.

In an explorative phase we have investigated fresh frozen tissue samples of consecutively operated NSCLC patients and obtained comprehensive molecular landscapes by the use of array technology, sequencing methods, tissue microarrays and immunohistochemistry. The combined clinical, histopathologic and molecular data set represents the largest single institute cohort of this kind worldwide and forms the vantage point for translational studies. We have identified specific aberrations on genomic and transcriptomic levels that are strongly associated with clinical outcome. Using immunohistochemistry these molecular changes were confirmed on the protein level in independent cohorts, thus, have potential for use in clinical diagnostics (Micke et al., 2011; Botling et al., 2013).

In addition to epithelial tumor cell characteristics, stromal components were identified and correlated to relevant patient characteristics and survival (Edlund et al., 2012). Immunoglobulin light chain expression and plasma cell infiltration were demonstrated as powerful prognostic markers in NSCLC and other human solid tumors (Lohr et al., 2013; Schmidt et al., 2012). The results highlight the impact of the host’s immune response in tumorigenesis. To identify potential immunogenic targets we applied RNA sequencing technology on 204 NSCLC tumor samples. This analysis provided unexcelled resolution of gene expression, including splice variants and mutations. The combination of our NSCLC data set with 32 different normal tissues (Lindskog et al., 2014) allowed characterization of lung cancer specific gene expression. Based on this data we were able to define the landscape of cancer testis antigens in NSCLC on the transcriptomic and proteomic level, hopefully, providing new cancer specific targets for immunotherapeutic intervention (Micke et al., 2014).

Over the last years the lung cancer genome has been characterized by whole genome and exome sequencing on fresh-frozen tumor tissue. Based on this knowledge we have established and optimized an 82-gene panel for characterization of “real life” NSCLC samples, i.e. small formalin-fixed paraffin embedded (FFPE) biopsies, by next generation sequencing. The data will be used to define the genetic characteristics of our established surgical patient cohorts in relation to biomarker profiles, as well as for selection of prospective patients for targeted treatment.

Translation to diagnostic molecular pathology

A key effort of our group is to translate knowledge and established technology into routine diagnostics. To this end, mutation assays of KRAS, NRAS, BRAF, EGFR and PIK3CA in lung cancer, colon cancer and melanoma have been set up at the clinical Department of Pathology. Our group now leads the Solid Tumor Work Package in the national Clinical

Sequencing Platform (Science for Life Laboratory). An important milestone was met during 2014 as we were able to launch, as the first laboratory in Sweden, a multiplex mutation assay (targeted NGS) for targeted treatment prediction in cancer patients in routine health care.

Group members during 2014

Patrick Micke, associate professor, group leader

Dijana Djureinovic, PhD student

Millaray Marincevic, researcher

Johanna Mattsson, PhD student

Johan Botling, associate professor, group leader

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Funding 2014

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Regional Research Council (Uppsala-Örebro region), 750 kSEK

Swedish Cancer Society, 500 kSEK

Central ALF Uppsala, 600 kSEK

Johan Botling

Swedish Cancer Society, 500 kSEK

ALF, 400 kSEK

Vinnova, 500 kSEK (together with P. Micke)

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Amyloid Research

Per Westermark

The assembly of proteins into amyloid fibrils as cause of disease is attracting increasing attention, not only in systemic disorders and in connection with neurodegenerative conditions but also associated with other diseases such as type 2 diabetes. We have a broad interest in the nature, pathogenesis and impact of a number of amyloid diseases, both systemic and localized.

Together with researchers in Umeå we have found that there are two distinct phenotypes in Swedish familial transthyretin (TTR)-derived amyloidosis and that these are characterized by differences in posttranslational processing of the protein. We can distinguish between the two with the aid of a simple subcutaneous adipose tissue biopsy. This is important since one of the phenotypes carries a big risk of progressive cardiomyopathy also after liver transplantation, which is the main treatment today. While the Swedish type of mutation (V30M) is characterized by the two different phenotypes, most other TTR mutations are associated with a risk for cardiomyopathy. We have recently shown that spinal stenosis may be a manifestation of TTR-amyloidosis, both of wildtype and of mutation-associated type.

The possible transmission of amyloid diseases by a prion-like mechanism is one of our main interests. We are, in collaboration with researchers at SVL and SLU, Uppsala, performing studies on the possibility that AA-amyloid may be present in our environment and act a putative risk factor for development of the disease in animals and human. Together with G.T. Westermark, Department of Medical Cell Biology, we have found that seeding, cross-seeding and transmission of localized amyloidoses are possible, such as those consisting of A β and IAPP.

Localized amyloid has been identified as important actors in Alzheimer's disease and type 2 diabetes. We are currently investigating the possibility that amyloid deposits also are important in some other major diseases, particularly aortic aneurysm and atherosclerosis. Amyloid in atherosclerotic plaques is an overlooked phenomenon and our hypothesis is that toxic protein aggregates are mechanistic in the pathogenesis of atherosclerotic lesions. We are evaluating a candidate protein for the atherosclerotic amyloid fibril.

Our laboratory is also working in association with the University Hospital and is performing amyloid diagnostic work within the hospital. As systemic amyloidoses are increasingly identified as clinical problems particularly in elderly, we are receiving an increasing number of biopsies each year. Our laboratory is devoted to development of existing methods to determine type of systemic amyloidosis. For this, we are also developing new antibodies for clinical use and are planning to introduce mass spectrometry.

Group members during 2014

Per Westermark, professor em., group leader
Ellahe Charkhkar, lab technician

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Clinical and experimental pathology

The objectives in different research projects carried out within the field of clinical pathology or cytology is to increase knowledge regarding disease related alterations observed in tissue. One goal is to improve diagnostics to make it more informative, another is to identify potential targets to be used for the development of new treatment strategies. The alterations to be looked for can be seen as morphological changes, changes in protein expression or on the DNA or RNA level.

