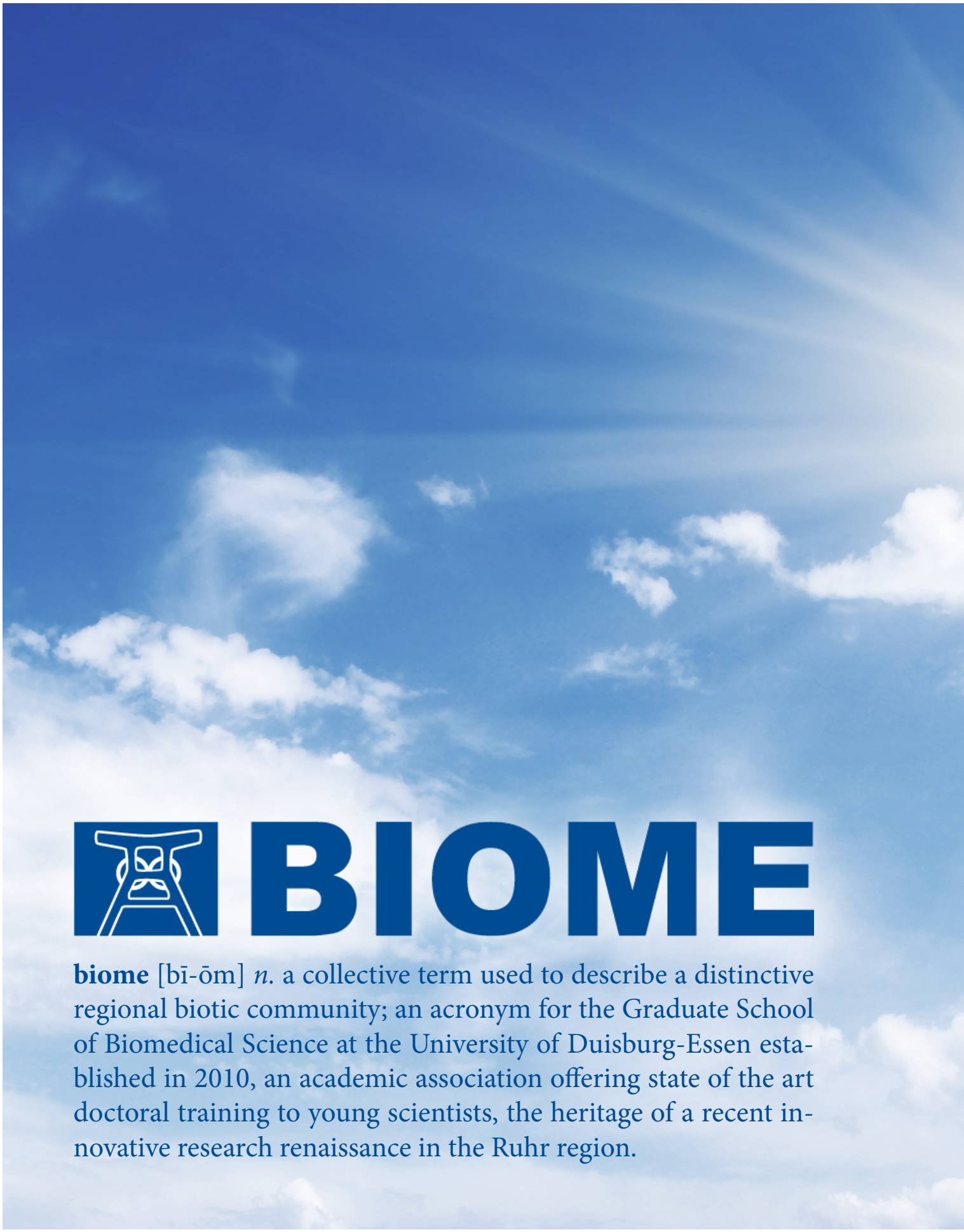
UNIVERSITÄT
DUISBURG
ESSEN

Open-Minded

BIOME

Graduate School of Biomedical Science

Beginnings: 2010-2011



BIOME

biome [bī-ōm] *n.* a collective term used to describe a distinctive regional biotic community; an acronym for the Graduate School of Biomedical Science at the University of Duisburg-Essen established in 2010, an academic association offering state of the art doctoral training to young scientists, the heritage of a recent innovative research renaissance in the Ruhr region.

Contents

Bernd Sures, Dean of Biology

Message from the Faculty of Biology

The Faculty of Biology considers BIOME a successful model for integrating graduate education and research in biomedicine. The BIOME approach of generating synergies at multiple levels - within and between specific core fields of research, between graduates and researchers - is just wonderful. It strengthens the research community at our university by a rich choice of local seminars, and links young graduates with international top researchers by high profile workshops.

A key factor of BIOME's success is to give responsibility to young people and to take them and their research seriously, all in way that simultaneously encourages self-reliance and collaboration, and that provides valuable feedback for personal and scientific development.

We are grateful to the dedicated team that has worked out the BIOME concept and organised a successful start, and we are proud that many groups of our faculty have helped to make BIOME really take off. We trust that BIOME will have a wonderful future and that it will keep its high standard. May BIOME thrive and keep its dynamics for many years to come.

Bernd Sures and **Daniel Hoffmann**
Dean of Biology *Vice Dean for Research and Young Academia*

Michael Forsting, Dean of Medicine

Message from the Faculty of Medicine

The Graduate School BIOME started in summer 2010, and I am very proud of the presentation of the first annual report 2010-2011.

Currently BIOME has well over 170 members counting principal investigators, staff and graduates alike. They represent the full spectrum of our main research areas: cardiovascular disease, cancer, and organ transplantation as well as the bridging disciplines immunology and infectious diseases, and genetic medicine. Recent graduate numbers are 125, which will go up to about 150 once the graduate course "Molecular determinants of the cellular radiation response and their potential for response modulation" (GRK 1739) and the new core on Transplantation Medicine start in 2012. New applications for membership arrive on a weekly basis, so the total numbers given here are constantly increasing.

