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Department of Immunology, Genetics and Pathology

Annual Report
2013

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2013

Department of Immunology, Genetics and Pathology

Uppsala University

Introduction

The Dept. Immunology, Genetics and Pathology (IGP) hereby presents its activities during 2013. This has been a year with profound changes for the department. Two prominent researchers together with their groups moved to the department during 2013, Taija Mäkinen from London Cancer UK and Christer Betsholtz from the Karolinska Institutet. I take the opportunity to thank them both for choosing IGP as their base.

On the other hand, Mats Nilsson accepted a professorship at Stockholm University, but remains affiliated with Uppsala University and IGP. We are happy that Mats and certain of his group members remain at the department. IGP moreover announced several lectureships and are happy to welcome Lars Feuk, Ola Söderberg and Taija Mäkinen in such positions.

Another dramatic development for the department was the move of most of the Medical Genetics and Genomics groups, the Molecular Tools group and the SciLife platform Genome Center from the Rudbeck Laboratory to the Biomedical center. The move resulted in that the department now is split in two locations. It also involved the duplication of many of the department's shared function rooms and considerable investments in new equipment. Moreover, the move allowed the creation of a very strong Science for Life Laboratory environment in genetics/genomics at the Biomedical Center. We see both opportunities and risks with the split physical location of IGP. It is essential that the very fruitful IGP research environment is maintained.

Further changes in the departmental life are to come with the rebuilding and construction of additional annexes to the Rudbeck laboratory, which will be going on for the coming two years.
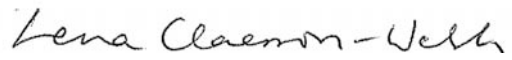
Research at IGP remains organized in seven research programs; Cancer and Vascular Biology, Clinical Immunology, Hematology, Medical Genetics and Genomics, Molecular and Morphological Pathology, Human Protein Atlas and Molecular Tools which interact closely with the three affiliated clinics (Clinical Genetics, Clinical Immunology and Transfusion Medicine and Clinical Pathology and Cytology). The Department moreover hosts several strategic research programs (U-CAN, EXODIAB, and StemTherapy) and five SciLife-supported platforms; Genome Center, BioVis, Tissue Profiling, the PLA, and the Clinical Sequencing platforms. It has been a privilege to see the strategic programs develop and the imprint they make on a national level. The platforms are very impressive in their ambition and ability to serve an ever-increasing number of customers.

IGP continued to grow financially during 2013, which has been a trend now for the last five years. Our scientists attracted considerable, very competitive grants from the Cancer Society, the Söderberg Foundation, the Swedish Research Council, the Knut and Alice Wallenberg foundation, and from the EU. There are currently three ERC grants (two senior grants to Christer Betsholtz and Ulf Landegren and one junior grant to Lars Feuk) at the department. Linda Holmfeldt received a Wallenberg Academy Fellow award. Fredrik Swartling was named the Ragnar Söderberg-researcher in medicine, in 2013. Other prominent grants to the department this year include the Astra Zeneca SciLife grants. Several of the IGP PIs belonged to consortia that received these very substantial supports and Sven Nelander is PI for one such consortium. Ulf Gyllensten was the recipient of an impressive grant from the Swedish Foundation for Strategic Research. Magnus Essand's research on the use of oncolytic virus for the treatment of neuroendocrine tumors received media attention resulting in the creation of a donation fund enabling initiation of clinical trials. The list of special grants and attention to IGP researchers is by necessity incomplete and obviously all grants are welcome and will be put in the best of use.

The overriding common goal of IGP's research activities is to improve prevention, diagnostics and treatment of diseases such as cancer and diabetes. As Head of Department, I have strived to support this endeavor to the best of my capacity, by providing service to the

scientists. I gratefully acknowledge the many who help me with these efforts: the IGP administrative staff, the medicine/pharmacy discipline project coordinators, the grants office, the legal affairs division and the central administration.

Uppsala 2014-04-29

Lena Claesson-Welsh

Contents

Employees during 2013

Abramsson Malin
Adler Jeremy
Adlerteg Tom
Agarwal Prasoon
Ahlander Anders
Ahlgren Viktoria
Ahlstav Suzanne
Alafuzoff Irina Ingeborg
Al-Amin Abdullah
Alemayehu Groom
Ali Muhammad Akhtar
Allen Marie
Al-Walai Somar
Alzrigat Mohammad
Ameur Adam
Anagandula Mahesh Kumar
Anand Manivel Vivek
Andersson Kallin Sandra
Andersson Linda
Andersson Magdalena
Andersson Sandra
Andrae Johanna
Aronsson Maria
Arslan Ahmed
Asif Sana
Asp Michaela
Asplund Anna
Backeryd Lindström Anna
Banski Piotr
Barbu Andreea
Baskaran Sathishkumar
Bergman Julia
Bergström Tobias
Betsholtz Christer
Bhoi Sujata
Bjerke Mia
Blokzijl Andries
Bolin Sara
Bondeson Marie-Louise
Boox Pirkko
Broström Ulrika
Brännström Johan
Bunikis Ignas
Bus Magdalena
Bäck Jennie
Caglayan Demet
Cahill Nicola
Cancer Matko

Cane Gaëlle
Cavalli Marco
Chen Lei
Chmielniakova Jana
Christiansson Lisa
Chugunova Elena
Claesson-Welsh Lena
Classon Christina
Clausson Carl-Magnus
Cortese Diego
Cui Tao
Dahl Niklas
Danielsson Angelika
Danielsson Louise
Darmanis Spyros
Davies Hanna
Davoodpour Padideh
Delgado-Vega Angélica
Dimberg Anna
Djureinovic Dijana
Djureinovic Tatjana
Dohlmar Ulf
Dumanski Jan
Ebai Tonge Brunhilda
Edlund Karolina
Edqvist Per-Henrik
Edvinsson Åsa
Ekberg Elin
Ekvall Sara
Elfineh Lioudmila
Elshafie Amir
Emanuelsson Hanna
Enroth Stefan
Espinosa Fernandez Belen
Essand Magnus
Falk Sörqvist Elin
Ferletta Maria
Feuk Lars
Flamourakis Georgios
Folkers Kelly
Fonnaland Karin
Forsberg Lars
Forsberg-Nilsson Karin
Fransson Moa
Frisk Gun
Fristedt Duvefelt Charlot
Fromell Karin
Gallant Caroline

Galli Joakim
Georganaki Maria
Grannas Karin
Gremel Gabriela
Grånemo Joakim
Gu Jijuan
Gustafsson Birgitta
Gustafsson Erika
Gustavsson Inger
Gyllensten Ulf
Gängel Konstantin
Haglund Mikael
Halvardson Jonatan
Hamad Osama
Hammer Joanna
Hammond Maria
Hansson Tony
Hartman Karin
He Liqun
Hede Sanna-Maria
Hedlund Lindberg Julia
Hedlund Marie
Heldin Nils-Erik
Hellström Ann-Charlotte
Hellström Mats
Henriksson Kerstin
Hermansson Annika
Herthnek David
Hertz Ellen
Herö Johanna
Hillerdal Viktoria
Hodik Monika
Hong Jaan
Honjo Satoshi
Huang Hua
Häggqvist Susana
Höijer Ida
Ilbäck Carolina
Jarny Mika
Jeansson Marie
Jernberg Wiklund Helena
Jiang Yiwen
Jin Chuan
Johanneson Bo
Johansson Patrik
Johansson Sebastian
Johansson Swartling Fredrik
Johansson Åsa

Jonasson Inger
Juko-Pecirep Ivana
Juter John
Kalushkova Antonia
Kamali-Moghaddam
  Masood
Kampf Caroline
Karlsson Hannah
Karlsson Marie
Karlström Therese
Kastemar Marianne
Keller Annika
Kermani Shila
Kesti Dennis
Kiflemariam Sara
Klaesson Axel
Klar Joakim
Koos Björn
Korsgren Olle
Kuhnemund Malte
Kundu Snehangshu
Kundu Soumi
Kunze Elene
Kutschan-Bunikis Sabrina
Källström Lillemor
La Fleur Linnea
Landegren Ulf
Larsson Chatarina
Larsson Erik
Larsson Jimmy
Lavina Siemsen Barbara
Leja Jarblad Justyna
Lembring Maria
Li Xiujuan
Lidström Malin
Liljenfeldt Lina
Lindau Cecilia
Lindblom Susanne
Lindholm Carlström Eva
Lindskog Bergström Cecilia
Lindström Anne-Christine
Ljungström Viktor
Loskog Angelica
Lundin Erika
Löf Liza
Löfgren Eir
Lööf Johan
Magnusson Christina
Magnusson Kristina
Magnusson Peetra

Mangsbo Sara
Mansouri Larry
Marinescu Voichita
Marques Souza de Oliveira
Martinsson Pernilla
Mathot Lucy
Mattsson Johanna
Micke Patrick
Mickelsen Jansson
  Annlouise
Moens Lotte
Molnar Matyas
Morin Eric
Mulder Sara
Mäkinen Taija
Naboulsi Rakan
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
Nyberg Linnea
O´hurley Gillian
Oelrich Johan
Ohlin Elisabet
Olerud Johan
Olsson Cecilia
Olsson Ingmarie
Oskarsson Linda
Pacholsky Dirk
Pan Gang
Pandey Gaurav Kumar
Paul-Wetterberg Gabriella
Persson Skare Tor
Peterson Pia
Petersson Sara
Petri Anna
Pettersson Ulf Gösta
Polajeva Jelena
Pontén Fredrik
Pääbo Svante
Ramachandran Mohanraj

Ramezani Mehrafarin
Rasi Chiara
Raykova Doroteya
Razzaghian Hamid Reza
Reddy Bysani Madhusudhan
Regierer Babette
Rendo Verónica
Roche Francis
Rosenling Therese
Rosenquist Brandell Richard
Rung Johan
Russell Camilla
Röjerås Ellen
Salih Tagrid
Sandberg Charlotta
Sandin Linda
Sandström Johanna
Savov Vasil
Schenström Maria
Schuster Jens
Simu Tuulikki
Singh Umashankar
Sjöblom Tobias
Sjöstedt Evelina
Sjösten Anna
Skog Oskar
Smith Ross
Sohrabian Azita
Stamatopoulos Konstantino
Stenbeck Funke Lillemor
Stoimenov Ivaylo
Strand Ann-Sofi
Strid Stina
Sutton Lesley Ann
Svensson Emma
Söderberg Ola
Taberman Sofie
Tararuk Tatiana
Taussig Mike
Tellgren-Roth Christian
Teramura Yuji
Testini Chiara
Tibbling Gunilla Birgitta
Tötterman Thomas
Uhrbom Lene
Ullbors Anna-Maria
Ullerås Erik
Wadelius Claes
van Hooren Luuk
Vannemreddy Yugandhar

Weishaupt Holger
Wester Kenneth
Westermark Bengt
Weström Simone
Wicher Grzegorz
Wik Lotta
Wikner Charlotte
Williams Nina
Vinnere Pettersson Olga

Wu Di
Vuu Jimmy
Wyöni Per-Ivan
Xiong Anqi
Yaka Cane
Yan Junhong
Yu Di
Yuan Xie
Zaghlool Ammar

Zhang Lei
Zhao Jin
Zieba Agata
Åberg Gunilla
Åberg Petter
Åslund Lena
Öberg Fredrik
Öncü Delal