The assessed tissues are obtained from humans, i.e., biopsies, surgical specimens or autopsy specimens and the methods implemented are numerous. All studies on human tissue are carried out following the current legislation in Sweden; [The Act](#) (2003:460) and the statute (2003:615) concerning the Ethical Review of Research Involving Humans; [the statute](#) (2007:1069) with instructions for Regional Ethical Review Boards; the statute (2007:1068) for the Central Ethical Review Board. The translations of the Act (2003:460) and the Statute (2003:615) are updated with changes that came in to force 2008).

Inflammation, transplantation and cardiovascular pathology

Erik Larsson, Anna-Carin Wallgren

These projects are aiming at a better understanding of the mechanisms involved in acceptance of transplanted organs, mainly kidneys. We focus on changes in blood vessels and in the interstitium. The techniques used are different immunological and molecular biological methods and our goal is to prolong the lifetime of a well functioning graft. The work is performed in collaboration with the departments of transplantation, nephrology, microbiology and immunology.

Human endogenous retroviruses (HERVs) are estimated to make up roughly 9 per cent of the human DNA. Many of these sequences are inactivated and can't be expressed. However several have conserved parts of their genome and can be expressed also on protein level. There is growing evidence that HERVs have made a significant contribution to human evolution, development and physiology as well as playing possible role in initiation and progression of human diseases.

We aim to create a map of different expressed HERV encoded proteins in both normal and neoplastic tissues by using antibody based proteomics. This work is performed in collaboration with the human protein atlas project (HPA).

Hematopathology

Rose-Marie Amini, Christer Sundström, Maysaa Abdulla

Inflammatory cells are in close proximity to all kinds of malignant tumours, where the innate immunity (myeloid) acts as the first line of defence. The innate immunity includes macrophages, granulocytes (neutrophils, eosinophils, basophils), mast cells, dendritic cells and NK cells. The adaptive immunity (lymphoid) is the acquired immunity consisting of B- and T-cells.

An increasing interest in the "tumour microenvironment" has been shown lately since new treatment modalities like "targeted therapy" with cytotoxic T-cells and against tumour associated macrophages have been developed. Prediction of survival in follicular lymphoma was recently shown to be based on molecular features of the tumour infiltrating immune cells.

Our group studies the microenvironment in malignant lymphomas with a special focus on the inflammatory cells in Hodgkin lymphoma and diffuse large B-cell lymphomas. The presence and function of the surrounding immune cells are correlated in large population-

based patient cohorts to clinical data like patient characteristics, treatment outcome and survival.

We also focus on the pathogenetic mechanisms in B-cell lymphomas affecting the spleen (splenic marginal zone lymphomas) and lymphomas primarily affecting the central nervous system. We and others have observed an increase in the incidence of primary CNS lymphomas (PCNSL) and these lymphomas may differ regarding tumour characteristics compared to those primarily affecting lymphoid tissue only.

Tissue arrays for the identification and validation of new diagnostic and prognostic tumour markers in histopathological thyroid tumour diagnosis.

Malignant melanoma

Margrét Agnarsdóttir

The incidence of cutaneous malignant melanoma has increased dramatically in Caucasians in the last few decades, an increase that is partly explained by altered sun exposure habits. For the individual patient, with a localized disease, the tumour thickness of the excised lesion is the most important prognostic factor. However, there is a need to identify characteristics, especially for patients with thin melanomas (< 1mm) that can place patients into certain risk groups.

The protein expression of multiple proteins in malignant melanoma tumours was studied, with the aim of identifying potential new candidate biomarkers. Representative samples from melanoma tissues were assembled in a tissue microarray format and protein expression was detected using immunohistochemistry. Two cohorts were used and for a subset of proteins the expression was also analysed in melanocytes in normal skin and in benign nevi. Furthermore the cohorts were employed to develop an automated algorithm to identify melanoma cells in the tissue samples. The immunohistochemical staining was evaluated manually and for the majority of proteins also with the automated algorithm. It has been difficult to identify new single prognostic protein markers that have a stronger predictive value than the thickness but some of the markers were described for the first time in melanomas. However, combining results for a few markers employing the automated algorithm has revealed interesting combination of markers that we are still working with.

Pituitary adenomas – predictive and potentially therapeutic biomarkers

Olivera Casar-Borota

Pituitary adenomas comprise about 10 to 15% of all intracranial neoplasms in adults. They cause serious symptoms related to hormonal hypersecretion from the adenoma tissue and/or related to the effects of the intracranial tumour mass. The cornerstones of the treatment of pituitary adenomas are surgery for non-functioning pituitary adenomas (NFPAs), growth hormone (GH)-producing and adrenocorticotroph hormone (ACTH)-producing adenomas, medical treatment with dopamine receptor agonists for prolactinomas and with somatostatin analogues and GH-receptor antagonist for GH-producing adenomas, and radiotherapy (gamma-knife or conventional) for postoperative residual tumours, aggressively growing adenomas or histologically atypical adenomas.

Unfortunately, a considerable proportion of the patients are not cured even following combined surgical/medical/radiotherapy. This indicates a need for defining tumour biomarkers that can predict the clinical response to the established medical treatment, as well as biomarkers that could indicate new, potentially effective medical therapeutic options, especially in patients with NFPAs and ACTH-producing adenomas.