BIOME supports the effort of our Medical School to combine basic research with the clinical sciences. The benefits of interdisciplinary exchange and of working at the interface of basic and clinical science improve the skills of MDs and PhDs alike.

BIOME is also a connecting link between the Medical School and the Faculty of Biology, which push the research activities of both faculties.

Michael Forsting
Dean of Medicine

Universitätsklinikum Essen

Inauguration of BIOME with Robert Huber on 5 July 2010

Graduate School of Biomedical Science (BIOME)

2010-2011: The Beginnings of the BIOME Story

It has been astonishing and rewarding for BIOME's initiators to watch what started as a mere notion of a graduate school germinate into a concrete concept, take root and develop into a flourishing and increasingly popular network of fascinating thematic diversity uniting young academia, senior scientists, physicians and faculty at the stimulating interface of basic and clinical research.

It has become increasingly apparent in the exceedingly competitive global academic world in recent years that the traditional "*im stillen Kämmerlein*" form of German doctoral education - whereby a graduate researches mainly alone and independently under the supervision and guidance of just one doctoral supervisor - no longer adequately prepares young academics for the demands of the job market and the realities of their future career after the conferral of their doctoral degree. Nowadays young postdoctoral researchers are expected not only to be competent specialists in their particular field of expertise, but also to possess a broad, fundamental understanding of the interdisciplinary aspects of their subject. In addition to this, it is required of these young professionals that they are well-equipped with excellent English language oral and written presentation skills, are team-oriented and aware of the ethics of good scientific practice, know how to acquire research funding and are adept at networking.

Nationwide experience has shown that graduates are best able to attain such pooled knowledge and translational skills by participating in a structured educational programme during their MD or PhD studies which has led to an absolute boom in the establishment of graduate schools at German universities in the last few years, especially in the area of biomedical research. In fact, the availability of such programmes has become such a standard as to be a vital criterion as to whether a candidate accepts a position at a particular university or not and, in the strong regional intervarsity struggle to attract the best of minds, is a decisive and long-term determining factor which cannot be underestimated.

Thus, the Graduate School of Biomedical Science (BIOME) was formally inaugurated on 5 July 2010 in Essen to offer our local graduates just such equalising opportunities. The guest of honour gracing this event was Prof. em. Robert Huber, the Nobel Laureate in Chemistry.

The BIOME Concept

The main aims of our graduate school are to incorporate the vast majority of PhD or MD graduates conducting research in the biomedical field into integrated lecture programmes with thesis-related themes, to offer them close and extended interaction with visiting keynote speakers through regular meet-the-expert forums, and to expose them to opportunities for career-oriented networking. To this end, we have created a strong interdisciplinary research and training graduate school, a joint cooperation between the Faculty of Medicine (University Hospital Essen) and the Faculty of Biology (University of Duisburg-Essen, Campus Essen).

These faculties have two successful and flourishing (closed admission) graduate courses funded by the German Research Foundation (DFG) which form the scientific basis of BIOME:

- *GRK 1045: Host-Pathogen Interaction (est. 2004)*
- *GRK 1431: Gene Transcription (est. 2006)*

As from April 2012 a third DFG-supported graduate course is to join these ranks:

- *GRK 1739: Radiation Sciences*

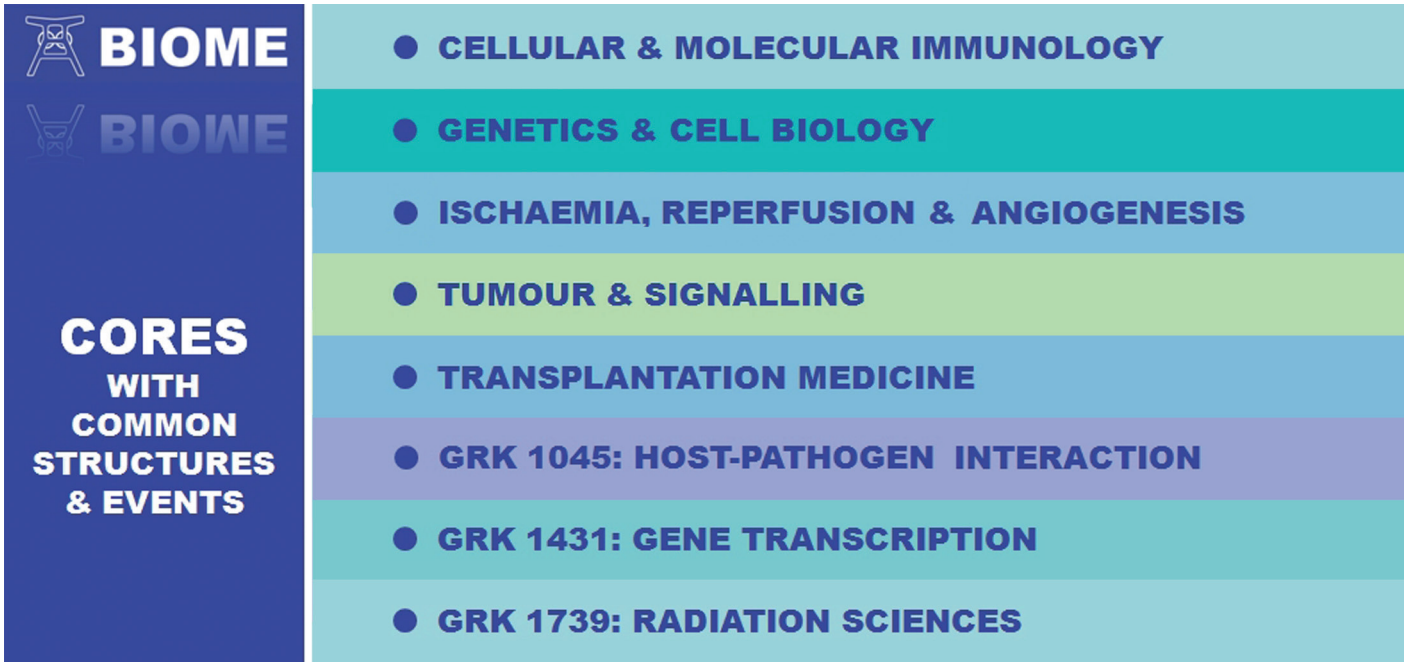
Stemming from the first two initiatives mentioned above, five cores of further expertise within the Graduate School of Biomedical Science have been identified, namely:

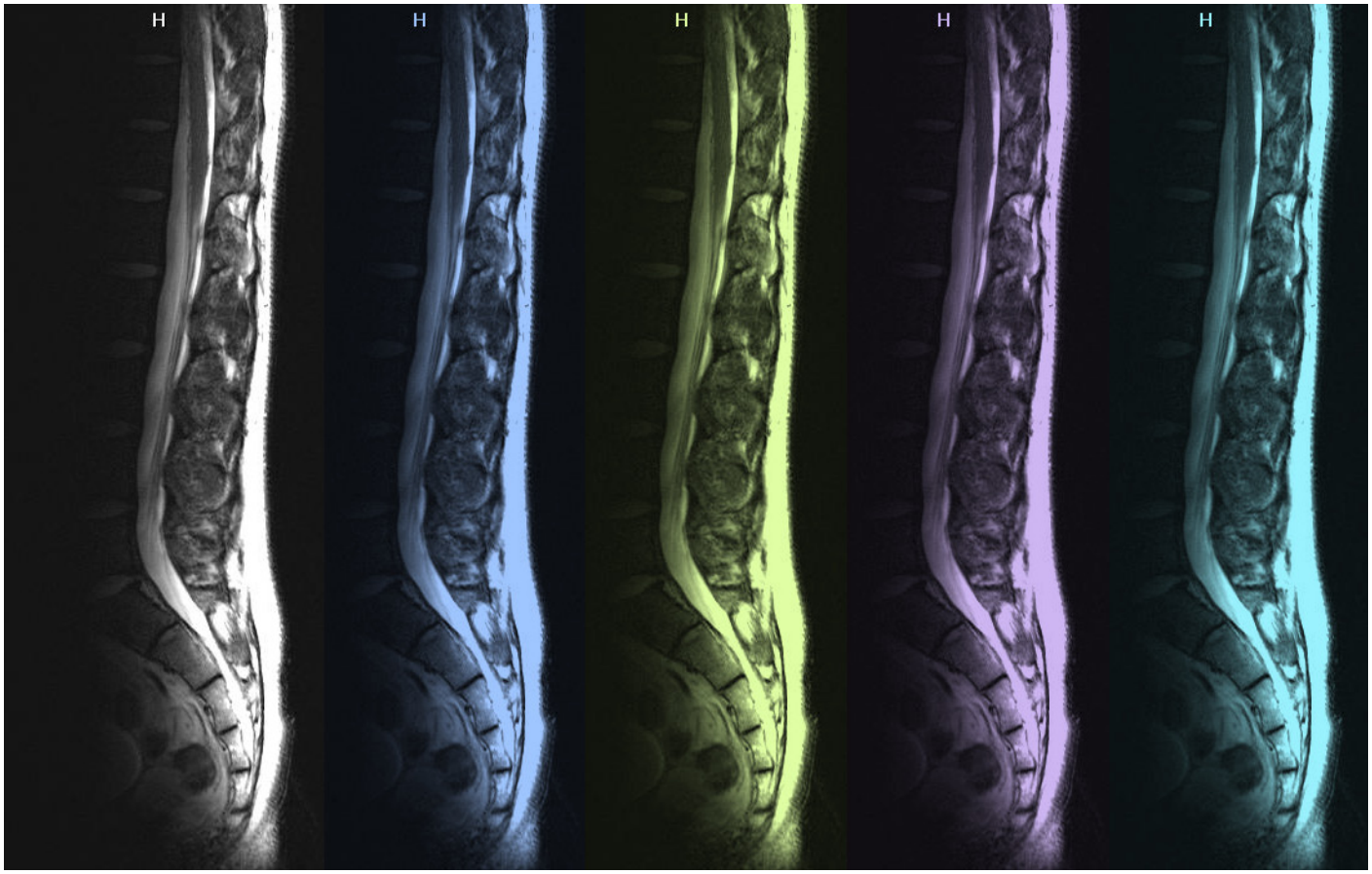
- *Cellular and Molecular Immunology*
- *Genetics and Cell Biology*
- *Ischaemia, Reperfusion and Angiogenesis*
- *Tumour and Signalling*
- *Transplantation Medicine (one-year course for medical graduates starting in April 2012)*

All the graduate courses and cores within BIOME reflect the university's following five outstanding and widely-recognised biomedical research themes:

- *Oncology*
- *Transplantation*
- *Immunology and Infectious Diseases*
- *Genetic Medicine*
- *Cardiovascular Disease.*

Due to the international nature of the scientific world, the working language of the graduate school is English. A dual rationale lies behind this decision: firstly, German-speaking participants are offered a platform for the intensive development of their English skills within a familiar working environment and, secondly, international graduates are in no way disadvantaged by a foreign language barrier when participating in the BIOME programme.





The backbone of BIOME's educational curriculum is its obligatory core lecture series consists of about 20 lectures a year with literature seminars or doctoral progress reports after each talk.

An integral part of the BIOME programme is that each of the newly-established cores holds individual and/or joint annual scientific retreats, a tradition which started with the GRKs and has been carried on by the BIOME modules in 2011. These larger events with their wider-reaching audience, higher public profile and intense networking opportunities are particularly important for the concentrated development of graduate public presentation and teambuilding skills. Wherever possible, joint symposiums are held to promote productive interdisciplinary exchange between a large number of members of the graduate school and the keynote speakers. Particularly noteworthy was the graduate school's hosting of the *IRUN Symposium on Immune Recognition of Pathogens and Tumours* with guests from affiliated universities around Europe in October.