Organization of the Department of Immunology, Genetics and Pathology
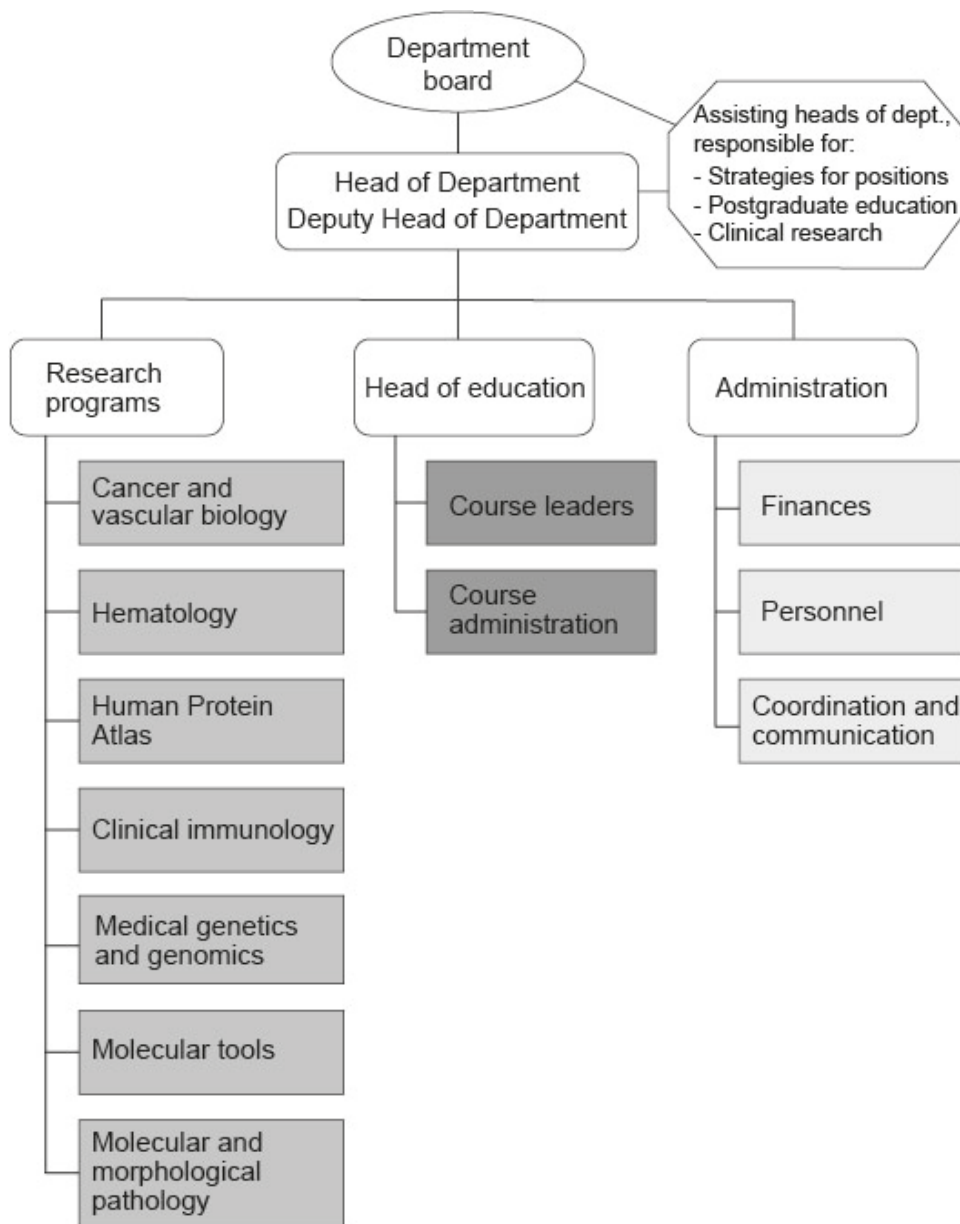
Head of Department

Lena Claesson-Welsh

Vice Head of Department

Ulf Landegren

Assistant Heads of Department

Anna Dimberg, graduate education
Karin Forsberg Nilsson, stratagies for postitions and recruitments
Bo Nilsson, clinical research

Department Board

Members during 2013

Lena Claesson-Welsh, Head of Department
Linda Andersson, graduate student representative
Sara Bolin, graduate student representative
Niklas Dahl, teacher representative, deputy
Emelie Ekstrand, undergraduate student representative
Lars Feuk, teacher representative
Karin Forsberg Nilsson, teacher representative
Lina Fredriksson, 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
Maria Lembring, graduate student representative, deputy
Viktor Ljungström, graduate student representative, deputy
Johan Lööf, undergraduate student representative, deputy
Fredrik Pontén, teacher representative
Fredrik Swartling, teacher representative, deputy
Ola Söderberg, teacher representative
Lene Uhrbom, teacher representative, deputy

Teaching organization

Nils-Erik Heldin, head of teaching
Viktoria Ahlgren, course administrator
Suzanne Ahlstav Hernadez, course administrator
Gunilla Tibbling, course administrator

Administration

Viktoria Ahlgren, course administrator
Suzanne Ahlstav Hernadez, course administrator
Anna Backeryd Lindström, accounting
Pirkko Boox, senior advisor
Ulf Dohlmar, accounting
Birgitta Gustafsson, financial coordinator
Mikael Haglund, accounting
Holger Henningsson, personnel
Therese Karlström, personnel
Shila Kermani, accounting
Barbro Nelson, accounting
Camilla Nilsson personnel
Christina Magnusson, administrator of postgraduate education
Sara Mulder, administrative assistant
Tuulikki Simu, accounting
Ulla Steimer, accounting
Gunilla Tibbling, course administrator

Strategic Research Projects

IGP participates in four projects that were funded in the governmental grant scheme for strategic research – SciLife Lab Uppsala, U-CAN, EXODIAB and StemTherapy.

For **SciLife Lab Uppsala** we host five core facilities that are described in more detail below.

The aim of **U-CAN** is to develop a biobank with tumor and blood samples from patients with different types of cancer. In addition, patient data and radiological images are also collected. The overall objective is to increase our knowledge about different types of cancer, and to develop treatment strategies that can be adapted to the needs of individual patients. What makes the project unique is the effort to collect the material before, during and after therapy, making it possible to study the effect of therapy on the tumor. Several researchers at the Department are involved in the U-CAN organisation and in research that includes material and information collected in the project. The U-CAN project is a collaboration between Uppsala University, Umeå University, Stockholm University and the KTH Royal Institute of Technology.

The strategic research area **EXODIAB** (Excellence of Diabetes Research in Sweden) is a joint effort between Lund and Uppsala University. One major focus of this multidisciplinary consortium in collaboration with industry and health care, goes towards building and providing a national infrastructure and national platforms to facilitate diabetes research in Sweden and to stimulate the interaction between academia and industry. The platforms include some of the best biobanks for diabetes research in the world and a human tissue laboratory, exploring human islet functioning as well as potential new diabetes therapies. Another central theme in EXODIAB focuses on future generations of outstanding diabetes researchers, by developing tailor-made education and offering career opportunities while integrating genetics, bioinformatics, physiology, cell biology, clinical, epidemiological and nutritional research. The overall aim is to develop new strategies for early risk assessment and novel therapies for prevention and treatment of diabetes and its complications.

The overall aim of **StemTherapy**, which is also a joint effort between Lund and Uppsala University, is to advance the development of stem cell-based cell replacement therapies for diabetes, stroke and hematological diseases. In order to build a strong base of knowledge about stem cells and disease mechanisms and thus pave the way for future efforts to devise new therapies, StemTherapy assembles very strong and inter-disciplinary competence in basic stem cell biology and clinical cell therapy. The establishment and coordinated use of forefront technology is instrumental for making major advances in this field and StemTherapy has established extensive collaborative ties with national and international institutions to increase diversity, quality and innovation. StemTherapy also puts emphasis on providing resources to facilitate the recruitment of new young promising researchers, to support new and existing junior group leaders and to encourage interactions between the clinic and basic research.

During 2013, the strategic research programs started to prepare for their final report to be submitted in 2014. We hope that there will be opportunities for the programs to both optimize and continue their work.

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 an 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 2013 the services offered by the BioVis were regularly used by 87 research groups, covering 137 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.

In the spring of 2013 the BioVis platform moved its services for image analysis, flow cytometry and cell sorting as well as microscopy from Rudbeck Lab to the MTC house to be more central to all users and to provide a better environment for it´s services. The electron microscopy unit is still situated in the Rudbeck Lab.

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.

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 massively parallel sequencing. It is one of three facilities constituting the national SciLifeLab platform for Clinical Diagnostics.

In the first three months of operation, the facility purchased its first Illumina HiSeq 2500 instrument and formed administrative routines for operations, and agreements between the different partners of the facility. A facility manager, Johan Rung, was recruited, and started in October 2013. The first external projects are expected in early 2014, and the first facility developed gene panel based tests for routine diagnostics taken into production. In 2014, two additional bioinformaticians and two molecular biologists will be recruited. Development projects in three areas (solid tumors, haematological malignancies, and inherited diseases) are ongoing in separate work packages. Two additional work packages are devoted to the development of bioinformatic analysis pipelines and data management, and policies and practises for ethics and reporting.

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

PLA-based Platform for Proteome Analyses

The PLA Proteomics facility that is part of the Affinity Proteomics platform of Science for Life Laboratory, was established in 2010 and provides services for the scientific community for sensitive and specific analyses of proteins using *in situ* proximity ligation assays (*in situ* PLA). Since 2012 the facility also provides help to establish solid-phase PLA for sensitive and specific detection of proteins in body fluids such as plasma, cerebrospinal fluids, etc. The PLA technology has been developed at the Department of Immunology, Genetics and Pathology, Division of Molecular Tools, and is an affinity-based technology in which target molecules are recognized by sets of probes, each consisting of an affinity binder (typically an antibody) equipped with an oligonucleotide. Upon recognition of target molecules the oligonucleotides are designed either to ligate to each other (for solution-phase PLA), or to be used to guide circularization of two accessory linear DNA molecules (for *in situ* PLA). The DNA molecules that form by ligation are amplifiable by real-time PCR for solution-phase measurements or by rolling circle amplification for *in situ* detection. The 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 2013 the facility's services were used by Swedish scientists both as a service for fee and in different collaborative projects. Services offered by the PLA Proteomics facility include visualization of individual proteins or their modifications and interactions using *in situ* PLA, and establishment of new solid-phase PLA for detection of protein in solution. Currently a list of more than 150 different *in situ* PLA-based assays have been established for analyses of proteins in cells and tissues at single cell and single molecule resolution. This list is continuously updated as new assays are developed. The facility also assists by establishing assays for new target molecules or by adapting established assay formats for new applications, by mutual agreement with users. The assistance also includes expert advice on design of experiment and on sample- and 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 2013 the facility preformed service for researchers that included the construction of more than 40 TMAs, over 13.000 cut tissue sections, over 2500 slide scannings and 2000 immunohistochemical stainings.

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 4100 TMAs containing over 226.000 tissue cores, 280 cell microarrays containing over 36.500 cell cores, stained over 208.000 slides for immunohistochemistry, scanned over 128.000 slides and cut over 780.000 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, facility director
Caroline Kampf, facility manager
Lillemor Källström, technician
Evelina Sjöstedt, research engineer
Cane Yaka, research engineer
Ann-Sofie Strand, technician

Uppsala Genome Center

Uppsala Genome Center (UGC) was established in 1998 by a grant from the Foundation for Strategic Research (Stiftelsen för Strategisk Forskning) as a national core facility for genotyping and DNA sequencing. 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 center is open to academic research groups in Sweden on a non-profit basis and provides a facility for rapid genome sequencing and qualified bioinformatics support. The center 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 AB SOLiD™, Ion Torrent (PGM™), Ion Proton™ systems from Life Technologies and on RSII from Pacific Bioscience.
- Sanger Sequencing Service
- Genotyping with STR-markers
- Service for separation of custom prepared samples by capillary electrophoresis on AB3730XL Genetic Analyzer

During 2013 a RSII instrument from Pacific Bioscience 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 20 kb. This technology is a complement to the established short reads instruments at NGI.

The different NGS technologies can be used for *de novo* sequencing, whole genome re-sequencing, targeted re-sequencing, gene expression studies and tag counting. RNA sequencing can be performed either as whole transcriptome analysis, or as sequencing of small RNA molecules. With the latest introduced technology from Pacific Bioscience UGC have now the capacity to offer different technologies for different type of projects, with variation in output of sequencing data from 10 Mbp till 90 000 Mbp per run and read length from 75 bp to 20 000 bp.

UGC has been taking part in projects in collaboration with the Uppsala Akademiska Hospital and the National Veterinary Institute (SVA) with the ambition to establish a workflow for rapid sequencing of bacteria and viruses from clinical samples. We have now a workflow where it's possible to deliver sequencing data within three days after sample delivery.

UGC has been accepted as "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 2013 UGC provided service for more than 145 Next Generation Sequencing projects on the NGS 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 Ameur, bioinformatician
Somar Al-Walai, research engineer
Michaela Asp, research engineer
Ulrika Broström, research engineer
Ignas Bunikis, bioinformatician
Nicola Cahill, research engineer
Susana Häggqvist, research engineer
Ida Höijer, research engineer
Sebastian Johansson, research engineer
Cecilia Lindau, research engineer
Anna Petri, research engineer
Christian Tellgren-Roth, bioinformatician
Olga Vinnere Pettersson, project coordinator
Nina William, research engineer

Prizes and awards

Anna Dimberg was awarded the prize **Flormanska belöningen** for her work with clarifying how the development of blood vessels affects tumours.

Anna Dimberg received a **Benzelius Award** for her research on blood vessels in tumours and how they interact with inflammatory cells.

Ulf Landegren was awarded Uppsala University's innovation prize **Hjärnäpplet**, for his achievements as entrepreneur.

Richard Rosenquist Brandell received the **Uppsala County Council research prize 2013** for his research on chronic lymphocytic leukemia.

Moa Fransson was selected as winner of the mentorship program **Mentor4research** in 2013.

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, 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 sixth semester of the Biomedicine program. About thirty-five students participated in the course.