Objectives: We have focused on protein biomarkers such as somatostatin receptors and E-cadherin as the predictors of GH-producing pituitary adenomas biological behaviour and

clinical response to medical treatment with somatostatin analogues. We have demonstrated that immunohistochemical analysis of somatostatin receptors in GH-adenoma tissue may be an important tool in the selection of the optimal and individualised pharmacological treatment of the patients with acromegaly. Further, we have demonstrated that reduced level of E-cadherin correlates with more aggressive clinical behaviour in ACTH-producing adenomas.

We have also studied how different morphological patterns in GH-producing adenomas (sparsely vs. densely granulated adenomas) determined by expression of cytokeratin Cam 5.2 in the tumour cells correlate with clinical features of the adenomas and with the response to the medical treatment. In addition, we have demonstrated correlation between the granulation pattern and radiological features of GH adenomas, which could be helpful in predicting the clinical behaviour of the adenomas before the surgery and histological examination.

On the gene level, we have examined how mutational status of the *gsp* gene influences the biological behaviour and response to medical treatment with somatostatin analogues in GH-producing adenomas, and how mutational status of the E-cadherin gene and HDAC-2 gene correlates with clinical behaviour of ACTH-producing adenomas. Regarding new potentially therapeutic biomarkers, we have focused on research of KIT protein and KIT gene in pituitary adenomas of different hormonal types.

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The Human Protein Atlas

The aim of the Swedish Human Protein Atlas is to systematically determine the distribution and relative abundance of all human proteins in defined tissues, cells and sub-cellular compartments. In addition to generating a map of protein expression patterns, the Human Protein Atlas provides a starting point for the discovery and identification of biomarkers.



The Human Protein Atlas

Fredrik Pontén, Mathias Uhlén

The Swedish Human Protein Atlas project has been set up to allow for a systematic exploration of the human proteome using an antibody-based proteomics strategy. This mapping effort can be viewed as an ambition to generate an additional "layer" of information on top of the human genome sequence data. By determining the localization and relative abundance of proteins in specific tissues, cells or subcellular compartments our general knowledge will increase. There is also a demand for new biomarkers, particularly in the field of cancer diagnostics where markers are needed to determine cellular differentiation, grade of malignancy and stratification of tumors with respect to prognosis and response to therapy.

Analysis of protein expression patterns is performed using immunohistochemistry on tissue and cell microarrays. These contain more than seven hundred spots of normal and cancer tissues as well as *in vitro* cultured cells. Immunohistochemically stained tissue microarray sections are scanned to obtain high-resolution images. Each image is manually annotated by pathologists to determine expression and localization profiles. Cells are annotated using an image analysis-based system.

In addition to generating antibody-based protein profiling data, the Human Protein Atlas has also performed transcriptomic (RNA-seq) analyses for the majority of tissues and cell-lines used in the project. This transcriptomic data is integrated to the atlas to provide an additional layer of information of gene/protein expression in our tissues, and furthermore serves as a tool to validate the proteomic data generated by antibodies.

All protein and RNA profiling data, including the underlying high-resolution images is presented in an anatomically comprehensive, publicly available protein atlas (www.proteinatlas.org). New data and more features are released in annual updates of the database. The current version 13 of the Human Protein Atlas includes protein profiles from close to 24.000 antibodies generated towards 17.000 unique proteins (corresponding to over 80% of the human protein encoding genes). All antibodies are used for protein profiling in normal human tissues from 144 individuals, where a defined set of normal cell types are annotated for each tissue, and in 216 different tumors representing the 20 most common forms of human cancer. In addition to the high throughput protein profiling core project, several projects with more specific objectives are run based on the resources gene-rated within the Human Protein Atlas. Below is a short description of such selected projects, in which the work of the technical staff headed by Ing-Marie Olsson (TMA- and IHC-groups), Evelina Sjöstedt (Microscopy-group) and Cecilia Lindskog-Bergström (Protein profiling) should also be acknowledged.

Cancer biomarkers

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In collaboration with Karin Jirström (MAS), Patrik Micke, Johan Botling, Irina Alafuzoff, Michael Bergqvist, Anja Smiths, Anca Dragomir, Bengt Glimelius (UAS), Dan Hellberg (Falu lasarett), Lars Holmberg (ROC), Monica Nistér, Georg Klein (KI), Jutta Huvila, Olli Carpén (Turku University), Irma Fredriksson (KI/KS), Anna Dimberg (UU), Liam Gallagher (UCD, Dublin), Gabriella Gremel (Manchester University, UK), Gillian O'Hurley (Oncomark, Dublin), Halfdan Sörbye (Bergen University Hospital, Norway), Camilla Qvortrup, Per Pfeifer (Syddansk universitet, Denmark), Gerrit Meijer, Meike de Wit (VUMC, Amsterdam), Mathias Uhlén (SciLifeLab).

In several projects the aim is to further analyze the role of proteins identified as potential cancer biomarkers in the screening effort performed within the Human Protein Atlas project. Tumor material from well-defined patient cohorts with tumors representing all major forms of human cancer are being collected and assembled into tissue microarrays. In addition to tumor material, clinical data is also collected to create databases allowing for testing and validation of protein expression patterns of importance for diagnostics, prognostics and functional tumor biology studies. There are special emphasis on i) colorectal cancer for the identification of markers that can stratify patients into groups of high or low risk for recurrent disease, ii) breast cancer in young women based on a large national cohort and extensive clinical database of >1000 patients where the focus is to understand why this patient group has such poor prognosis, and iii) gynecologic cancers for evaluation of novel prognostic biomarkers. Other collaborative biomarker projects include lung cancer, melanoma, high and low grade gliomas, cervical cancer and prostate cancer.