Additionally, the smaller, core-specific meet-the-expert forums which take place after the keynote speakers' talks during the regular lecture series are also an essential part of our programme. These unique sessions enable the participating graduates direct and in-depth interaction with internationally recognised specialists in their research fields in an intimate, informal environment which is conducive to vigorous and highly informative debate. In the pilot graduate courses (GRK 1045 and GRK 1430), these sessions have been evaluated as immensely beneficial and fruitful by both the guest speakers and the doctorates, and are reciprocally regarded as highlights.

Another aspect important to BIOME is the integration of graduates in the design and organisation of core events and selection of speakers. Doctoral initiative, self-sufficiency and engagement are encouraged and welcomed as part of the educational framework.

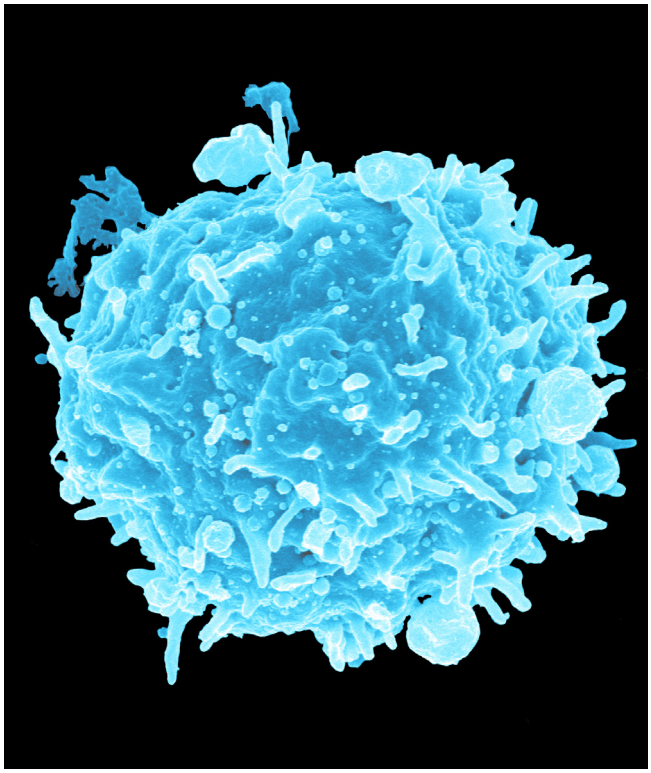
Regular workshops for the targeted training of career-related soft skills are an extended part of the BIOME programme. Topics include scientific writing, presentations, good scientific practice, acquisition of funding etc. To date, these seminars have been provided by sister organisations within the university such as ProFor (a broad-based doctoral forum), the West German Tumour Centre (WTZ) and MediMent (a mentoring programme).

BIOME Research Themes

Cellular and Molecular Immunology

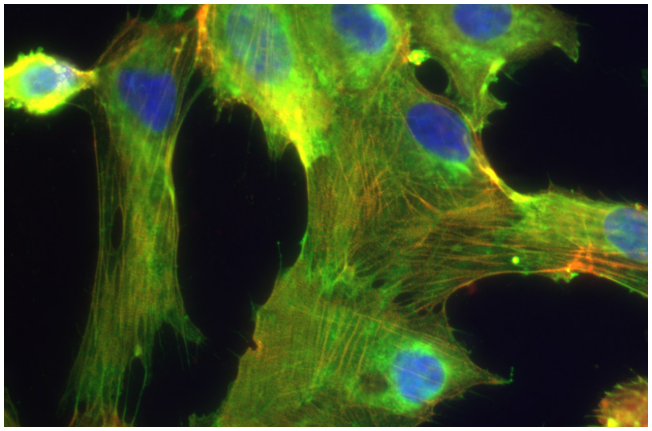
At the end of 2011 the core Cellular and Molecular Immunology had 22 graduate members. Eight graduates work in clinical departments and 14 perform their studies in institutes of the Faculty of Medicine. We are very happy to see that, consequently, the research topics of those graduates cover a wide range of projects combining clinical, translational and experimental immunology in this core. Examples of such graduate projects include the analysis of immune cells in kidney transplantation and multiple sclerosis (clinical), projects on cancer immunotherapy (translational), or the development of an artificial human haematopoietic stem cell niche *in vitro* (experimental). While many projects analyse various aspects of T cell biology (the classical immunologist's favourite), we also host projects on myeloid cells, NK cells, B cells and haematopoietic stem cells.

The lectures are held on a fortnightly basis and are currently being hosted jointly with the core Tumour and Signalling, with each core holding separate graduate seminars after each talk.



Genetics and Cell Biology

The BIOME core Genetics and Cell Biology was established in summer 2010 under the guidance of Hemmo Meyer, Perihan Nalbant, both University Campus Essen, and Verena Jendrossek and Bernd Giebel, both University Hospital Essen. It aims at providing PhD graduates from the Faculties of Medicine and Biology with the opportunity of participating in a three-year multidisciplinary training in various basic, translational and clinical aspects in the fields of molecular and cell biology as well as genetics and to gain insight into modern technology and recent developments in the field. At present, a well-balanced mixture of PhD graduates of the University Campus Essen (10) and the University Hospital Essen (10) take part in the programme, nicely reflecting the representation of this important basic and clinically-oriented research area at both locations. The graduates attend a monthly lecture series throughout the year which is coupled to a graduate seminar where the graduates present the results of their own experimental work to the whole group. In May 2011 the course organised a first annual retreat together with the GRK 1431. On the occasion of this excellent two-day meeting, the graduates presented their work in formal presentations having the opportunity to discuss their research with leaders in the fields of cell migration, cell division, regulation of transcription as well as ageing and stem cells. In 2012, the graduates plan to organise the annual retreat by themselves. The first group of PhD graduates are expected to have graduated by the end of 2012.