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. Approximately fifty students attended. 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 forty students attended each course.

Physiotherapy

A course in Pathology, within the Physiotherapy program, was given twice during 2013. The course was for 1.5 credits and around forty-five 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 2013. 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 Basic Forensic Science, Forensic Medicine and Forensic Genetics, 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. The program was given for the first time as an international master program during the fall 2013. 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, 15 credits, and Advanced Techniques in Molecular Medicine, 15 credits, are given on the first semester of the program. Courses in Epigenomics, Biomarkes, Bioimaging and Regenerative Medicine are also included in the program. The master programmes attracted many applicants.

Postgraduate Education at IGP

In 2013 the Department had 75 students registered for a postgraduate education. Sixteen PhD students defended their PhD theses and two students obtained licenciate 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 2013 three 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.

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 2013, 32 seminars were given in the series. All postgraduate students also attend a pedagogical course given at Uppsala University.

The department organized the first PhD-student retreat in Romme Alpin in March 2013. 41 students attended the conference. All attending students presented their work through either an oral presentation or a poster presentation.

Scientific reports

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.
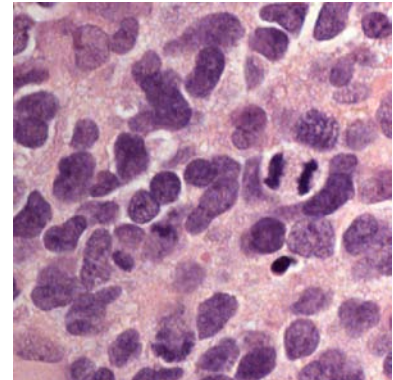
The formation of new blood vessels, angiogenesis, is normally a strictly controlled process, which is a prerequisite for embryonic development as well as for growth and repair of tissues after birth. Moreover, several diseases, such as cancer, involve exaggerated angiogenesis, leading to disorganized and dysfunctional vasculature that helps to propagate the disease. Our research focuses on how angiogenesis is regulated in health and disease. We study the role of stimulating growth factors and negatively regulating proteins that control 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 angiopoietins during embryonic and postnatal development, as well as in pathological processes in the adult organism, such as fibrosis, 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 Angpiopoietin-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-Siemsen, 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, Radiosa Gallini

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 PDGFRa. Generally viewed PDGFRa is expressed by mesenchymal and glial cells, whereas adjacent epithelial, muscle or neuronal 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 PDGFRa? 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 PDGFRβ, 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, PDGFRβ-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 2013

Christer Betsholtz, professor, group leader
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Johanna Andrae, researcher
Marco Castro, PhD student
Lwaki Ebarasi, post doc
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Helene Leksell, biomedical analyst
Colin Niaudet, researcher
Marta Oliveira, student
Elisabeth Raschperger, research fellow
Michael Vanlandewijck, post doc

Funding during 2013

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

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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 in cancer and eye disease, by histidine-rich glycoprotein

Satoshi Honjo, 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). We presently have two major goals with our research on HRG: To identify the receptor mechanism, and to manage the transition to a clinically applicable therapeutic for retinopathies and for caner.

Vascular endothelial growth factor (VEGF) receptor in regulation of vascular permeability

Kelly Folkers, Marie Hedlund, Simone Weström, Eric Morin, Xiujuan Li, Elisabet Ohlin, Narendra Padhan, Shujing Shi, 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 n 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 and mutation from Y949 to F949 in VEGFR2 results in a block in VEGF-induced vascular permeability. We study the effect of the block in vascular permeability on angiogenesis and on a number of disease models such as cancer

(melanoma, glioblastoma, insulinoma) and myocardial infarction (in collaboration with Prof. Jan Borén, Sahlgrenska Academy). The signal transduction properties of the mutants are studied by systemic delivery of VEGF followed by tissue lysis and immunoblotting. Alternatively, we employ the proximity ligation assay to study phosphorylation evens and complex formation *in situ*. In related project, we examine the biology of other VEGFR2 phosphotyrosine sites such as the 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 our 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.

Cancer fingerprints

Narendra Padhan

By 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. The strategy allows the identification of all posttranslationally modified versions of a particular signal transducer with one reagent, which saves time and resources. The goal is predict therapy resistance in cancer by identifying signal transduction pathways associated with tumor hypoxia and tissue metabolism (i.e. cancer fingerprints) in human cancer tissue such as colorectal cancer and chronic lymphatic leukemia.

Group members during 2013

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Jeremy Adler, research engineer
Kelly Folkers, research assistant
Hannah Feringa, guest student
Daisuke Fukuhara, post doc
Marie Hedlund, research engineer
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Funding during 2013

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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 microenviroment.

αB-crystallin in tumor angiogenesis and inflammation
Lothar Dieterich, Hua Huang and Anna Dimberg

αB-crystallin/HspB5 is a molecular chaperone with pro-survival properties that is expressed in many types of cancer. We have shown that αB-crystallin is expressed in a subset of tumor vessels in human tumors, and that it regulates survival of endothelial cells during tumor formation. The established role of αB-crystallin in tumor cells and vessels suggest that this protein may be a potential target for tumor therapy.

However, αB-crystallin has also been suggested to have an anti-inflammatory function. This implies that loss or targeting of αB-crystallin in a tumor setting may increase inflammation, and possibly enhance tumor growth. We have shown that αB-crystallin affects tumor-related inflammation through several distinct mechanisms, by regulating expression of adhesion molecules during endothelial activation and through modulating expansion and recruitment of immature myeloid cells during tumor progression.

Molecular regulation of vascular abnormalization in glioblastoma
Elise Langenkamp, Sofie Mellberg, Lei Zhang, Hua Huang, Johan Lööf, Wesam Bazzar, Marzieh Shirali 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 are currently investigating how growth factor signalling and differential gene expression contributes to aberrant vascular function and tumor progression 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 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. We are presently investigating how pro-angiogenic signalling affects endothelial activation and how this influences the response to immunotherapy.

Group members during 2013

Anna Dimberg, researcher, group leader
Wesam Bazzar, master student
Maria Georganaki, PhD student
Hua Huang, PhD student
Elise Langenkamp, post doc
Johan Lööf, teaching assistant
Sofie Mellberg, post doc
Marzieh Shirali, master student
Luuk van Hooren, PhD student
Lei Zhang, PhD student

Funding during 2013

Publications 2011-2013

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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. 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

Extracellular matrix interactions of importance for glioma formation and neural development

Soumi Kundu, Anqi Xiong, Tobias Bergström, Grzegorz Wicher, Annika Hermansson, Tanja Paavilainen, Eli Burell, Argyris Spyrou, Yuyuan Xiong, Lulu Rama Haseeb

The focus of this project is the "glioma 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 glioma 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 heparanse. In this project we address HS proteoglycan biosynthesis and degradation in clinical brain tumor samples, human glioma cell culture as well as mouse and human models of glioma. 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 glioma growth. Furthermore, we examine the role for heparanase in neural differentiation.

The postnatal and the adult brain have different ECM composition in the neurogenic subventricular zone, which influences neural progenitors. The interactions with the ECM, the surrounding cells and blood vessels govern differentiation, proliferation and self-renewal. We therefore explored characteristics of the early postnatal neurogenic niche, because of its potential implications for tumors that occur during childhood (Bergström et al, 2014)

Human glioma cell cultures as a new experimental platform

Tobias Bergström, Annika Hermansson, Grzegorz Wicher

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, Grzegorz Wicher, Tobias Bergström

The signaling pathways mutated in glioma are in general important neural stem cell pathways that regulate proliferation, differentiation, migration and survival. Mutations in these pathways are associated with brain tumor development and thus present therapy targets. The PDGF pathway is one of these, and we investigate the role for different PDGF isoforms and their receptors in neural differentiation.

We previously found that malignant brain tumors and neural stem cells share a common transcriptional signature (Demoulin et al, 2006). Nuclear receptor binding protein 2 (NRBP2) was selected for further study based on the high level of regulation, its potential survival-conferring effect in progenitors (Larsson et al, 2008) and because of its regulation in pediatric brain tumors (Xiong et al, in preparation). Our work aims to identify the function of NRBP2 and its role in CNS and tumor development.

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

Grzegorz Wicher, Grigorios Kyriatzis

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.

Group members during 2013

Karin Forsberg Nilsson, professor, group leader
Tobias Bergström, PhD student
Eli Burell, student
Annika Hermansson, research engineer
Suomi Kundu, post doc
Grigorios Kyriatzis, student
Lulu Rahma Adil Haseeb, student
Tanja Paavilainen, Erasmus student
Argyris Spyrou, student
Grzegorz Wicher, post doc
Anqi Xiong, PhD student
Yuyuan Xiong, student

Dissertations during 2013

Tobias Bergström, Modeling Neural Stem Cell and Glioma Biology, September 25, 2013.

Funding during 2013

Publications 2011-2013

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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

Jimmy Larsson, 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 (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 recently published 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 *x99384*.

Kinases belong to an important drug target class in oncology, which strongly suggests that our studies on *x99384* will contribute to the understanding of kinase/phosphatase signaling in general and angiogenesis/tumor angiogenesis in particular.

Group members during 2013

Mats Hellström, researcher, group leader
Jimmy Larsson, post doc
Anja Nitzsche, PhD student
Chiara Testini, PhD student

Publications 2011-2013

1. Kalén M, Heikura T, Karvinen H, Nitzsche A, Weber H, Esser N, Ylä-Herttuala S, Hellström M. Gamma-secretase inhibitor treatment promotes VEGF-A-driven blood vessel growth and vascular leakage but disrupts neovascular perfusion. PLoS One. 2011, 6(4):e18709.

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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 in a variety of diseases that affect a large number of people worldwide, including obesity, autoimmunity, atherosclerosis, hypertension and cancer.

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

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)

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 2013

Taija Mäkinen, senior lecturer, group leader
Ines Martinez-Corral, researcher
Lukas Stanczuk, researcher
Maria Ulvmar, researcher
Andrea Taddei (post doc, Cancer Research UK London Research Institute)

Funding during 2013

Publications 2011-2013

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

1. Mellor RH, Tate N, Stanton AWB, Hubert CE, Mäkinen T, Smith A, Burnand KG, Jeffery S, Levick JR, Mortimer, PS. Mutations in FOXC2 in humans (lymphoedema distichiasis syndrome) cause lymphatic dysfunction on dependency. J Vasc Res. 2011, 48, 397–407.
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14. Martinez-Corral I, Makinen T. Regulation of lymphatic vascular morphogenesis: Implications for pathological (tumor) lymphangiogenesis. Exp Cell Res. 2013, 319:1618-1625.

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 unique 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:

- Development of a high resolution molecular map of the HGCC compendium (Voichita Marinescu).
- Comparative network modeling of HGCC vs other neural cancers using cancerlandscapes technology (Maja Olsson)
- Systematic testing of predicted targets at the SciLifeLab facilities (Linnéa Schmidt, Caroline Hansson, Sathishkumar Baskaran)

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

Cancerlandscapes: an interactive, global map of regulation in human cancer

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.

New mathematical models of brain tumor progression and invasiveness

Philip Gerlee, Sven Nelander

Brain tumors are characterized by invasive growth, which makes surgical resection inefficient. In this project, we develop new mathematical simulations of brain tumor growth to understand the principles of brain tumor invasiveness, and to determine new strategies to inhibit invasive growth. The models should be applicable to designing relevant cell screens for glioblastoma and cytometry-based patient prognostics.

Group members during 2013

Sven Nelander, Associate Professor, group leader
Satishkumar Baskaran, PhD student
Ludmila Elfineh, research engineer
Lukasz Huminiecki, researcher
Patrik Johansson, PhD student
Marianne Kastemar, technician
Cecilia Krona, researcher
Santhanam Kulasekara Subramoniam Muthulakshmi, student
Voichita Marinescu, post doc
Torbjörn Nordling, research assistant

Part of the group is working in Gothenburg
Teresia Kling, PhD student
Maja Olsson, post doc
Linnea Schmidt, PhD student

Funding during 2013

Publications 2011-2013

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Childhood Brain Tumor Development and MYCN Protein Stabilization

Fredrik Swartling

MYCN is a transcription factor and a potent mitogen with essential roles in normal brain development. Misexpression of MYCN occurs frequently in medulloblastoma, the most common malignant childhood brain tumor of the cerebellum. Amplification of MYC proteins (like MYCN and MYC) in medulloblastoma is strongly correlated with poor prognosis suggesting MYC proteins as 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 brain tumor 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 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-lifes. 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 have showed how FBW7 loss leads to more aggressive tumors. 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, 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 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 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 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 (Bandopadhayay 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 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, 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. 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.

Group members during 2013

Fredrik Swartling, researcher, group leader
Sara Bolin, PhD student
Sanna Hede, post doc
Čančer Matko, PhD student
Rashmi Prakash Chowath, student
Vasil Savov, PhD student
Holger Weishaupt, post doc

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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 due to their extremely poor prognosis and lack of efficient treatment strategies.