Tissue specific proteomes defined by RNA-seq and antibody-based protein profiling

Cecilia Lindskog-Bergström, Sandra Andersson, Dijana Djureinovic, Linda Oskarsson, Evelina Sjöstedt, Angelika Danielsson, Per-Henrik Edqvist, Anna Asplund, Agata Zieba, Caroline Kampf, Julia Bergman-Larsson, Fredrik Pontén

In collaboration with Uppsala Akademiska Hospital, Dept. of Clinical Pathology, Linn Fagerberg, Björn Hallström, Jan Mulder (SciLifeLab), Åsa Sivertsson (KTH), Gabriella Gremel (Manchester University, UK), Karolina Edlund (Ifado, Tyskland), Mathias Uhlén (SciLifeLab)

The large-scale RNA-seq effort of multiple human normal tissues undertaken by the Protein Atlas project has facilitated the systematic comparison among tissues with the aim of defining the “tissue-specific proteome” for each tissue. The project is focused on identifying the highest abundant tissue-enriched or group-enriched transcripts (for highly similar tissues) and comparing these across all other tissues or tissue-groups. The antibody-based IHC protein profiling data is included in these analyses to provide a spatial resolution of where the gene is expressed on the protein level with respect to different cell types/ sub-compartment/layers, etc. On a global scale, over 20 such tissue- or tissue-group specific proteomes have been defined to date.

Protein signatures related to pancreatic islet cell biology

Angelika Danielsson, Cecilia Lindskog, Fredrik Pontén

In collaboration with Olle Korsgren, Lars Johansson (UU), Mathias Uhlén (SciLifeLab)

This project aims to develop tools for *in vivo* imaging of beta-cells by the identification of new surface cell markers specifically expressed on beta-cells. Specific tissue microarrays are

generated and following searches in the Human Protein Atlas database, candidates showing selective expression patterns in Langerhans islets are further validated and characterized. Candidate proteins showing surface expression and availability of epitopes *in vivo* are used as templates to generate small molecules, affibodies and/or diabodies that can further be utilized in the development of PET tracers for *in vivo* use in humans. The overall goal is to develop a system for measuring beta cell mass in humans as a tool to study anti-diabetic drugs and development of diabetes.

Antibody validation, performance and characterization.

Anna Asplund, Sandra Andersson, Agata Zieba, Caroline Kampf, Per-Henrik Edqvist, Fredrik Pontén

In collaboration with Ulf Landegren, Karl Andersson/Lars Gedda, Tobias Sjöblom (UU), Cecilia Williams (University of Houston), Mathias Uhlén (SciLifeLab)

The objective of this technical development project is to develop assays and strategies that can validate and verify protein expression data obtained using antibodies in cells and tissues, i.e. immunohistochemistry. For instance, methods for simultaneous detection of multiple epitopes on a common antigen are developed. Primarily, enzyme based, light microscopical immunohistochemistry and immunofluorescence is used, but also proximity ligation. In addition, *in situ* hybridization and immunohistochemistry on consecutive sections are also explored as a tool for antibody validation. For technical antibody validation transient knock-down of expression, real-time measurements of antibody binding as well as simultaneous stainings using multiple antibodies are employed.

Correlation studies on global RNA transcript and protein expression levels in cell-lines

Anna Asplund, Karolina Edlund, Per-Henrik Edqvist, Caroline Kampf, Fredrik Pontén

In collaboration with Uppsala Akademiska Hospital, Dept. of Clinical Pathology, Emma Lundberg, Linn Fagerberg, Björn Hallström (SciLifeLab), Åsa Sivertsson (KTH), Mathias Uhlén (SciLifeLab)

The enormous amount of IHC data available within the HPA project enables large-scale comparative studies of RNA and corresponding protein levels on a global level. Data from 17 cell lines, analyzed with RNAseq as well as with IHC using approximately 10 000 antibodies, revealed that 18% of the antibodies generated IHC staining patterns correlating with RNA with a Pearson correlation coefficient of $>0,5$. This antibody-based proteomics approach also aims to elucidate the protein signatures inflicted by *in vitro* growth conditions and map cell type specific protein signatures to understand how different cell lines can be utilized as model systems to study different tumor types.

Novel diagnostic tools for determining the origin of cancer metastases

Julia Bergman, Dijana Djureinovic, Per-Henrik Edqvist, Fredrik Pontén

In collaboration with Karin Jirstrom (MAS), Patrick Micke (UAS), Gabriella Gremel (Manchester University, UK), Mathias Uhlén (SciLifeLab)

The use of antibodies that target proteins that are tissue- or cell type specific are crucial diagnostic tools in clinical pathology where they are used in immunohistochemistry-applications for the characterization of cancer. Such specific diagnostic antibodies can be used to determine from which original tissue the cancer has developed and to sub-classify the tumor type. The vast amount of data in the Human Protein Atlas is screened for cell and tissue specific proteins. Identified candidates are further validated and characterized for sensitivity and selectivity of specific target binding. Selected antibodies are used to analyze the

expression pattern in a large TMA (over 900 cases) containing mainly metastases and primary tumor tissue from tumor types where additional diagnostic markers are needed. The aim is to find and define panels of diagnostic markers to be used in clinical pathology.

Protein profiling using highly characterized antibodies towards cancer proteins

Caroline Kampf, Fredrik Pontén

In collaboration with Gordon Whitely, Stephen Hewitt (NCI-CPTC program), Mathias Uhlén (SciLifeLab)

In an effort to generate highly characterized monoclonal antibodies towards proteins suggested to be involved in cancer growth and spread, the NCI initiated the CPTC program to drive the development of a central community core that would help accelerate biomarker discovery and validation, cancer diagnostics development, and therapeutics monitoring. As part of this effort CPTC antibodies are tested and used for protein profiling using Human Protein Atlas strategies including immunohistochemistry and immunofluorescence.