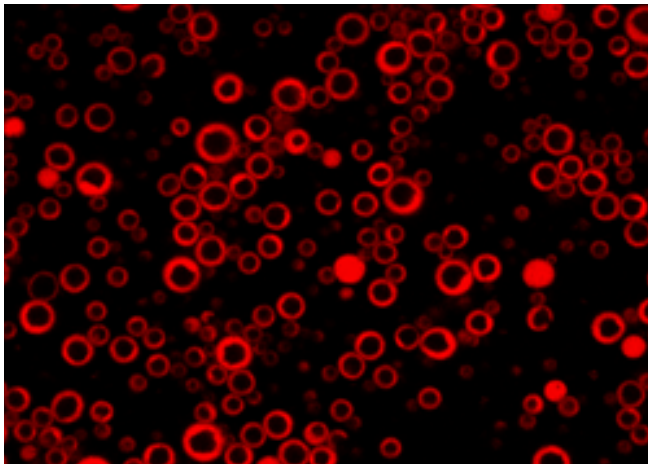
Ischaemia, Reperfusion and Angiogenesis

Injury due to insufficient perfusion and/or due to reperfusion of a previously ischaemic tissue (ischaemia-reperfusion injury) is the underlying cause or is decisively involved in many diseases including heart attack, stroke or various kinds of shock. Ischaemia-reperfusion injury is central to two of the core areas of the Faculty of Medicine, the cardiovascular system and transplantation, but is also related to the other research focus, oncology.

The course does not only cover mechanisms and consequences of cell and tissue injury during ischaemia-reperfusion of various organs including the heart, brain, intestine, liver, and muscle but also regenerating processes following ischaemia-reperfusion such as angiogenesis, and protective/therapeutic measures such as pre- and post-conditioning and pharmacological treatment.

Lectures and seminars are held on a fortnightly basis on the mechanisms of apoptotic and necrotic cell death, signal transduction cascades, inflammatory responses, growth factors, local progenitor and stem cells, and on strategies used to prevent cell and tissue injury induced by ischaemia-reperfusion in different organs and to support regeneration.

Members of the course (current total is 17) are given the opportunity to obtain insights into subcellular (mitochondrial), cellular and small/large animal models of ischaemia-reperfusion injury and to get into contact with clinical and translational research on the subject. A wide analytical spectrum is presented including e.g. cell and molecular biology (analysis of cellular dedifferentiation and transcriptional and post-transcriptional regulation), conventional histology, immune staining (immunofluorescence), electron microscopy, confocal 3D imaging, protein-protein interaction analysis, angiogenesis assays, and *in vivo* imaging.



Tumour and Signalling

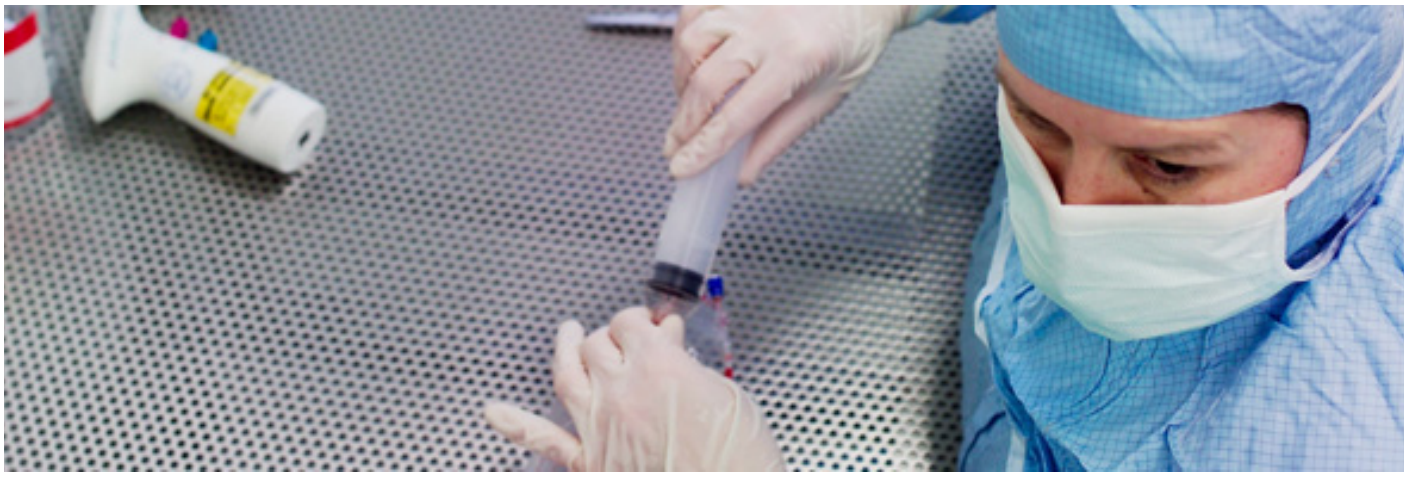
The course introduces young scientists (PhD and/or MD graduates) to the cell and molecular biology of tumourigenesis and medical oncology. The fortnightly lectures of the three-year course focus on signalling in tumours, intracellular signalling as well as the communication within the tissue environment. The course does not recapitulate textbook knowledge of signalling but gives insight into aberrant signalling and communication pathways during tumourigenesis and metastasis. Special emphasis is placed on new therapeutic concepts interfering with tumour-specific signalling. The course imparts knowledge on modern laboratory tools such as cell imaging, array technologies, proteomics and flow cytometry. Furthermore, the aim is to convey knowledge about up-to-date *in vivo* cancer models utilising transgenic mouse technology. The lectures are accompanied by literature seminars to train members in the critical discussion of recent papers of interest, or seminars where graduates present their own results, thus furthering the extension of their presentation skills and the development of their own scientific projects. In addition, scientific guests are regularly invited to share their expertise with the doctorates within the framework of these lectures and seminars. The molecular and cell biological signature of the most important tumour entities are presented and correlated to clinical aspects. To achieve translational research, members are given the chance to participate in defined clinical grand rounds. This core currently has 18 registered graduates.