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 relevant and unique platform for our basic and pre-clinical glioma research.

Projects

- **Building the HGCC biobank of cultured glioblastoma cells**.
  Yuan Xie, Yiwen Jiang, Smitha Sreedharan, 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, Caroline Haglund and Malin Jarvius (UU)

- **The interplay between cell of origin, oncogenic activation and developmental age in glioma development**.
  Smitha Sreedharan and Yuan Xie

- **Mechanisms of SOX5 induced tumor suppression**.
  Smitha Sreedharan and Yiwen Jiang

- **Role of LGR5 in glioma stem cells**.
  Yuan Xie and Yiwen Jiang, in collaboration with Voichita Marinescu, Sven Nelander, Andries Blokzijl and Ulf Landegren (UU)
- **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

Group members 2013

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Yiwen Jiang, post doc
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Smitha Sreedharan, post doc
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Human Malignant Glioma – from Oncogenic Mechanisms to Treatment

Bengt Westermark

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

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

Another project focuses on the involvement of inflammatory cells, specifically mast cells (MCs), in gliomagenesis.

CGGBP1 in cell cycle checkpoint regulation and telomere protection

Umashankar Singh, Bengt Westermark

We have found that the transcriptional regulator CGGBP1 is involved in DNA damage response, localizes to midbodies, regulates abscission and prevents tetraploidy. A novel role of CGGPB1 as a protector of teleomeres is studied in human diploid fibroblasts. Expression of a mutated form of CGGBP1, in which an ATR phosporylation 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. Nuclear transport and function of CGGBP1 requires phosphorylation on Tyr20.

Search for candidate drugs for the treatment of malignant glioma

Anna Segerman, 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 cell death, growth inhibition or differentiation as endpoints, we will analyze the response to single or combinations of known cytokines and cytokine inhibitors. 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. On the contrary, over expression of Sox21, a known Sox2

antagonist, induce progenitor cells to differentiate into neurons. 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-signaling pathway and the JAK/STAT-signaling pathway. In addition we will study if theses signaling pathways or if the Sox2/Sox21 axis can be inhibited by low molecular weight inhibitors to prevent brain tumor progression.

The wide distribution of Sox2 and Sox21 in glioblastoma and the suppressor role of Sox21 make the Sox2/Sox21 axis a very interesting target for new potential therapy.

The role of mast cells in gliomagenesis

Elena Chugunova, 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. Importantly, proliferating MCs were detected, suggesting that the MC accumulation was caused by local expansion of the MC population. In line with these findings, strong expression of stem cell factor (SCF), i.e. the main MC growth factor, was detected, in particular around tumor blood vessels. Additional signaling axis involving CXCL12 and its receptor CXCR4 play a role in the invasive nature of glioma.

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.

The Role of Plasminogen Activator Inhibitor-1 (PAI-1) in Mast Cell Recruitment to Glioblastoma is LRP1 Dependent

Elena Chugunova, Ananya Roy, Antoine Coum

Recently the focus of the cancer research community on the diversified types of cells populating brain tumor has dramatically increased. We were first to report the presence of mast cells (MCs) in the glioma microenvironment and to demonstrate the mechanisms of MC

recruitment, in a grade-dependent manner. 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). Previous publications demonstrated that overexpression of PAI-1 in glioblastoma (GBM) is significantly correlated with shorter survival. In addition, the serum PAI-1 level, was shown, could be attributed as a marker for the prediction of glioma grade. We found that antibodies that neutralized PAI-1 attenuated infiltration of MCs. Furthermore, to address the potential implication of LRP1 in this process, we demonstrated the expression of LRP1 in LAD2 cells but also revealed the presence of LRP1 in MCs in human high-grade glioma tissue.

To properly assess the role of LRP1 in MC migration, we used LRP1 antagonist, receptor-associated protein (RAP), and demonstrated the attenuation of MC migration upon the blocking. Moreover, the activation of potential PAI-1/LRP1 axis with purified PAI-1 in MCs promoted differential phosphorylation of a number of signaling molecules which are, as previously been shown, regulated by LRP1-inducing signaling.

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

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 2013

Bengt Westermark, professor, group leader
Nils-Erik Heldin, associate professor
Marianne Kastemar, lab technician
Mia Niklasson, researcher
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Elena Chugunova, researcher
Antoine Coum, student
Ananya Roy, researcher
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Maria Ferletta, researcher
Maria Karoutsou, student
Erika Lundin, research assistant

Funding during 2013

Publications 2011-2013

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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 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.
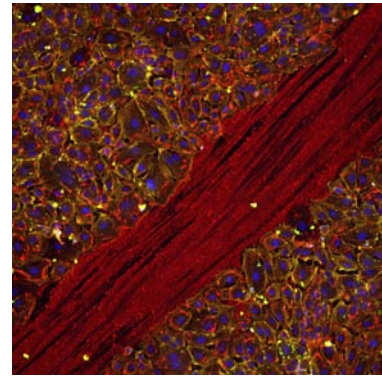
Genetic Modification of Viruses and T Cells for Cancer Therapy

Magnus Essand

Viruses are modified to selectively kill tumor cells. Virus infectivity is altered through modification of the virus capsid so that the virus targets molecules that are over-expressed on tumor cells. Virus replication is altered by introduction of regulatory elements into the virus genome to specifically control viral gene expression to tumor cells. We are primarily working with oncolytic adenovirus but we also have research on Semliki Forest virus and Vaccinia virus.

T cells are modified to express novel T cell receptors (TCR) or chimeric antigen receptors (CAR) that recognize antigens that are expressed and presented by tumor cells. This way the tumor antigen-directed T cells specifically kill tumor cells. We also introduce microRNAs into the T cells in order to knock down certain receptors and make the T cells resistant to suppressive factors in the tumor microenvironment. We mostly use lentiviral vectors to genetically modify T cells. In addition, we are developing new protocols to isolate and expand T cells that are resistant to immunosuppressive factor.

Oncolytic Virus Therapy for Cancer

Di Yu, Mohanraj Ramachandran, Chuan Jin, Justyna Leja, Berith Nilsson, Abdul Haseeb, Christian Tebid Tebid

Oncolytic viruses are emerging therapeutic agents for cancer. Adenovirus has the capacity to infect tumor cells, replicate therein, lyse the cells and release progeny virus, which infect neighboring cells, thus amplifying the initial inoculums. The lytic cell death induced by the 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.

We are developing genetically engineered viruses for cancer therapy. We focus our efforts on prostate adenocarcinoma, neuroendocrine carcinoma and neuroblastoma. Virus replication is controlled by insertion of a tumor- or tissue-specific promoter and further restricted by introduction of tissue-specific microRNA target sequences in the viral genome. The

infectivity is altered through genetic modification of virus capsid to favor infection of tumors cells. In order to target metastatic sites we are evaluating various cell types as vehicles to deliver virus at the tumor site. We are in particular looking at tumor antigen-restricted T cells, which target tumor cells directly and macrophages, which are attracted to the inflammatory environment of the tumor.

T Cell Therapy for Cancer and Virus Infections

Chuan Jin, Di Yu, Victoria Hillerdal, Vanessa Boura, Grammatiki Fotaki, Mohanraj Ramachandran, Berith Nilsson, Alex Karlsson-Parra

Adoptive transfer of antigen-specific T cells in combination with host immune-depletion has during the last years emerged as an effective therapeutic approach to combat severe virus infections and cancers. It is particularly effective when T cells from cancer patients are isolated, engineered with a new receptor and then transferred back to the patient.

We have recently cloned a T cell receptor (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 T cells expressing chimeric antigen receptors (CARs) for antigens associated with prostate cancer and neuroblastoma. The killing efficacy of the genetically engineered T cells is evaluated both *in vitro* and *in vivo*.

We have long experience in isolating and expanding antigen-specific T cells from peripheral blood and we have recently developed a rapid protocol for simultaneous activation and expansion of cytomegalovirus (CMV)-directed cytolytic and helper T cells. The protocol has great potential to treat transplant patients who suffers from CMV disease due to their immunosuppressive medication.

We also have long experience with T cells directed against tumor-associated antigens and we have shown that prostate cancer patients have an increased frequency of prostate antigen-specific T cells in their blood. We are now continuing our efforts on the development of T cell expansion protocols to generate effector T cells that are highly resistant to immunosuppressive factor.

Group members during 2013

Magnus Essand, Professor, group leader
Alex Karlsson-Parra, Assoc. prof., chief physician
Berith Nilsson, project leader
Justyna Leja, post doc
Di Yu, post doc
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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 (TID) 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 TID and to pave the way for development of new strategies for prevention and cure of TID.

The work is organised in five projects with the following objectives:
  a) Unravel the etiology of TID.
  b) Halt or prevent TID in newly diagnosed patients by transplanation 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 TID, experimental and clinical studies.
  e) Induction of immunological tolerance: Regulatory T cells for treatment of transplantation induced immune reactions

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Anna Wiberg, PhD student

Dissertations during 2013

Monika Hodik, Enterovirus Implications in Type 1 Diabetes, September 13, 2013.

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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. In this project we use the knowledge gained from our studies of how tumor cells prevent attacks by immune cells and explore such preventions to block MS. Our goal is to develop immunosuppressive cells that can suppress the unwanted immune reactions locally in the nervous system. Further, we are investigating the immunological mechanisms of hematopoetic stem cell transplantation for MS patients.

Immunostimulatory gene therapy for cancer

Hannah Karlsson, Emma Svensson, Stina Söderlund, Ioanna Milenova, Wictor Gustafson, Berith Nilsson, Lina Liljenfeldt, Lisa Christiansson, Camilla Lindqvist, 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 have just initiated a clinical trial using CD19-targeting chimeric antigen receptor (CAR) T cells for lymohoma 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, Emma Svensson, Kenneth Wester, 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 sheet 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 hematopoetic 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 performed together 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 2013

Angelica Loskog, assoc. professor, group leader
Joachim Burman, PhD student, specialist in neurology
Lisa Christiansson, PhD student
Hannah Karlsson, PhD student
Lina Liljenfeldt, PhD student
Emma Svensson, PhD student
Camilla Lindqvist, post doc
Stina Söderlund, resident in hematology
Kenneth Wester, researcher
Berith Nilsson, project coordinator (part time)
Wictor Gustafson, student
Yoanna Milenova, student
Pooja Vijay Ghopal, student

Dissertations during 2013

Lisa Christiansson, Myeloid-Derived Suppressor Cells and Other Immune Escape Mechanisms in Chronic Leukemia, May 17, 2013.

Funding during 2013

The Swedish Cancer Society, 500 kSEK

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Islet Engraftment and the Role of the Endothelium in Type 1 Diabetes

Peetra Magnusson

Type 1 diabetes demands life long treatment by exogenously administered insulin but still a small group of type 1 diabetes patients suffer from severe blood sugar level fluctuations. The possibility to cure the insulin-deficiency by transplantation of islets of Langerhans is very appealing for these patients.

Our research is focused on processes to improve islet survival and engraftment after transplantation. As strategies to improve islet engraftment we use supportive components such as protein scaffolds and different types of helper cells. Another line of research in our group is to understand the effect upon the islet vasculature at early onset of type 1 diabetes and the effects of ischemia reperfusion injury upon endothelial cells.

MSC, EC and supportive proteins

Moa Fransson, Johan Brännström, Sofia Nordling

Collaboration with Katarina LeBlanc, Karolinska Institutet, and Olle Korsgren

In the process of revascularization multipotent mesenchymal stem/stromal cells (MSC) can support endothelial cells (EC) by the production of growth factors and matrix proteins. MSC also produce proteases enabling vessels to migrate into the surrounding tissue during angiogenesis. Due to the immunosuppressive capacity the MSC are used in the clinic to treat graft versus host disease.

We have access to human MSC from healthy volunteers and we have results showing that the cells can respond towards a simulated rejection response by islets exposed to allogeneic lymphocytes, which make them candidate cells in islet transplantation. Furthermore, we are investigating the contribution of the endogenous islet EC in the revascularization of transplanted human islets and combining biomaterials with MSC and islet.

Blood EC chamber

Sofia Nordling

Collaboration with Bo Nilsson, Jaan Hong and Rolf Larsson

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 EC and blood cells, we have a system of blood chambers combined with cultured EC, a blood EC 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 EC, symbolizing an activated and inflammatory state of disease.

Endothelium in early onset of diabetes

Sofia Nordling, Johan Brännström

Collaboration with Gun Frisk and Olle Korsgren

Several inflammatory and immunological diseases are linked to an affected endothelium. In diabetes the secondary effects of the disease are coupled to the endothelium leading to nephropathy or retinopathy. Detailed information concerning the effect and the possible role of the islet endothelium at the initial phase of type 1 diabetes is sparse, mainly due to the lack

of available human material. Through access to unique donated material we have the possibility to investigate the endothelium in the pancreas at early onset of type 1 diabetes.