Building up tissue-specific genome-scale metabolic models based on RNAseq and immunohistochemical data

Caroline Kampf, Anna Asplund, Fredrik Pontén

In collaboration with Adil Mardinoglu, Jens Nielsen (Chalmers University)

The work constitutes a reciprocal collaboration, in which the HPA provides prof. Jens Niensens group with transcript and protein data for build-up and validation of their in-house generated tissue-specific metabolic models, and the models in return enable us to biologically interpret the massive amount of data generated within the HPA project. The metabolic models also makes it possible to identify potential drug targets, which can be validated using immunohistochemistry in tissue cohorts or functional assays in cell lines. This ongoing collaboration has so far rendered nine publications, where six are in high impact journals.

Group members during 2014

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Funding during 2014

Knut och Alice Wallenbergs stiftelse, 13 800 kSEK
ALF, 600 kSEK

Swedish Cancer Society, 500 kSEK
EU (Fast-Path), 380 kSEK
SSF, 300 kSEK
EU (Affinomics), 150 kSEK
Vinnova, 130 kSEK
Erik, Karin och Gösta Selanders stiftelse, 100 kSEK

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Molecular Tools

The development of new molecular detection strategies is fundamental to progress in biomedical research and diagnostics. The Molecular Tools research program has pioneered a broad range of technologies for measuring and characterizing DNA, RNA and protein molecules in solution phase or in situ. Examples of techniques that we have developed in the past include oligonucleotide ligation assays, padlock and selector probes, and circle to circle amplification for nucleic acid analyses; and proximity ligation assays in solution phase or in situ for investigating proteins or protein complexes. We apply our methods, often in collaboration with colleagues, to gain new biomedical insights and to identify diagnostic opportunities in a broad range of diseases including cancer, neurodegeneration, and infectious and cardiovascular disease.



We also very actively seek to make our techniques generally available as services from SciLifeLab, and through licenses to leading biotech companies, or by spinning out companies that can further develop these technologies. In addition, there is a steady stream of new, powerful technologies currently being developed in our research program, including UnFold and ExCirc probes, proxHCR, super rolling circle amplification (sRCA), and RCA reporters. Finally, we note that our lab has been a good breeding ground for young scientists, launching strong careers in academia or in the biotech industry.

Advanced Molecular Techniques in Genomics, Proteomics and Medicine

Ulf Landegren

Molecular medicine in research and clinical practice crucially depends on tools to accurately and comprehensively measure molecules. Such methods can provide entirely new biological insights, reveal disease processes, and serve to monitor responses to therapy. Our group has a very strong tradition of developing molecular tools for measuring and imaging DNA, RNA, protein molecules in biological samples such as blood and tissues. Methods we have pioneered include the oligonucleotide ligation assays, padlock, and proximity probes, as well as the novel nFold and ExCirc probes and super rolling circle amplification, currently under development in our lab. We apply these methods together with collaborating partners in a wide range of biomedical analyses with some focus on malignancies, neurodegeneration, cardiovascular disease, autoimmunity and infectious disease.

The probes we use typically include elements with affinity for specific nucleic acid or protein molecules, along with unique identifier DNA sequence elements that serve as a code for the recognized target molecules. The information content of the DNA strands that form in detection reactions is recorded after amplification by PCR, rolling circle amplification, or via next generation sequencing to identify the recognized target molecules. These very general procedures permit highly specific solution-phase or localized analyses of large sets of target molecules, extending even to the single-copy level to evaluate the molecular heterogeneity in cells and tissues, and the techniques are promising for a new generation of high-performance point of care analyses. Some of our ongoing projects are highlighted below.

New generation probing technologies to chart the molecular composition of cells

Rachel Nong

In this project we aim to construct a new class of probes to simultaneously detect or image most or all kinds of molecular events at improved kinetics, sensitivity, and specificity.

We have demonstrated the performance of these so called nFold probes and ExCirc probes in proof-of-concept experiments, and we currently investigate possibilities of applying them for simultaneously imaging large numbers of mRNA and miRNA molecules and their interactions with other molecules in tissue sections. In this context, we are establishing a new approach for molecular engineering of advanced molecular probes via directed evolution using parallel massive array DNA synthesis and next generation sequencing technologies in collaboration with Dr. Tarjei Mikkelsen at the Broad Institute.

Development of super rolling circle amplification

Lei Chen

The aim is to produce rolling circle amplification products at several micrometers in size on solid support or in solution and apply this technique on biological samples for efficient and rapid detection. We have successfully generated rolling circle products on the solid support with diameter in several micrometers. These new approaches for signal amplification will be important both for localized and digital detection and they may also enable rapid analyses at the point of care.

Development of specific and sensitive drug-protein interaction detection methods

Abdullah Al-Amin

Structural similarities in active sites lead to lack of selectivity and unwanted side effects in rational drug design. Methods are needed to study direct, more selective binding and correct localization of candidate drug and its target interaction in relevant clinical specimen during important steps in drug development in preclinical study. In a first phase of the study, we have described very sensitive and specific *in situ* drug-target interaction detection methods, where DNA-linked kinase inhibitors interaction were visualized by rolling-circle amplification (RCA) and via proximity ligation assays (PLA) within breast cancer tissue sections, cancer cell lines and in chronic myeloid leukemia (CML) patient samples. Another on going strategy is to combine thermal shift assay with multiplex proximity extension assays (PEA) for a quantitative drug proteins physical interaction measurement in cell extracts.