Transplantation Medicine

The University Hospital Essen is among the leading institutions in Europe in the field of organ transplantation. To improve organ transplantation and to overcome shortages of organ donation and limitations in patient and graft survival after transplantation, it is our aim to strengthen the research endeavour "Basic Science in Transplantation Medicine". In a close cooperation between surgeons, physicians and basic scientists, the main research topics are (I) organ protection and organ regeneration, (II) inflammation and (III) immunity in transplantation.

Starting in April 2012, the core has a one-year curriculum. Fortnightly lectures in the first semester of this course focus on basic knowledge and the clinical aspects of transplantation and, in the second, on various aspects of basic science in transplantation such as preservation injury, tubular injury induced by immunosuppressive medication, monocyte/macrophage-mediated organ- and recipient inflammation, natural killer cell functions after transplantation, tolerogenic dendritic cells, genetic modification of stem cells, induction of T cell tolerance, modification of T lymphocyte functions due to CNS-immune system interaction, as well as risk assessment and monitoring in kidney transplantation. Following the lectures during the first part of each semester, the MD/PhD students will thereafter present and discuss their own results in a graduate seminar.

Although this course is specifically aimed at meeting the needs of MD graduates for a structured research programme, PhD graduates are also most welcome to sign up for this core for a one-year period. Thereafter these doctorates (i.e. PhD or MD/PhD candidates) may join one of BIOME's other cores for a further two years in order to meet the full three-year participation requirements.



GRK 1045: Host-Pathogen Interaction

The PhD graduate course "Modulation of host cell functions to treat viral and bacterial infections" in the Rhine-Ruhr area of Germany offers young scientists an excellent opportunity to further their fundamental education in the science of immunology and infection. A high competence in the fields of infections, immunmodulation and signaltransduction has been established by a number of departments at the Universities of Bochum, Düsseldorf and Duisburg-Essen. New strategies are being developed for the intervention of acute or chronic infections in which the focus of therapy is not the pathogen itself but rather the function of host cells or cells of the immune system. The different working groups of the graduate course are located at ten different institutes and these provide the 28 participants (13 scholarship holders, one postdoctoral fellow and 14 associated members including graduates from the Transregional Collaborative Research Centre TRR 60) with a sound academic education in the subjects of the graduate course. Lectures are held on a fortnightly basis with graduate presentations after each talk. The DFG has supported this course with close to € 5 million for a nine-year period.

GRK 1431: Gene Transcription

The goal of the graduate research training programme GRK 1431 "Transcription, chromatin structure and DNA repair in development and differentiation" is to understand how cells read the genetic information (DNA) that is stored in the cell nucleus and how they make it available for the development of a whole organism with different tissues and cell types. In doing so, the programme offers 14 scholarship holders in the areas of biology, biochemistry and medicine the opportunity of obtaining a qualified education in biomedical research. A further five associated doctorates are also participants on this course, which comprises of about 20 lectures a year.

The DNA is organised and packaged into chromatin within the nucleus, which controls its accessibility during DNA replication, reading of the DNA (transcription) and DNA repair. This GRK investigates how changes in the chromatin influence these processes. Such changes include specific modifications of the chromatin without altering the DNA sequence itself (epigenetics). It is important to understand these mechanisms, because they can explain diseases, for instance cancer, as well as developmental perturbations that result from erroneous gene activity.

This central research idea is pursued by a group of research specialists at the Centre for Medical Biotechnology (ZMB) of the University Duisburg-Essen, with members belonging to the Faculties of Biology, Chemistry and Medicine. It thus concentrates the relevant expertise in the area of gene regulation at this university. Approved funding for this programme is € 2.9 million for 4.5 years.

GRK 1739: Radiation Sciences

Radiotherapy belongs to the three main therapy options for malignant tumours. To improve the therapeutic gain of radiotherapy, researchers aim to develop effective strategies for a biology-based optimisation of treatment protocols combining ionising radiation and targeted drug therapy. Both the realisation of these efforts and the successful translation of novel basic insights into clinical application require scientists and clinical oncologists with a strong background in radiation sciences. Our GRK helps to meet these needs by providing comprehensive multidisciplinary training for MD, MD/PhD, and PhD graduates in basic, translational, and clinical research aspects in radiation sciences. The research programme aims to achieve a better mechanistic understanding of key molecules that determine the cellular response to ionising radiation and thus radiation sensitivity with the goal of providing a scientific basis for effective response modulation. The activities of the GRK will ensure long-term progress in this important research area that is already particularly strong at the University of Duisburg-Essen and has been further strengthened recently by the participation in the BMBF-funded German Consortium for Translational Cancer Research and by the completion of the West German Proton Centre.

Starting in April 2012, excellent scientific projects covering cutting-edge topics in radiation biology and experimental radiation oncology form the basis of a multidisciplinary education in the field. Project-oriented laboratory training is complemented with training in general and specific methods of radiation biology and related fields, and with lectures in radiation oncology and radiology, radiation biology, molecular, cellular, and tumour biology, and cell signalling. An extensive visiting researcher programme, regular seminars, and self-organised courses will promote in-depth knowledge in the key aspects of radiation sciences, as well as in project presentation, project development, and scientific discussion skills. Additional courses will convey essential information in bio-ethics, good scientific practice, and will enhance other important soft skills useful for a scientific career. To advance networking and mobility, the graduates will be encouraged to participate in national and international meetings and to arrange internships with other groups of the GRK and prominent laboratories abroad. Each of the 11 graduates will be regularly supervised by two mentors and a thesis committee will monitor the doctorate's progress and advise on research strategies and career perspectives. The central goal of the GRK is to educate the graduates to become independent scientists who are optimally prepared for a future scientific career in the areas of radiation biology/radiation oncology and biomedical sciences. DFG funding for the initial 4.5 year period is € 3.8 million.