Group members during 2013

Peetra Magnusson, researcher, group leader
Johan Brännström, research engineer
Moa Fransson, post doc
Sofia Nordling, PhD student
Fredrik Edin, PhD student
Zeinab Naime Ali, student
Pere Gíne i Gras, student
Wisam Abu Hashim, student
Linus Sanner, student
Yuk Ting Siu, student

Funding during 2013

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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 part of the healing process and is triggered by the humoral innate immune system, which primarily consists of the cascade systems of the blood. These subsequently activate endothelial cells, leukocytes and platelets, finally resulting in thrombotic and inflammatory reactions. Thromboinflammation is also an important pathophysiological mechanism in several clinical conditions and treatments:

1. The innate immune response in cell and cell cluster transplantation and therapies.
2. Ischemia-reperfusion injury in whole organ transplantation (also involved in 3, 4 and 6)
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 miscelles, polymers, virus vectors etc.
6. Biomaterials implants (joint replacements, scaffolds for tissue engineering etc), extracorporeal treatments (hemodialysis, cardiopulmonary bypass)

Cross-talk between the cascade systems and activated platelets

Huda Kozarcanin, Osama Hamad, 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. 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.. Platelet-bound C3(H2O) acts as a ligand for leukocyte CD35 and CD11b/CD18, potentially enabling platelet-leukocyte interactions.

In addition, activated platelets and fibrin leads to the activation of the lectin pathway enzymes enzymes, MASP-1 and -2 without complement activation. Thus, in addition to their traditional role as initiators of secondary hemostasis, platelets also act as mediators and regulators 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, Bo Nilsson, Kristina Ekdahl

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 an ESRD patient 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 severe

adverse reactions that cause damaging thromboinflammation, triggered by the intravascular innate immune system, which lead to poor results and non-function.

The overall aim of this project is to clarify the mechanisms and identify nature's own specific control points of regulation in these adverse reactions in order to be able to significantly improve the quality of hemodialysis devices and kidney grafts by applying these concepts of regulation in hemodialysis and kidney transplantation. We envisage that conveying a novel soluble complement inhibitor to the clinical stage via phase 1/2a clinical studies, creation of nano-profiled surfaces with low activating properties and generation of easy-to-apply one step-coatings for treatment of biomaterials (hemodialysis) and endothelial cell surfaces (kidney grafts) will revolutionize the treatment modalities of ESRD. The feasible hemodialysis treatment periods are anticipated to be extended, combined with an improved quality of life and in kidney transplantation attenuation of innate immune reactions will prolong the life expectancy of the graft and make kidneys more accessible for transplantation.

All the novel techniques can be applied to other types of implantations, extracorporeal treatments and transplantation and in the future be used in xenotransplantation and stem cell therapies.

Thromboinflammation induced by nanoparticles

Jaan Hong, Padideh Davoodpour, Bo Nilsson, Kristina Ekdahl

Nanoparticles (NP) and nanostructured materials are used in more and more applications and their use is expected to increase dramatically in the future. We have found that NP induce thromboinflammation, and our aim is to apply the technology that we developed for elucidating the biocompatibility of biomaterials in contact with blood, in order to characterize the biological responses and toxicity of NP in contact with the tissue fluid / blood plasma / whole blood. The project will help to clarify the mechanisms of toxicity, and help to develop techniques for measuring the toxicity of present and future NP materials that are disseminated in our environment.

Group members during 2013

Bo Nilsson, professor, group leader
Sana Asif, research engineer
Andreea Barbu, researcher
Padideh Davoodpour, researcher
Karin Fromell, researcher
Osama Hamad, researcher
Jaan Hong, researcher
Huda Kozarcanin, PhD student
Susanne Lindblom, research engineer
Kristina Nilsson Ekdahl, professor
Lillemor Stenbeck-Funke, research engineer
Yuji Teramura, visiting professor

Funding during 2013

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Immune Complexes in Rheumatic Diseases and Chronic Infections

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).

Some of the questions that we try to answer in our basic research are:
1. How can IC be characterized, and what qualities of IC are associated with IC-stimulated production of different kinds of Inflammation-promoting and inflammation-suppressing substances?
2. What cell types respond to IC stimulation, and what types of receptors are triggered in this stimulation?
3. What substances are produced by cells stimulated by different kinds of IC?
4. Is there a need for interaction between different types of cells for the production of certain substances. How do these different cell types communicate?
5. How can the structure of IC be changed so that IC-mediated cellular effects are altered in a desired way?
6. Which functional *in vitro* tests are best suited to investigate different pathologic processes in different patient groups?

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.

Some of the questions that we try to answer in our clinically oriented research are:
1. Are there connections between the appearance of certain signs and symptoms (at the time of IC measurement or later) and the propensity of IC to stimulate or suppress inflammation *in vitro*?
2. In which body compartments can we find IC inducing harmful effects measured with our functional *in vitro* tests?
3. Are our functional IC-induced responses *in vitro* associated with the patients' prognosis?
4. What autoantibodies and autoantigens are parts of the IC isolated from different patient groups?
5. Can functional IC-induced responses *in vitro* be used to define new subgroups of patients in traditional criterion-based diseases like RA and SLE and can such functionally defined patient subgroups also be distinguished genetically?
6. Can the prognosis of IC-associated diseases be predicted by analysis either of functional IC tests *in vitro*, or by analysis of different IC-associated autoantibodies?

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 nothig 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 worlds' highest rates of stillbirths is 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 prognosis for APS pregnancies has improved with modern treatment.

SLE is known to be more common and more severe in blacks. Whether this also is the case for APS is not known. There is no litterature on SLE, APS or APS-related pregnancy complications in Sudan or West Africa.

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 will use Swedish SLE and APS patients from Karolinska Hospital and Sudanese women with oncomplicated pregnancies.

Group members during 2013

Johan Rönnelid, assoc. prof., senior consultant in clinical immunology, group leader
Amir Elshafie, researcher
Vivek Anand Manivel, PhD student
Sahwa Elbagir, PhD student
Linda Mathsson, affiliated researcher
Barbro Persson, physician
Azita Sohrabian, research engineer

Dissertations during 2013

Amir I. Elshafie, The Immunopathology of Rheumatoid Arthritis and Leishmania donovani Infection in Sudan, September 18, 2013.

Funding during 2013

Swedish Research Council, 1 170 kSEK
Swedish Rheumathism Association, 225 kSEK
King Gustav Vth 80-year foundation 150 kSEK
ALF, 500 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).

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 disappearence, 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 partners.

Local immunotherapy of cancer using monoclonal antibodies
Cancer Vaccines

Sara Mangsbo et al

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 also 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.

Recently we received funding from Bio-X (Vinnova) to take the vaccine strategy into clinical use. We will together with our collaborators in at LUMC, the Netherlands, develop a therapeutic vaccine based on the identified B cell epitope described above.

Myeloid cells in the tumor micro environment

Sara Mangsbo et al.

We have recently initiated a study in collaboration 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 wish to further explore 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 started 2012 and will continue until 2016.

Group members during 2013

Thomas Tötterman, professor, group leader
Sara Mangsbo, researcher, assisting group leader
Joachim Burman, MD, PhD student
Lina Liljenfeldt, PhD student
Erika Gustafsson, PhD student
Luuk van Hooren, PhD student
Ann-Charlotte Hellström, technician

Gabriella Paul-Wetterberg, engineer
Jonas Gustavsson, student
Frida Lindqvist, student

Dissertations during 2013

Linda Sandin, Immunomodulatory Therapy of Solid Tumors: With a Focus on Monoclonal Antibodies, December 13, 2013.

Funding during 2013

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Hematology

In addition to exhibiting an abnormal growth pattern, tumor 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 tumor 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 tumor 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.

The Control of Survival and Apoptosis in Human Multiple Myeloma

Helena Jernberg Wiklund

Our research focuses on multiple myeloma (MM). This is an incurable tumor where malignant plasma cells with complex genetic alterations grow in the bone marrow. The tumor is insensitive to apoptotic signals, for instance signals induced by the immune system. Regulation of cell death, apoptosis, is important for the manifestation of many diseases such as cancer, autoimmune diseases, neurodegenerative diseases and aids. The long-term goal of this research is to identify targets essential for tumor cell survival and analyze whether their function can be blocked in parallel signaling pathways. Such blocking could potentially restore the apoptosis process in tumor cells and might be used to increase the efficiency of tumor selective therapies.

The established model of MM *in vitro* constitutes of a large panel of well characterized cell lines representing the major genetic subtypes of the MM disease, magnetic bead sorted malignant tumor cells from MM patients, and non-neoplastic B cells and plasma cells from normal donors. *In vivo*, immuno-competent murine models, clinically, biologically and genetically highly relevant for the human disease, are operational for biological and therapeutical validation. The models have successfully been used in our group for identification of new targets amenable for potential therapeutic intervention in MM survival pathways i.e. the IGF-1R.

Molecular consequences of intervening with the IGF-1R signal

Charlotte Fristedt, Prasoon Agarwal, Pernilla Martinsson

A critical link between insulin like growth factor (IGF)-1 signaling and human cancer, exemplified by the mandatory role for IGF-1 receptor in oncogene transformation, makes the IGF-1 receptor an excellent target for inhibition of survival circuits in tumors. The significant prolongation of life by targeting the IGF-1R was published by us in a number of sequential papers 2005-2007 in Blood and Int J Cancer. However, the multiple myeloma tumor cells remained in vivo and caused relaps after treatment, warranting validation of combinatorial therapy. Molecular

consequences, alternative pathways and resistance mechanisms affected by the RTK inhibitors, the role of IGF-1 in gene silencing by histone modifications, the effects of the RTKi on gene activation by affecting such epigenetic modifications, and therapeutically relevant combinatorial regimens *in vitro* and *in vivo* has been evaluated.

Initially, we embarked on the resistance mechanisms by analyzing persistent clones of solid tumors and MM cell lines by CGH arrays in conjunction with gene expression arrays and functional studies (Hashemi et al 2011). We have recently described novel roles for IGF-1 as a) a nuclear receptor also in MM cells (Deng H et al 2011) and b) in regulating gene expression by epigenetic silencing of target genes, thereby maintaining survival in MM (De Bruyne et al 2012). These findings shed novel light on the molecular mechanisms of maintaining survival in MM and resulted in a widened approach of the present research program to map IGF-1R dependent and/or independent epigenetic silencing in MM.

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 also described drug resistance and underlying mechanisms (Dimberg et al 2012). In parallel, 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, manuscript 2014) (Maes, manuscript 2014).

In our present research program a panel of MM cell lines subgrouped to different usage of constitutive NF-kB 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, manuscript 2014).

A novel epigenetically regulated gene signature in MM

Antonia Kalushkova, Mohammad Alzrigat, 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 know 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 tumor 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 underway. 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

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 2013

Helena Jernberg Wiklund, professor, group leader
Prasoon Agarwal, PhD student
Mohammad Alzrigat, PhD student
Maria Eriksson, teaching assistant
Charlotte Fristedt, PhD student
Antonia Kalushkova, PhD student
Pernilla Martinsson, research engineer
Kenneth Nilsson, professor emeritus

Charlotta Sandberg, research engineer
Fredrik Öberg, associate professor
Mårten Fryknäs, associated bioinformatician

Dissertations during 2013

Antonia Kalushkova, Epigenetic gene regulation in multiple myeloma and mood disorders, June 12, 2013.

Funding during 2013

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Molecular Hematology - Chronic Lymphocytic Leukemia

Richard Rosenquist

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 treatment in this incurable disease.

CLL is the most common adult leukemia in Western countries. It is a biologically and clinically heterogeneous malignancy with varying clinical course. Many patients survive for years 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 70 years. At present two prognostic scoring systems are used in clinical practice (Rai and Binet), but 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, Diego Cortese, 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 have high intra-subset homogeneity both regarding clinical outcome as well as biological features.

We have further characterized many of these subsets with "stereotyped" BcRs that appear to share clinico-biological features. For instance, *IGHV3-21*-utilizing CLLs show strikingly similar BCRs (subset #2) and a very poor prognosis regardless of *IGHV* gene mutational status. Interestingly, we recently demonstrated that subset #2 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.

Novel prognostic markers in CLL

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

Next-generation sequencing (NGS) studies have revealed a number of novel recurrent mutations in 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 in 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, Viktor Ljungström, Larry Mansouri, Richard Rosenquist

Using next generation RNA sequencing, we have generated data from complete transcriptomes for eight CLL cases (four subset #4 and four subset #1). Analysis revealed that 156 genes (e.g. *WNT9A*) and 76 non-coding RNAs were differentially expressed between the two subsets. In addition, we identified more than 400 novel splice variants that were predominantly expressed in the poor-prognostic subset#1. Moreover, we detected 16-30 missense mutations per sample and mutations were found in genes (e.g. *ATM* and *E2F4*) with a strong potential in CLL evolution. Larger numbers of patients are currently under investigation using RNA-sequencing.