A platform for sensitive protein detection

Tonge Ebai

There is a great need for protein detection at improved sensitivity. We are developing assay formats that can improve specificity of detection, reduce nonspecific background, and permit strongly amplified detection signals while using standard assay formats. In one approach, proteins are captured from biological samples via antibodies immobilized in microtiter wells. The proteins are then detected via two more antibodies that have been modified with oligonucleotides, such that they can template the formation of a circular reporter DNA strand for amplified detection via rolling circle amplification. This assay offers increased specificity of detection while ignoring some sources of nonspecific detection and permitting enhanced detection signals.

Single cell proteomics

Caroline Gallant

The ability to investigate biological phenomena at the level of single cells is attracting increasing interest as a means to characterize cellular heterogeneity and explain biological responses by individual cells. We are developing miniaturized protein assays from single cell lysates for parallel, sensitive protein detection via proximity extension assays. The assays are used to characterize differences among, and responses by neural cells at the level of single cells.

Precise mapping of cell signaling pathways

Peter Lönn

In this project we are combining new molecular tools with classical biochemical methods to map dynamics of cell signaling pathways. We are especially interested in the multifunctional signaling cytokine TGF- β and are investigating the roles this pathway plays during carcinogenesis.

One part of the project is to optimize *in situ* PLA and PEA to enable high-throughput read-out of specific post-translational modifications and protein-protein interactions. The purpose is to use such assays to screen libraries of small molecular inhibitors, siRNAs, or bioactive peptides. The goal is to bring new insights about how post-translational modifications and interactions are regulated during carcinogenesis and furthermore to identify putative biomarkers and therapeutic targets/molecules/peptides.

RCA reporters - increasing the size of regular RCA products and potentially a new method for rapid point of care diagnostics

Johan Björkesten

In this project we aim to add pre-prepared protected circles to the sample together with RCA mix (including e.g. Phi29 polymerase and dNTPs). The circles are protected in such a way that they will only be amenable for RCA upon recognition of a certain target molecule which can be single stranded DNA or RNA. The circle together with a protection forms the RCA reporter. If the RCA reporters are designed to recognize an RCA product they can be used for a next generation of RCA growing blobs in a similar way as sRCA (developed in the group by Lei Chen). RCA reporters will be a very easy way to achieve micrometer sized blobs although it will probably not include the great specificity of SNP detection compared to sRCA. If RCA reporters are designed directly to e.g. viral RNA present in the blood they can potentially be used for rapid point of care diagnostics.

We have demonstrated in a synthetic model that RCA reporters can recognize a single stranded DNA target and upon recognition initiate RCA. The balance between protecting the circles from spontaneous RCA initiation and efficient activation upon target recognition has to be established. This will probably be done via directed evolution using parallel massive array DNA synthesis and next generation sequencing technologies.

PLA for protein analyses and biomarker validation

Masood Kamali-Moghaddam et al.

In the proximity ligation assay, specific proteins as well as their interactions and modifications, can be analyzed by translating detection reactions to DNA sequences. In this method protein binding reagents are modified by conjugation to DNA oligonucleotides. When two or more of these modified binders recognize a target molecule or a pair of interacting

proteins, the free ends of the attached oligonucleotides are brought in proximity and can be joined by DNA ligation. The ligation products are then amplified by PCR. The PLA technique can be carried out in solution – requiring very small amounts of materials to be tested – or on a solid phase whereby the target molecules to be detected can be first immobilized via affinity probes, while other materials are removed by washes. In a yet another format of PLA (*in situ* PLA) that can be used for protein analyses in cells and tissues the oligonucleotides are designed to guide circularization of two accessory linear DNA molecules. The DNA molecules that form by ligation are amplifiable by rolling circle amplification and visualized using epi-fluorescence or confocal microscopes.

The combination of the use of two or more binding reagents and efficient DNA amplification provides high sensitivity and specificity of detection, surpassing conventional protein detection methods. PLA can therefore provide a powerful molecular tool for protein measurements at extremely low concentrations.

We continuously improve methods for sensitive proteome analyses, aiming for further improved sensitivity of detection and for simultaneous detection of proteins in highly multiplexed formats. In addition to sensitive detection of soluble proteins, different variants of the technology has been used to establish assays for detection of immune complexes, aggregated proteins, fusion proteins and micro vesicles. For instance, in the field of neurodegenerative disorders we have developed a sensitive assay for specific detection of protein oligomers that plays a central role in diseases such as Alzheimer, prion and Parkinson diseases. Using the *in situ* PLA, we have established extremely specific and sensitive assays to study protein interactions and posttranslational modifications such as phosphorylation of Tau protein, which plays a central role in development of Alzheimer's disease. In addition, we have developed a multiplex PLA in which up to 47 proteins are analyzed simultaneously using very small amount of patient samples. The Multiplex PLA has, for instance, been used to screen blood samples from patients with chronic pain, and cerebrospinal fluid samples from patients with amyotrophic lateral sclerosis, and we have identified several biomarker candidates in the latter disease.

We have also developed a new form of PLA (4PLA) that requires binding by five different antibodies for specific detection of more complex target molecules. Using this sensitive assay form we have for the first time been able to detect prostasomes in blood plasma – establishing these as a member of a new class of biomarkers generally referred to as microvesicles/exosomes. 4PLA-based detection of prostasomes revealed elevated levels of these microvesicles in samples from prostate cancer patients, and the analysis also demonstrated that the concentration of prostasomes better reflects disease aggressiveness than the currently used PSA test.

Currently, we utilize various multiplex proximity assays to identify and characterize a large number of microvesicles originated from different organs – such as prostate, lung and breast – in order to establish new sensitive and reliable diagnostic and prognostic tests using this novel class of biomarker candidates.