BIOME Symposia in 2011

GRK 1431: Gene Transcription together with the core Genetics and Cell Biology

The 2011 annual meeting of the GRK 1431 was organised as a joint meeting with the BIOME core Cell Biology and Genetics. It took place from 3 - 4 May in Dormagen/Zons. As in previous annual meetings, presentations were given by the PhD graduates, as well as by invited speakers. The guest speakers included Martin Eilers (Würzburg), Markus Löbrich (Darmstadt), Christian Reinhardt (Cologne), Andrea Musacchio (Dortmund), Walter Witke (Bonn), and others.

The sessions covered all research areas of the two graduate themes, for example tumour biology, DNA repair, cell division, as well as regulation of transcription. Those graduates who did not give talks presented their work in a poster session. The retreat enabled lively discussions not only between the graduate members of the two participating school cores with each other, but also with the principal investigators of the two cores and the invited speakers.

In September 2012, instead of a retreat as an annual meeting, the GRK 1431 will co-organise together with the German Society for Genetics a scientific conference in Essen on the topics of the GRK 1431. Room will be given during the conference to present the research of the graduate school.

17th Xanten Workshop "Cell and Tissue Damage: Mechanisms, Protection and Treatment"

The annual workshop in Xanten on the Lower Rhine has a long tradition. In 2011 it took place from 24 to 26 November at Hotel van Bebber.

Since 2002 it has been predominantly organised by the Institute of Physiological Chemistry (Herbert de Groot). Considering its relatively small number of participants (about 60, ca. 40 lecturers) this workshop is highly interdisciplinary, including varying basic topics such as inflammation, regeneration, and animal stress physiology, as well as applied clinical aspects (e.g., ischaemia/reperfusion, trauma/haemorrhagic shock, artificial oxygen carrier, and tissue protection).

Contributions to this workshop consist mainly of short lectures (7 minutes), but there are also selected plenary talks (20 minutes). There is always a clear focus on the discussion of the lecture contents for which the majority of time is granted.

Both young scientists as well as seasoned researchers are invited to participate actively through lectures and talks. For this reason, the Xanten workshop is also a suitable platform for medical and graduate students to present their latest findings to a critical and experienced audience.

In 2011, the annual meeting of the BIOME core Ischaemia, Reperfusion and Angiogenesis was included within the workshop for the first time. Of the 17 graduates currently constituting this core, most took part, four presenting aspects of their theses. Invited guest speakers reported on, e.g., "Copper metabolism in astrocytes" (Ralf Dringen, Bremen), "Artificial receptor molecules against protein aggregation" (Thomas Schrader, Duisburg-Essen), "Effects of alcohol during pregnancy" (Ernst van Faassen, Leiden) and "Problems in diagnosis of mesenteric ischaemia" (Bernd Luther, Krefeld). For personal discussions plenty of time was available during the communal evenings as well as on the tour through the "Museum Nibelungen(h)ort" in Xanten which was visited as a cultural programme. It was a rewarding experience for all participants.

The date for the 18th Workshop in Xanten has already been fixed for 29 November to 1 December 2012.

IRUN Symposium

IRUN Symposium on Immune Recognition of Pathogens and Tumours

In autumn 2011 the Graduate School of Biomedical Science at the University of Duisburg-Essen hosted an exciting new symposium in the Ruhr Metropolis, Germany. The congress was aimed at sharing the latest knowledge about infection and cancer and establishing closer contact between biomedical graduates and scientific researchers across Europe. The initiative was a collaboration between the Nijmegen Centre for Molecular Life Sciences (NCMLS), Radboud University Nijmegen and the BIOME cores Cellular and Molecular Immunology, Tumour and Signalling, and the GRK 1045: Host-Pathogen Interaction. Also participating in this endeavour were IRUN partner keynote speakers from the Glasgow Biomedical Research Centre, University of Glasgow as well as from the Center for Molecular Biology of Inflammation (ZMBE) at the University of Münster. The symposium was opened by Jan Buer, the Faculty of Medicine's Vice Dean of Research and Young Academia and was closed by Jörg Schröder, the University of Duisburg-Essen's Vice Rector for Research, Junior Academic Staff and Knowledge Transfer.

From 20 – 21 October 2011 eighty European scientists and graduates gathered to discuss in lectures and poster sessions such cutting edge issues as "What have we learnt from the 2009 swine flu pandemic?", "Exactly how is the immune system suppressed in infection and cancer?", "How is inflammation controlled, and how are inflammatory signals received and processed?", "In which ways can we activate the immune system to fight infection and cancer?" and, "Can we develop an effective vaccine against cancer?". Guest keynote speakers were Gosse Adema, Irma Joosten, Jurjen Tel, Gerard Graham, Stephan Ludwig, Andreas Krueger and Reinhard Fässler.

The event was a great success as the participants came to realise that colleagues at affiliated institutions are working on similar problems but are using fascinatingly different approaches to try to solve them. During the course of the two days a growing awareness of possible reciprocally beneficial collaborations emerged as a stimulating and promising concept. Thus, a new tradition of lively, mutual scientific exchange and dialogue between the strong life science research centres and graduate schools within IRUN has been born and it is planned to follow this up with regular symposia every two years to be hosted at a different IRUN location each time. Discussions concerning the concrete design of a successor meeting start in spring 2012 in Münster.

Developments and Prospects

Since its foundation in 2010, the graduate school initiative has been met with an extremely positive acceptance by both staff and doctorates alike, and currently has well over 170 members, a total which is rising almost daily. This is about 75% of all graduates at the biomedical faculties. Most of the staff are members of the Centre for Medical Biotechnology (ZMB), an interdisciplinary scientific centre of the University of Duisburg-Essen, integrating medical research at the University Hospital and the natural sciences at Essen's main campus.