Due to recent technical advancements, it is now possible to investigate several 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 demonstrated the applicability of targeted next-generation sequencing of the *ATM* and *TP53* gene in a well-characterized CLL cohort. Considering the increasing number of prognostic genes in CLL, we foresee that this new approach will be applied to include all such genes for genetic screening in CLL.

Group members during 2013

Richard Rosenquist Brandell, professor, group leader
Panagiotis Baliakas, PhD student
Sujata Bhoi, PhD student
Diego Cortese, PhD student
Anastasia Hazidimitriou, guest researcher
Viktor Ljungström, PhD student (also in Tobias Sjöblom's group)
Larry Mansouri, researcher
Kostas Stamatopoulos, guest professor
Christer Sundström, professor
Lesley-Ann Sutton, researcher
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Funding during 2013

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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 analyses are developed to increase sensitivity and allow smaller amounts of DNA to be analysed.

New Tools and Methods for 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 evidence samples. New methods are important since forensic samples often have been subjected to harsh environments at crime scenes and are therefore also commonly degraded. 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 an extremely sensitive analysis due to a high copy number per cell, while the autosomal markers in very short fragments will give a high discrimination power and Y-chromosome markers allow resolution of mixed samples.

Several new assays based on pyrosequencing, microarrays, real-time quantification or Sanger-sequencing have been developed and used successfully in analysis of evidence material in forensic cases. Now, a combination of traditional methods and next generation sequencing (NGS) technologies will be used 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 and is based on a capture technology with high sensitivity. Finally 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 improves relationship analysis, prediction of visible characteristics and individual identification. The new identification assays allow smaller amounts of DNA to be analysed, thus enabling the use of DNA testing forensic cases with challenging samples. In addition, the novel techniques are used in genetic investigations of historical samples.

Saint Birgitta (Saint Bridget of Sweden) lived between 1303 and 1373 and was designated 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. The origin of the two skulls was assessed first by analysis of mitochondrial DNA (mtDNA) to confirm 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 2013

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

Dissertations during 2013

Maria Lembring, Application of Mitochondrial DNA Analysis in Contemporary and Historical Samples, December 14, 2013.

Funding during 2013

Brottsoffermyndigheten, 1 000 kSEK
Vinnova, 800 kSEK

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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 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:

Sara Ekvall, Christian Wentzel, Cecilia Soussi Zander, Göran Annerén,

In collaboration with Annika Englund, 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 perform studies of growth in DS patients, especially in relation to growth hormone (GH) levels and the function of the thyroid gland and GH therapy. We have shown that adult persons with DS do not suffer from GH deficiency, that early GH therapy in young children with DS improves mental development and head circumference in adulthood. Another finding is that high neonatal TSH levels in DS do not predict thyroid disorders later in life.
- To study mortality and morbidity in patients with DS. Our present results demonstrate an increased average lifetime in patients with DS where death not only is associated with dementia but also with arteriosclerosis and circulation problems.
- To perform international studies on the prevalence of newborn children with DS in relation to the maternal age, prenatal diagnosis, termination of trisomy 21 pregnancies and following-up studies on the introduction of new screening methods such as CUB.
- 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 progession 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 RASopaties.

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

Marie-Louise Bondeson, Sara Ekvall, Eva Kimber, 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 2013

Marie-Louise Bondeson, professor, clinical molecular geneticist, group leader
Göran Annerén, professor, senior staff physician
Berivan Baskin, assoc. prof., clinical molecular geneticist
Sara Ekvall, researcher
Carina Frykholm, staff physican
Patrik Georgii Hemming, clinical genetics resident

Cecilia Soussi Zander, staff physican
Ann-Charlotte Thuresson, assoc. prof., clinical molecular geneticist
Christian Wentzel, staff physican

Dissertations during 2013

Christian Wentzel, Molecular and Clinical Characterization of Syndromes Associated With Intellectual Disability, May 8, 2013.

Funding during 2013

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Exploring Mendelian traits using next-generation sequencing technologies and iPSC 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 bysingle 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 targets for intervention in these and similar disorders.

Next generation sequencing for the identification of gene mutations and gene-rearrangements associated with specific 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 specific phenotypes. We are using whole genome sequencing, targeted sequencing, exome-sequencing and transcriptome sequencing as on selected patient samples. As an example, we have utilized the SOLiD and Ion Proton sequencing platforms (Uppsala Genome Center) in 40 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 shown either 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.

We have characterised a number of unique phenotypes/disorders and additional clinical entities are continuously identified through collaborators. Different imaging technologies and model systems are used in order study the pathophysiological mechanisms caused by novel genes/gene variants, e.g. confocal microscopy 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. We circumvent this using induced pluripotent stem cell (iPSC) technology. Human skin fibroblasts derived from patients with clinically well-defined neurodevelopmental disorders are reprogrammed to iPSC using a non-integrating vector system. The iPSC constitute a source to recapitulate neurogenesis and to generate different neuronal cells.

Homogeneous populations of early differentiated neuronal stem cells are analysed for e.g. growth, migration, neurit outgrowth, global expression pattern, global protein 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, Mowat-Wilson syndrome and Lissencephaly.

Group members during 2013

Niklas Dahl, professor, group leader
Angêlica Delgado Vega, physician
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Feria Hikmet Noraddin, student
Muhammed Jameel, PhD student
Ayda Khalfallah, post doc
Joakim Klar, researcher
Laureanne Pilar Lorenzo, researcher
Doroteya Raykova, PhD student
Jens Schuster, researcher
Maria Sobol, post-doc

Funding during 2013

Publications 2011-2013

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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.

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

Lars 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 Forsberg, Hamid Razzaghian, Chiara Rasi, Jan Dumanski

In collaboration with: Wojciech Zegarski, (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 2013

Jan Dumanski, professor, group leader
Lars Forsberg, Ph.D., investigator
Hanna Davies, research engineer
Chiara Rasi, research assistant
Hamidreza Razzaghian, PhD student
Niklas Malmqvist, research assistant

Dissertations during 2013

Razzaghian, Hamid Reza, Post-zygotic Genetic Variation in Health and Disease, April 23, 2013.

Funding during 2013

Publications 2011-2013

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Genetic Variation in Human Evolution and 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 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 Zhang, 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) 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 Cavelier and Adam Ameur at IGP), we use RNA sequencing to study transcription in tissues from humans and primates to identify novel functional elements and to characterize differences between the species. 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 2013

Lars Feuk, associate professor, group leader
Eva Lindholm Carlström, researcher
Jonatan Halvardson, PhD student
Vs Ganapathi Varma Saripella, student
Ammar Zaghlool, PhD student/Post-doc
Jin Zhao, PhD student

Dissertations during 2013

Ammar Zaghlool, Genome-wide Characterization of RNA Expression and Processing, November 29, 2013.

Funding during 2013

Foundation for Strategic Research, 1 500 kSEK
Swedish Research Council (Medicine), 1 500 kSEK
Swedish Research Council (Natural sciences and engineering), 800 kSEK
European Research Council Starting Grant, 3 000 kSEK

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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 be modeled on the proteome, glycome and lipidome?

Our second project concerns the genetics and 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, trnascriptome, 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, Joanna Hammer, Erik Wilander, 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 Riin 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 is 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 2013

Ulf Gyllensten, professor, group leader
Dan Chen, post doc
Tao Cui, post doc
Louise Danielsson, research assistant
Angelica Delgado Vega, PhD student
Victoria Engström, research assistant
Stefan Enroth, researcher
Erik Gustavsson, project assistant
Inger Gustavsson, research engineer
Julia Hedlund Lindberg, research engineer
Bo Johanneson, researcher
Oscar Johansson, student
Åsa Johansson, researcher
Ivana Juko, PhD student

Dissertations during 2013

Angélica Delgado Vega, Dissecting the Genetic Basis of Systemic Lupus Erythematosus: The Pursuit of Functional Variants, April 26, 2013.

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Phosphorylation Profiles, Biomarkers and the Adenovirus Replication Cycle

Ulf Pettersson

The total tyrosine phosphorylation profile of a cell is determined by the balancing activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Both PTKs and PTPs are implicated in the development of cancer. We are developing a system for studying cell type-specific phosphorylation "fingerprints". The phosphorylation profiles of tumor cells can potentially be used to determine the aggressiveness of a tumor and to better predict and assess the efficacy of a particular anti-tumor treatment. A better understanding of the global picture of phosphorylation could also aid rational drug design where any of the PTKs or PTPs could be targeted.

Many important discoveries have been made using human adenovirus as an experimental system. Today adenoviruses have become of great interest as gene delivery vectors in gene therapy and as oncolytic viruses in the use of cancer treatment.

We have initiated a project the aim of which is to further study the basics of adenovirus replication. One of the goals is to perform a complete biochemical characterization of the adenovirus particle. We have found that at least 11 adenovirus proteins are modified and the nature of the modifications has been determined. So far 28 phosphorylation sites were identified at the amino acid level. An unexpected finding was that two proteins in the capsid contain phosphorylated tyrosine residues. Using masspectrometry several novel adenovirus encoded proteins have been discovered.

Another project aims at a detailed characterization of the adenoviral transcriptome and the transcriptome 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 more than 100 novel splice sites. An adenovirus landmark map showing splice and polyadenylation sites has been constructed. In addition we have identified a set of micro RNAs, which are up- and downregulated during an adenovirus infection.

Moreover, we study tyrosine phosphorylated proteins and their dynamics in adenoviral infected cells. Our findings will reveal new insights into how viruses utilize cellular pathways to optimize their replication.

Altered regulation of post-translational modifications of proteins is important in cancer and could be potential markers for bladder and prostate cancer. We have developed an optimized method for studying protein markers, where urine samples from patients and controls are analyzed. This means that markers for urinary tract cancer diagnosis can be detected in a non-invasive and reliable way.

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 remarkably 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) (unpublished). 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 2013

Ulf Pettersson, professor, group leader
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Funding during 2013

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Genome-Wide Analyses to Identify Mutations that Cause Cancer

Tobias Sjöblom

We aim at finding and understanding mutated genes that cause common human cancers, particularly cancers of the breast and colon. 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 novel methods for early tumor detection, improved diagnosis, and targeted cancer chemotherapy. Cancer drugs that inhibit specific oncogenes are already in clinical use for treatment of several types of cancers, and somatic mutations can cause acquired resistance to such therapies.

Integrated patient sample collection in clinical cancer care

Tony Hansson, Lucy Mathot, Sara Kiflemariam

Identification of mutated genes that cause cancer or resistance to cancer therapies requires systematic sample collection from cancer patients. We therefore coordinate a longitudinal collection of patient data, tissues, and imaging before, during, and after cancer therapy at Uppsala Academic Hospital (www.u-can.uu.se). At the end of 2013, more than 5 500 patients with cancers of the colorectum, brain, prostate, ovaries, lymphoma and haematological malignancies had been included in U-CAN (Tobias Sjöblom, Tony Hansson, clinical partners). A major constraint on cancer genomics is the lack of samples from the large patient cohorts required to gain knowledge about infrequently mutated genes. We have therefore developed a technology for scalable serial extraction of DNA and RNA from tissue samples (Mathot et al, 2011). This procedure has been automated for use in routine pathology and linking extraction to automated storage repositories to enable facile access to samples (Mathot et al, 2013). More than 1000 tissue samples have been successfully processed using this automated technology and it is now being established at Uppsala Biobank. The technology was awarded a Bio-X grant for commercialization of diagnostic technologies and a company, ExScale Biospecimen Solutions AB (www.exscalebio.com), was founded in May 2012 and completed the first round of funding in Q4 2013. Further, a scalable genotyping technology for cancer biobanks based on multiplex detection of small indel polymorphisms has been developed (Mathot et al, 2012).

Mutational studies of candidate cancer genes

Tom Adlerteg, Lucy Mathot, Viktor Ljungström

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 novel candidate breast cancer genes previously discovered by us, encompassing 130 kb of protein-coding sequence, in a panel of 96 breast tumors and identified novel mutations in 12 genes, including DIP2C, ADAM12, GLI1, NOTCH1 and several other putative breast cancer genes (Jiao et al, 2012). Several of these genes are being subjected to functional studies in the group. In a collaborative effort, we validate and scale the HaloPlex target enrichment technology for re-sequencing of cancer genes and exomes in clinical samples. We have developed analysis tools for rapid and accurate statistical analysis of deep sequencing data from tumors with high content of normal genomes and are currently performing deep mutational analyses of 672 genes in cancer pathways in 107 colorectal cancers (Tom Adlerteg, Lucy Mathot).