Group members during 2014

Ulf Landegren, professor, group leader

Abdullah Al-Amin, PhD student

Andries Blokzijl, researcher

Johan Björkesten, PhD student

Lei Chen, PhD student

Christina Classon, research engineer

Tonge Ebai, PhD student

Elin Ekberg, administrative assistant
Caroline Gallant, post doc
Joakim Galli, project coordinator
Johanna Herö, research engineer
Peter Lönn, researcher
Johan Oelrich, systems developer
Lisa Steidle, student
Mike Taussig, researcher
Erik Ullerås, project coordinator
Rachel Yuan Nong, post doc

Member establishing independent research group

Masood Kamali Moghaddam, researcher
Liza Löf, PhD student
Felipe de Oliviera, PhD student
Anne-Li Lind, PhD student
Lotta Wik, post doc
Di Wu, PhD student
Junhong Yan, PhD student
Agata Zieba Wicher, researcher

Dissertations during 2014

Di Wu, Proximity Ligation and Barcoding Assays: Tools for analysis of proteins and protein complexes, April 25, 2014.

Funding during 2014

Swedish Research Council (Medicine and Health), 1 100 kSEK; 2 500 kSEK
Swedish Research Council (Science and Engineering), 980 kSEK
Swedish Research Council (Infrastructure, PI: J. Dillner), 1 200 kSEK
Swedish Research Council, 430 kSEK (to Peter Lönn)
SSF, 1 650 kSEK
EU FP7, DiaTools, 2 000 kSEK
EU FP7, Affinomics (PI: M. Taussig), 700 kSEK
EU FP7, BBMRI-LPC (PI: M. Perola), 850 kSEK
EU FP7 Marie Curie ITN, BioMaX (PI: J. Lammertyn), 1 100 kSEK
EU FP7 Marie Curie ITN, GastricGlycoExplorer (PI: N. Karlsson), 400 kSEK
ERC, ProteinSeq, 4 500 kSEK
IMI OncoTrack (PI: Hans Lerach), 3 000 kSEK

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Molecular Diagnostics

Mats Nilsson

The main aim of our research is to develop improved techniques for molecular analyses. We aim to develop techniques that enable determination of quantities and location of specific nucleic acids in situ; amplified single-molecule detection in solution; and sequence composition of DNA samples.

The basic molecular devices that are used are the padlock- and selector probes, that are both acting through a strictly target dependent ligase-mediated DNA circularization reaction. These reagents have a specificity matching that of PCR, but can unlike PCR be deployed in highly multiplex analyses.

An important objective is to apply these techniques in collaborative projects to solve fundamental research questions and to serve unmet clinical diagnostic needs. In a multidisciplinary approach, dedicated micro and nano devices employing the molecular detection techniques will be developed, to enable rapid, sensitive and cost-effective point-of-care diagnostics.

Mats Nilsson is currently visiting professor at IGP and has since the second half of 2012 his main laboratory at Science for Life Laboratory in Stockholm, being professor at the Department of Biochemistry and Biophysics, Stockholm University (<http://www.dbb.su.se/en/?p=researchgroup&id=237>). His current group at IGP is mainly engaged in two projects, but he is also engaged in numerous collaborations with other groups at IGP.

Amplified single-molecule detection and biosensors

David Herthnek, Camilla Russell, Malte Kühnemund

An ideal diagnostic analysis device should be able to detect specific biomarkers with single-molecule sensitivity, exquisite specificity, a wide linear quantitative range, high quantitative precision, in a multiplex format, cost effectively and user-friendly. Depending on the purpose, different requirements will apply for such devices, e.g. cheap and simple devices for use in the field in developing countries, simple devices for consumer self diagnostics, rapid and accurate point-of-care devices, and high-throughput - high performance central laboratory devices.

Examples of applications that require extreme sensitivity include the detection of biomarkers leaking from an affected organ into the circulation for early diagnosis of disease, and infectious diagnostics where a single pathogen may be sufficient to cause disease. Moreover, both these applications typically require parallel analyses of large sets of biomarkers.

Sensitive biomolecular analysis requires a highly selective identification reaction coupled to signal amplification that does not introduce background signal noise. Present biosensor and diagnostic devices are limited in one or more of the desired analytic properties. For protein biomarkers, multiplexing and sensitivity are typically limited, while for nucleic acid biomarkers, sensitivity has to be sacrificed to gain multiplexing and the analysis devices are expensive and not very user friendly. We now aim to develop diagnostics concepts by deploying our molecular tools in biosensor devices by utilizing nano- and micro engineering.

We are collaborating with M Strömme and P Svedlindh at the Ångström Lab, Uppsala University, to develop a simple magnetic sensor that could be integrated in a hand-held device to detect amplified molecules labeled with iron nanobeads. The basic concept has been shown to work and promising sensitivity has been achieved. We are further exploring a simple and

sensitive electric read-out within the Berzelii Technology Center for Neurodiagnostics, where we published a proof-of-concept paper recently (see list of publications).

Targeted multiplex genome analysis

Elin Falk-Sörqvist, Lotte Moens, Lucy Mathot

We have developed the selector probe technique, for targeted re-sequencing applications. Our aim is to enable high-performance selective target enrichment as a sample preparation step for next-generation sequencing instruments.

By focusing the sequencing power to the genes and chromosomal regions that are most likely to be relevant for a particular disease, a lot more DNA samples can be sequenced for a certain amount of research funding. In addition, the quality of the sequence can be improved since greater sequencing depth can be afforded, and the data analysis is greatly facilitated compared to sequencing whole genomes. Our main focus is to develop the technology for applications in clinical diagnostics.