Functional studies of novel candidate cancer genes

Muhammad Akhtar Ali, Tatjana Djureinovic, 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 of cancer gene status. One approach to perform such analyses is through knock-in or knock-out of the mutated allele(s) in human cancer cells. We have successfully targeted both alleles of the putative breast cancer gene DIP2C and obtained evidence for a phenotype (Chatarina Larsson, Muhammad Akhtar Ali). We have also generated knock-ins of colorectal cancer genes (PRDM2, MLL3, and KRAS) that are currently characterized by us and used by collaborators in drug discovery efforts (Muhammad Akhtar Ali, Tatjana Djureinovic). In collaboration with Horizon Discovery Ltd, we develop computational tools for designing gene targeting constructs to all genes in the human genome (Ivaylo Stoimenov). We have developed technology for forward genetics by transposon mutagenesis in human cells, and applied it to probe and map the KRAS pathway in human colorectal cancers resulting in assignment of 160 new genes to the pathway (Snehangshu Kundu, Muhammad Akhtar Ali).

Mutational and expressional analyses of cancer genes *in situ*

Sara Kiflemariam

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 tissue arrays. For validation purposes, we have analyzed 17 genes chosen for their tissue specific expression patterns or potential as novel cancer biomarkers in collaboration with the Human Protein Atlas project (Kiflemariam et al, 2012). Further, we have evaluated the expression patterns of the tyrosine kinome and the tyrosine phosphatome in ~40 normal tissues and 6 tumor types, totalling 37 000 tissue specimens, leading to the discovery of novel vessel biomarkers in human cancers (Kiflemariam et al, manuscript). We have also 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, submitted).

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Veronica Rendo, PhD student
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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 use massive parallel sequencing (ChIP-seq), which allows us to interrogate the whole genome. We have developed the ChIP-seq technique for the SOLiD platform together with Applied Biosystems and developed new efficient protocols for the Illumina platform.

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 collaborate closely with informatics experts. The methods can be used to reveal the mechanisms for common diseases and cancer. We have started to explore this in the liver cell line HepG2, in normal tissue and in various cancers.

A global view of gene regulation in metabolic and other diseases

Gang Pan, Marco Cavalli, Helena Nord, Madhusudhan Reddy Bysani

In collaboration with Susanne Bornelöv, Marcin Kruczyk,Umer Husen, 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.

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 find several factors that bind next to each other at individual regulatory elements. 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 on type 2 diabetes and other common metabolic diseases. We read chromatin signals in relevant tissues to find regulatory elements and test polymorphic variants in cell-based expression systems. This is done in collaboration with scientists in Uppsala, Sweden and the rest of Europe.

So far we have detected ~10 functional SNPs and in some cases the molecules that bind differentially between alleles. By layering additional large-scale in-house information we have detected hundreds of SNPs that are likely to be functional and they are associated to common diseases and intermediary phenotypes. We have started to assay them using a newly developed high-throughput system.

Group members during 2013

Claes Wadelius, professor, group leader
Madhusudhan Reddy Bysani, PhD student
Marco Cavalli, researcher
Helena Nord, post doc
Gan Pan, post doc
Emelie Wallén Arzt, student

Dissertations during 2013

Madhusudhan Reddy Bysani, Genome-Wide Studies of Transcriptional Regulation in Human Liver Cells by High-throughput Sequencing, June 10, 2013.

Funding during 2013

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Molecular and Morphological Pathology

Research in the field of surgical pathology incorporates in addition of the assessment of conventional morphology, assessment of proteomics and genetics. The scientific projects conducted at the department of "clinical pathology" are based on studying tissue samples obtained by clinicians due to suspected pathological alterations. The ongoing projects included studies on brain, lung, prostate, skin and gastrointestinal tumors among others as well as on degenerative and infectious alterations.

One of the major tools in pathology is microscope but in addition digital images are processed. In the image seen by the microscope or as a digital image alteration in the tissue, cells or cell compartments are identified and systematically analyzed applying various visualization techniques. Development of new techniques including advances in proteomics and genetics has added new dimensions to the scientific work carried out by pathologists.

Neuropathology

Irina Alafuzoff

The oldest old persons are the fastest growing group of the population worldwide. Parallel with this increase in survival, and ageing being the main risk factor for cognitive impairment/dementia in most developed societies, cognitive impairment/dementia are becoming a major health burden. During the last decades we have witnessed standardisation of clinical criteria and read numerous reports dealing with the incidence, prevalence and natural history of dementia. Since 1990, also the diagnostic neuropathology has been standardized and updated when needed.

In collaboration with an EU funded (until 2009) Network of Excellence (http://www.brainnet-europe.org) neuropathological diagnostic criteria for the most common neurodegenerative disorders have been standardised, including rigorous regional assessment and applying immunohistochemical (IHC) techniques.

Based on current knowledge the most common form of dementia is Alzheimer's disease (AD). The hallmark neuropathological alterations in this disease are accumulation of hyperphosphorylated tau (HPTau) and beta-amyloid. The significance of "other pathologies" for cognitive impairment/dementia has also been noted to be of importance as it has been shown that only a subset of subjects suffers from pure AD. AD commonly co-exists with alterations such as alpha-synuclein (alpha-S), vascular brain injury (VBI), primary HPtauopathy and TAR DNA binding protein 43 (TDP43). These co-morbidities are also seen in a "pure" form and cause cognitive impairment on their own (Lewy Body Dementia by alpha-S, FrontoTemporal Lobar Degeneration by TDP43, Argyrophilic Grain Disease, Progressive Supranuclera Palsy and CorticoBasal Degeneration by HPtau and Vascular dementia).

It is currently difficult, if not impossible, to judge based on the tissue alteration seen at autopsy which pathology had the most significant impact on the cognitive status of a given patient i.e., did the subjects suffer of AD with alpha-S pathology or of LBD with concomitant AD-related pathology? Furthermore, it has also been speculated that a certain type of

pathology predispose to another, i.e., AD-related pathology might predispose to alpha-S in amygdale body or TDP43 pathology in hippocampal granular cell layer. What is however well acknowledged is that the most common alteration seen in the brain tissue at autopsy (in close to all) in an aged subject is HPtau.

In 2011-2012 pathology of tau protein received special attention when two studies (one ours), independently performed in two different laboratories, revealed that HPtau is seen in the brain of young and middle-aged cognitively unimpaired individuals. Accumulation of the HPtau was detected in subcortical nuclei without any involvement of cortex. Moreover, no accompanying a-beta pathology was observed in the vast majority of samples. Thus it seems that the neurodegenerative process is initiated in subtentorial neuroanatomical regions and progresses from these regions to neocortex. This progression seems to be cell type dependent and thus predictable. The proteins that are altered are known (HPtau, a-beta, alpha-S, TDP43) whereas the proteins involved in the process of progression of the protein alteration are currently unknown. Further it is not known whether the process of progression is influenced by conditions such as hypertension, diabetes, cardiovascular disease etc, diseases that have been implicated as risk factors for neurodegenerative disorders. Another issue that has become of grates issue is the propagation of the protein alteration. Cell culture studies as well as studies carried out on transgenic animals have raised the option that tauopathies and synucleinopathies progress in a similar way as prions.

The role of transport proteins involved in intracellular trafficking in progression of neurodegeneration is debated. A set of transport proteins is to be systematically assessed applying IHC methods in a well characterised human post-mortem material on cellular level (compartment) and neuroanatomical level (regions). Furthermore, alterations of these transport proteins related to diseases such as diabetes, hypertension, cardiovascular diseases etc will be elucidated.

Group members during 2013

Irina Alafuzoff, professor, group leader
Adila Elobeid, PhD student
Svetlana Popova, researcher

Funding during 2013

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The Molecular Pathology of Non-small Cell Lung Cancer

Johan Botling – Patrick Micke

Non-small cell lung cancer (NSCLC) represents a histologically mixed group of highly aggressive tumors. In subsets of patients, distinct genomic abberations 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 for 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 and immunohistochemistry - whole genome SNPs/gene copy number aberrations, gene expression patterns, mutation status, and tissue microarray data. 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 genetic and trancriptomic levels that are strongly associated with different clinical outcomes. With help of immunohistochemistry these molecular changes were confirmed on the protein level and serve as reliable tools for clinical diagnostics (Micke et al., 2011; Edlund et al., 2012; Botling et al., 2013). Furthermore, in addition to epithelial tumor cell characteristics stromal komponents were identified to correlate relevant patient characteristics and prognosis. 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).

Currently we extend our lung cancer cohort to include patients operated between 2006 and 2010 to finally include altogether 431 patients with available fresh frozen tissue and 724 patients with formalin fixed paraffin embedded (FFPE) tissue samples. These tumors are now analysed by NGS techniques including RNAseq and targeted deep sequencing techniques. High throughput profiling of FFPE cancer tissues will add a new dimension to clinical cancer research as large, complete, population-based patient cohorts become available for comprehensive molecular characterization. A key aim of our group is diagnostic translation in corporation with the clinical molecular pathology laboratory and the newly formed work package on solid tumors in the Clinical Sequencing Platform (SciLife Uppsala).

Group members during 2013

Patrick Micke, associate professor, group leader
Millaray Marincevic, researcher
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Johan Botling, associate professor, group leader
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Publications 2011-2013

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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. Together with G.T. Westermark, Department of Medical Cell Biology, we found in an experimental model of AA-amyloidosis that both natural and non-natural beta-sheet fibrils, including such designed for nano technology, can induce the disease. A test system for possible environmental risk factors is important and has now been developed in *C. elegans*. We are, in collaboration with researchers at 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.

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 an informal reference laboratory for amyloid diagnostics and we receive an increasing number of biopsies each year.

Group members during 2013
Per Westermark, professor em., group leader

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Clinical Pathology and Cytology

In research projects carried out within the field of clinical pathology and cytology protein expressions are analyzed in sections obtained during biopsy, surgery or autopsy. The tissue can be assessed as such or in tissue microarray setting. All studies on human tissue are carried out after appropriate approval from Ethical committee. The results obtained by pathologist are incorporated with the clinical observations, i.e, clinical presentation, radiology, treatment response etc requiring collaboration with clinician and a good infrastructure. Neoplastic, inflammatory and degenerative disorders are studied

Inflammation, transplantation and cardiovascular pathology

Erik Larsson, Anna-Carin Wallgren

These projects are aiming at better understanding mechanisms involved in acceptance of transplanted organs mainly kidneys. The objectives are on changes in blood vessels and in the insterstitium. The techniques used are different immunological and molecular biological methods. The goal is to prolong life time 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 are aiming at creating 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, Susanne Bram Ednersson

Inflammatory cells are in close proximity to all kind of malignant tumours where the innate immunity (myeloid) acts as the first line of defense including macrophages, granulocytes (neutrophils, eosinophils, basophils), mast cells, dendritic cells and NK cells and 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.

Gastrointestinal diseases

Alkwin Wanders

Crohn´s disease (CD) is an idiopathic bowel disease characterized by chronic relapsing inflammation in the gastrointestinal tract. The pathogenesis of CD is complex and the etiology of CD is unknown, but it is considered a polygenic disease, which develops in a complex interplay between environmental factors and aberrant immune responses in a genetically susceptible host. Recent data show that two of the most frequently detected genetic polymorphisms NOD2 and ATG16L are associated with generation of dysfunctional proteins normally involved in the immune response against viruses.

Our own research group could detect for the first time the significant presence of Human Enterovirus species B subspecies Coxsackie B virus and Echovirus, in ileocecal resections from children with advanced ileocecal CD. All patients had polymorphisms in NOD2 or ATG16L1. Human Enterovirus species-B are ssRNA viruseses with tropism both for the intestinal epithelium, and the nervous system. They were present in significant amounts in the mucosa and in the ganglion cells of the enteric nerve system. Control patients contained no or only minimal amount of virus in their intestine. Our data of the virus infection of the enteric nerve system in CD patients provide a possible explanation why CD can present in a segmental fashion. Viruses could travel via nerve fibres of the enteric nerve system and thus affect different segments of the innerved intestine.

An intact epithelial barrier is important for homeostasis and normal gut function. Tight junctions are membrane-associated protein complexes that control paracellular permeability between enterocytes. Viruses from several common RNA virus families with tropism for the human gut mucosa have the ability to interact with tight junction proteins, thus leading to an increase in paracellular permeability. First results showed presence of Human Enteroviruses species B at the onset of the CD disease in the lower gastrointestinal tracts. Two tight junction proteins known to function as a virus receptor, CAR and JAM-A, were specifically downregulated. Studies of tight junction in gastric biopsies showed a loss or downregulation of CAR that was associated with enterovirus infection.

Further studies can be expected to gain new insight into underlying basic pathophysiological mechanisms of CD and other diseases in the gastrointestinal tract.

Malignant melanoma

Margret Agnarsdottir, Fredrik Ponten

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

The protein expression of multiple proteins in malignant melanoma tumors 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. Multiple cohorts were used and for a subset of proteins the expression was also analyzed in melanocytes in normal skin and in benign nevi. The immunohistochemical staining was evaluated manually and for part of the proteins also with an automated algorithm.

The protein expression of STX7 was described for the first time in tumors of the melanocytic lineage. Stronger expression of STX7 and SOX10 was seen in superficial spreading melanomas compared with nodular malignant melanomas. An inverse relationship between STX7 expression and T-stage was seen and between SOX10 expression and T-stage and Ki-67, respectively. In a population-based cohort the expression of MITF was analyzed and found to be associated with prognosis. Furthermore the cohorts were employed to develop an automated algorithm to identify melanoma cells in the tissue samples. Currently this automated algorithm is being used to evaluate the expression of more than thirty proteins. The aim is to identify a group of proteins where the protein expression results might be of value in identifying patients with thin melanomas with aggressive tumors.

Pituitary adenomas – research on selected predictive and potentially therapeutic biomarkers

Olivera Casar-Borota

Pituitary adenomas constitute 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 tumors, aggressively growing adenomas or histologically atypical adenomas. Unfortunately, a considerable proportion of the patients are not cured even following combined surgical/medical/radiotherapy, which indicates a need for defining tumour biomarkers which can predict the clinical response to the established medical treatment, as well as biomarkers which 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 important tool in the selection of the optimal and indivídualised 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 tumor cells correlate with clinical features of the adenomas and with the response to the medical treatment. 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.
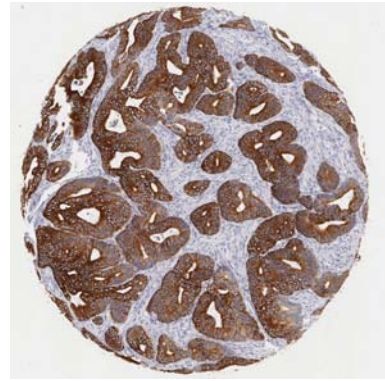
The Human Protein Atlas

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. Little or no information exists for a large proportion of all proteins encoded by the human genome. 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 preformed 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 12 of the Human Protein Atlas includes protein profiles from close to 22.000 antibodies generated towards >16.600 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, protein profiling is performed on a large set of cell lines and patient cell samples using an image analysis-based system.

Over 12 million high-resolution images have been generated and made publicly available on the Human Protein Atlas web portal. 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-group), Evelina Sjöstedt (IHC and Microscopy-group) and Cecilia Lindskog-Bergström (Protein profiling) should also be acknowledged.

Cancer biomarkers

Per-Henrik Edqvist, Gabriella Gremel, Gillian O'Hurley, Kristina Magnusson, Anna Asplund, Caroline Kampf, Anca Dragomir, Piotr Banski, Cecilia Lindskog, Julia Bergman-Larsson, Fredrik Pontén

In collaboration with Karin Jirström (MAS), Patrik Micke, Johan Botling, Irina Alafuzoff, Michael Bergqvist, Anja Smiths, 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), 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) on melanoma markers where image analysis algorithms are being explored for a more reproducible and objective assessment of immunohistochemical signals in melanoma samples. Other collaborative biomarker projects include lung cancer, endometrial cancer, 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, Jerker Linné, Angelika Danielsson, Karolina Edlund, Per-Henrik Edqvist, Gabriella Gremel, Anna Asplund, Agata Zieba, Caroline Kampf, Julia Bergman-Larsson, Fredrik Pontén

In collaboration with Uppsala Akademiska Hospital, Department of Clinical Pathology, Linn Fagerberg, Björn Hallström, Jan Mulder (SciLifeLab), Åsa Sivertsson (KTH), 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. Isolated Langerhans islets from humans intended for islet cell transplantation are collected and used together with various human samples of pancreatic tissues and endocrine tumors. 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. For identified proteins with beta cell-specific expression, monoclonal antibodies are generated and tested in animal studies and pancreatic explants. 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. A number of projects have been initiated aimed at generating more information on selected antibodies used in the Human Protein Atlas project, and to use existing detection techniques or to develop new techniques for antibody-based simultaneous studies of multiple proteins. 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. These techniques have different advantages and disadvantages regarding sensitivity, histological and intra-cellular resolution, implementation in high-throughput evaluations, etc. 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, Angelika Danielsson, Per-Henrik Edqvist, Caroline Kampf, Fredrik Pontén

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

The enormous amount of IHC data now available on tissues and cell lines within the HPA project enables large-scale comparative studies of RNA and corresponding protein levels on a global level. Methods include cell microarrays and immunohistochemistry combined with image analysis and bioinformatic tools to compare with RNA data from a range of tissues and cell lines analyzed for transcription profiles using RNAseq. Cell lines are suitable samples for comparative analysis of mRNA and protein levels, since each cell line constitutes a

homogenous collection of cells and unlike tissue does not represent a range of different phenotypes. 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

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

In collaboration with Karin Jirström (MAS), Patrick Micke (UAS), 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 (Chalrmers University)

The work constitutes a reciprocal collaboration, in which the HPA provides prof. Jens Nielsens 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 immunohistochemstry in tissue cohorts or functional assays in cell lines. This ongoing collaboration has so far rendered five publications in high impact journals.

Group members during 2013

Fredrik Pontén, professor, group leader
Groom Alemayehu, research engineer
Sandra Andersson, PhD student
Maria Aronsson, research engineer
Anna Asplund, researcher
Piotr Banski, post doc
Julia Bergman-Larsson, PhD student
Cecilia Lindskog Bergström, PhD student
Dijana Cerjan, lab technician
Dijana Djureinovic, research engineer
Anca Dragomir, physician
Per-Henrik Edqvist, project coordinator
Åsa Edvinsson, research engineer
Hanna Emmanuelsson, research engineer
Gabriela Gremel, post doc
Sofie Gustafsson, lab technician
Ann-Louise Mikkelsen Jansson, research engineer

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Eva Wahlund, lab technician
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Agata Zieba, post doc

Dissertations during 2013

Cecilia Lindskog Bergström, Tissue Microarrays for Analysis of Expression Patterns, January 25, 2013.

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Molecular Tools

Construction 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, circle to circle amplification for nucleic acid analyses and proximity ligation in solution phase or in situ for investigating proteins or protein complexes. We apply our methods, often in collaboration with colleagues, to gain new insights and diagnostic opportunities in a broad range of diseases including cancer, neurodegeneration, and infectious and cardiovascular disease.
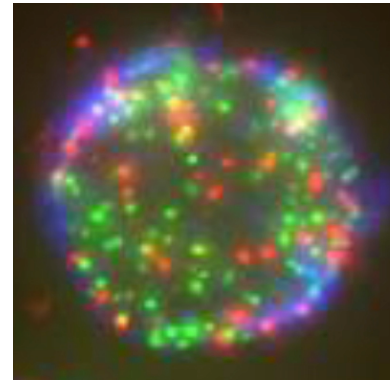
We also actively seek to make our techniques generally available by licensing them to leading biotech companies or spinning out companies from our lab, further developing these technologies. In addition, there is a steady stream of new technologies being initiated in our research program, including nFold probes for rapid and highly specific detection of proteins, nucleic acids, bacteria or viruses ExCirc probes and super rolling circle amplification (sRCA). 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.

Studies of infectious diseases

Caroline Gallant

We apply methods previously established in our lab and new hybrid approaches to study and detect infectious diseases. New methods being explored combine elements of nucleic acid and protein analysis for localized detection with improved efficiency (i.e. nFold probes), and they are combined with a novel approach to augment detection signals for ease of detection in diagnostic contexts, referred to as super rolling circle amplification (sRCA).

We apply our toolbox of methods on a number of platforms, including flow cytometry and microfluidic chips, and explore both research and point-of-care applications.

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.

Demonstration of the use of small molecules as affinity reagents in PLA

Abdullah Al-Amin

Structural similarities in active sites lead to lack of selectivity and unwanted side effects in rational drug design. More selective and efficient identification of molecules specifically binding proteins is of central importance in future medicine.

In a first phase of the project we seek to establish proof-of-concept for the use of small molecule based PLA in the screening and ranking of small molecule affinity reagents. The first envisioned applications are the visualization of small molecule – target protein interactions using *in situ* and solution phase PLA. The strategy is then to multiplex the assay using several different affinity reagents in the same sample. As a next step this application will be extended to studies in more complex environments such as cell cultures and blood samples.

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.

Characterization of sets of affinity reagents capable of jointly recognizing target proteins

Maria Hammond

Protein assays that depend on two or more affinity reagents can offer improved performance over single-binder assays. This is a consequence of the more stringent requirement for specific detection and the ability of rejecting detection signals from most nonspecifically bound reagents, but the identification of optimal combinations of affinity reagents represents a considerable challenge. We are using a variant of the PLA technique to allow large sets of affinity reagents to compete for binding to the same protein molecule, such that optimal pairs, trios or more affinity reagents for detecting the proteins may be read-out via tag sequences added to individual affinity reagents.

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 amyotorophic lateral sclerosis, and we have identified several biomarker candidates in the latter disease.

Multiplex PLA has also been used to analyze blood samples from patients with various malignant diseases. For instance a multiplex PLA with a panel of 36 proteins could distinguish patients with head-and-neck cancers from healthy controls, and the same panel of proteins was used to analyze blood samples from colorectal cancer patients, a study that confirmed increased levels of some currently known biomarker candidates in the patient samples compared to samples from healthy controls. We have also used multiplex PLA to screen cardiovascular patient samples, revealing significantly elevated levels both of known biomarks and also of some previously unknown ones that will have to be further investigated in larger studies.

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 refered to as exosomes. 4PLA-based detection of prostasomes revealed elevated levels of these micro vesicles 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.

Combinatorial protein compositions in subcellular modules and on cell surfaces

Di Wu

It is of crucial importance to identify combinations of proteins that tend to co-occur in subcellular complexes or on cell surfaces, since most biological functions are the effects of sets of cooperating proteins. Variants of the PLA technique are well suited to identify pairs of interacting molecules, and also trios have been successfully demonstrated. We are now developing other approaches that will expand the numbers of proteins whose interactions can be monitored, taking advantage of oligonucleotide-modified affinity reagents in novel analytic reactions.

Group members during 2013

Ulf Landegren, professor, group leader
Abdullah Al-Amin, PhD student
Lei Chen, PhD student
Spyros Darmanis, researcher
Tonge Ebai, PhD student
Elin Ekberg, administrative assistant
Georgios Flamourakis, research assistant
Caroline Gallant, post doc
Joakim Galli, project coordinator
Maria Hammond, PhD student
Johanna Herö, research engineer
Johan Oelrich, systems developer
Mike Taussig, researcher
Erik Ullerås, project coordinator
Jimmy Vuu, research assistant
Rachel Yuan Nong, post doc

Member establishing independent research group
Masood Kamali Moghaddam, researcher
Anne-Li Lind, PhD student
Liza Löf, PhD student
Felipe Marques Souza de Oliviera, PhD student
Lotta Wik, Post doc
Di Wu, PhD student
Junhong Yan, PhD student

Dissertations during 2013

Maria Hammond, DNA-Mediated Detection and Profiling of Protein Complexes, September 27, 2013.

Funding during 2013

Swedish Research Council, MH, 3 600 kSEK
Swedish Research Council, RFI, 1 200 kSEK
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EU; FP7, 2 050 kSEK
EU; FP7, 700 kSEK
EU; FP7, 850 kSEK
EU, ERC; 4 500 kSEK
EU; IMI, 3 000 kSEK
EU; FP7 Marie Curie, 1 100 kSEK
EU; FP7 Marie Curie, 520 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 2013

Mats Nilsson, professor, group leader

Group in Uppsala
Elin Falk Sörqvist, bioinformatician
David Herthnek, postdoc
Lotte Moens, post doc
Malte Kühnemund, PhD student
Lucy Mahtot, PhD student
Camilla Russel, PhD student

Group in Stockholm
Annika Ahlford, postdoc
Anna Engström, postdoc
Thomas Hauling, postdoc
Ronqin Ke, postdoc
Jessica Svedlund, postdoc
Pavankumar Ramachand, postdoc
Elin Lundin, PhD student
Anja Mezger, PhD student
Marco Mignardi, PhD student
Tomasz Kryzkowski, PhD student
Ivan Hernandez, PhD student

Funding during 2013

Vinnova/Swedish Research Council, 700 kSEK
Vinnova, 730 kSEK
EU/IMI, 580 kSEK
EU FP7, 360 kSEK

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Detecting Protein Interactions in Diseases

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 RAS 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 2013

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

Funding during 2013

Publications 2011-2013

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