Group members during 2014

Mats Nilsson, professor, group leader

Group in Uppsala

Megha Biradar, student

Elin Falk Sörqvist, bioinformatician

David Herthnek, postdoc

Malte Kühnemund, PhD student

Lucy Mathot, PhD student

Camilla Russel, PhD student

Group in Stockholm

Annika Ahlford, postdoc

Anna Engström, postdoc

Thomas Hauling, postdoc

Ivan Hernandez, PhD student

Ronqin Ke, postdoc

Tomasz Kryzkowski, PhD student

Elin Lundin, PhD student

Anja Mezger, PhD student

Marco Mignardi, PhD student

Pavankumar Ramachand, postdoc

Tagrid Salih, research assistant

Jessica Svedlund, postdoc

Dissertations during 2014

Lucy Mathot, From Tissue to Mutations: Genetic Profiling of Colorectal Cancer, November 7, 2014.

Funding during 2014

VINNOVA, 627 kSEK

EU/IMI, (PI H. Lerach), 400 kSEK

VINNOVA/VR: Berzelii centre, 537 kSEK

EU, Marie Curie ITN: BioMax (PI U. Landegren), 1 100 kSEK

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Molecular proteomics

Ola Söderberg

My ongoing research and future plans includes both methods development and application of these methods to solve biological and medical problems. These activities are highly interdependent: the need to answer new types of questions is the motivation for methods development, and the availability of novel methods provides opportunities to pursue new scientific challenges.

Although all information about both RNA and proteins is encoded in the DNA, the functional components of a cell are mainly proteins. At any given time point the proteome of each individual cell reflects both genetic and epigenetic information. However, the activity status of proteins is not encoded in the genome. Instead this is regulated by protein-protein interactions and post-translational modifications (PTMs), often as a result of external stimuli mediated by cell-to-cell contacts and binding of secreted proteins.

To deduce the influences of the cellular microenvironment analyses need to be performed of proteins, protein interactions and PTMs at a single cell level *in situ*, thus retaining information of the tissue architecture and positions of all individual cells within this. Targeted analysis utilizing affinity reagents, e.g. antibodies, has been used for decades in both research and for diagnostic purposes. To increase selectivity of affinity reagent based methods, multiple recognition events can be applied to overcome the problem with cross-reactivity, i.e. antibodies that bind to unintended targets. Detection of low abundant molecules requires either sensitive read-out instruments or powerful signal amplification.

Proximity ligation assay (PLA) combines multiple recognitions of affinity reagents with potent signal amplification, utilizing methods for DNA analysis to generate a signal that will be a surrogate marker of the targeted protein, PPI or PTM. The method is based on pairs of proximity-probes (i.e. antibodies conjugated to strands of DNA) to detect the proteins of interest. Only upon proximal binding of these probes can an amplifiable DNA molecule be generated by ligation, which enhance the selectivity of the method even further.

Since the development of *in situ* PLA in 2006 most of our efforts has been related to the use of *in situ* PLA and to further improve the method.

Tumor analysis

Karin Grannas, Linda Andersson, Axel Klaesson & Gaëlle Cane

A tumor does not consist of a homogenous population of cancer cells. Therefore, to understand cancer, the tumor microenvironment and the interplay between the different cell types present in the tumor has to be taken into account, and how this interplay regulates the growth and survival of the cancer cells.

The aim with this project is to use *in situ* PLA for simultaneous analysis of the activity status of multiple signal pathways at a single cell level. This will provide information on what pathways are active in cancer cells, and to what extent this varies depending on positioning within the tumor, and in addition it will reveal how the cancer cells affect the surrounding non-malignant cells in the tumor microenvironment. This knowledge will enable better diagnostics, improved prediction on response to therapy and possibly also act as an incitement to develop novel drugs that can modify the microenvironment to reduce cancer growth and ability to metastasize.

Within the project Karin, Axel and Gaëlle are developing assays to visualize activity status of pathways that are deregulated in colorectal cancers, such as WNT and EGFR pathways. The assays will be used investigate if analysis of signaling pathway activity in tumor tissue sections will provide better diagnostics and predictive power than conventional analysis.

These assays will also be used for high-content drug screening in primary cell cultures of colorectal cancer samples. Linda's part of the project is to develop assays that target signaling pathways in B cells, e.g. antigen binding by the BCR and cognate T cell stimulation via CD40, to monitor their activity status in Chronic Lymphocytic Leukemia.

Method development

Björn Koos, Carl-Magnus Clausson, Karin Grannas, Linda Andersson, Axel Klaesson, Gaëlle Cane

Although *in situ* PLA provides the mean to analyze protein interactions and PTMs, further improvements are required to increase the dynamic range, provide ability for multiplex analysis and for visualization of interactions between different types of biomolecules, e.g. proteins and nucleic acids. Carl-Magnus, Linda are focusing on increasing the performance on *in situ* PLA, by increasing the dynamic range and possibilities to perform parallel analysis, while Karin and Axel test novel designs to enhance efficiency. Björn and Gaëlle are developing methods for detection of RNA and protein-RNA interactions in fixed cells and tissues. In addition to these PLA based developments, we are developing completely new methods for analysis of protein interactions that will be more robust and inexpensive to facilitate automation and development of point-of-care devices for *in vitro* diagnostics.

Group members during 2014

Ola Söderberg, senior lecturer, group leader
Linda Arngården, PhD student
Gaëlle Cane, researcher
Carl-Magnus Clausson, PhD student
Elin Ekberg, adm. assistant
Karin Grannas, PhD student
Johanna Herö, research engineer
Karolina Hirvonen, student
Axel Klaesson, PhD student
Björn Koos, researcher
Erik Ullerås, project coordinator

Dissertations during 2014

Carl-Magnus Clausson, Making Visible the Proximity Between Proteins, March 28, 2014.

Funding during 2014

EU-FP7 (PRIMES), € 240 000

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