Another further development worth mentioning is the Faculty of Biology's unanimous decision in September 2011 to officially recognise BIOME's three-year graduate curriculum as a qualitatively-equivalent alternative pillar supporting the university's MD/PhD educational programme. This unique qualification enables freshly graduated and exceptionally talented young physicians with serious intentions of pursuing a vocation in biomedical research rather than in direct patient-oriented care to gain a second doctoral degree in Biology. The first candidate to opt for this career choice is in fact a BIOME member, and a number of other medical candidates are currently earnestly contemplating following in these pioneering footsteps.

With a total of 150 entries, five of the ten poster prize winners at the Faculty of Medicine's Research Day in 2011 were BIOME graduates, a significant objective indicator of the increasing quality of our doctorates' presentation skills.

The general coordinator is also happy to report that there has been a marked increase in the external awareness of the university's biomedical research generated by BIOME's public profile, a secondary spin-off bringing with it a myriad of unexpected opportunities for expansive scientific networking. Firstly, the number of national and international applicants drawn to our institution has risen substantially since the school was founded, as well as the amount of business-related interest coming from companies and organisations beyond our campus.

Thus, it is with a shared sense of accomplishment that BIOME is able to reflect back on an extremely successful establishment and early growth phase and look forward to the launches of the new GRK 1739: Radiation Sciences and the core Transplantation Medicine in April 2012. Moreover, we remain dedicated in striving to improve the academic environment in which we find ourselves and continue our deep commitment to strengthening long-lasting collaborations with research partners at similar centres of expertise located not only at our own institution, but also at others within Germany and across Europe.

BIOME Coordinators and Speakers

Chair

ULF DITTMER studied Biology at the University of Bremen from 1987 until 1992 and went on to do both his diploma and doctoral theses at the German Primate Center in Göttingen, receiving his PhD from the University of Göttingen in 1995. Ulf then headed the Viral Immunology group at the same German Primate Center for two years, after which he spent a further two years as a visiting scientist at the Rocky Mountain Laboratories, National Institutes of Health, Hamilton, USA. In 1999 he returned to Germany to obtain his *venia legendi* in Virology at Hannover Medical School and to head the Retroviral Immunology group at the Institute of Virology, University of Würzburg. In 2002 Ulf was appointed to the Institute of Virology, University Hospital Essen, firstly as an associate professor for Viral Immunology and then as a full professor for Experimental Virology in 2009. Since 2004 he has been closely involved with the GRK 1045: Host-Pathogen Interaction both as vice speaker (first term of funding) and later as main speaker (second financial phase). In 2009 the idea of BIOME took form and shape in Ulf's mind, and he became its chairman once it was established. During the first half of 2011 Ulf took sabbatical leave to collaborate with Kim Hasenkrug on developing a humanised mouse model for a proposed HIV vaccine trial at the Rocky Mountain Laboratories, NIH, Hamilton, USA, returning to Europe to take over the directorship of the Institute of Virology in Essen in October 2011.

Ulf's research focuses on the basic cellular and molecular mechanisms of immunity against retroviruses as well as vaccine development against retroviruses. He is also interested in immunotherapy against retroviral infections and virus-induced cancer, persistent viral infections and retrovirus-induced immunosuppression.

Chair

SVEN BRANDAU studied Biology in Hamburg and Los Angeles and conducted his PhD studies from 1993 until 1996 at the Bernhard-Nocht Institute for Tropical Medicine in Hamburg. From 1996 until 2007 he worked as a postdoctoral fellow and later as a senior scientist and independent group leader at the Research Center Borstel. During his habilitation period he started focusing on cancer immunotherapy and obtained his *venia legendi* in Immunology and Cell Biology at the University of Lübeck in 2003. In 2007 he moved to the University of Duisburg-Essen where he became an associate professor in 2009. Sven is the head of the Experimental Research Division of the Department of Otorhinolarnygology, University Hospital Essen, and co-chairman of the BIOME graduate school. He has received several awards for his work on experimental and translational aspects of tumour immunology.

Sven´s main research area is the immunological tumour-host interaction with a focus on myeloid cells. Additional research projects aim at developing novel immunotherapies for head and neck cancer and investigating the role of mesenchymal stromal cells in cancer and infection.

Chairs
Ulf Dittmer
Sven Brandau

General Coordinator
Delia Cosgrove

Scientific Coordinators
Cellular and Molecular Immunology
Sven Brandau, *Otorhinolaryngology*
Karl Sebastian Lang, *Immunology*
Cornelia Hardt, *Immunology*
Wiebke Hansen, *Medical Microbiology*

Genetics and Cell Biology
Verena Jendrossek, *Molecular Cell Biology*
Hemmo Meyer, *Molecular Biology*
Perihan Nalbant, *Molecular Cell Biology*
Bernd Giebel, *Transfusion Medicine*

Ischaemia, Reperfusion and Angiogenesis
Herbert de Groot, *Physiological Chemistry*
Frank Petrat, *Physiological Chemistry*
Kerstin Böngler, *Pathophysiology*

Tumour and Signalling
Elke Winterhager, *Molecular Biology, Tumour Research*
Joachim Göthert, *Internal Medicine, Haematology*
Alexandra Gellhaus, *Molecular Biology, Tumour Research*
Hannes Klump, *Transfusion Medicine*

Transplantation Medicine
Ursula Rauen, *Physiological Chemistry*
Oliver Witzke, *Internal Medicine, Nephrology*
Monika Lindemann, *Transfusion Medicine*

DFG Graduate Course Speakers
GRK 1045: Host-Pathogen Interaction
Ulf Dittmer, *Experimental Virology*

GRK 1739: Radiation Sciences
Verena Jendrossek, *Molecular Cell Biology*

GRK 1431: Gene Transcription
Ann Ehrenhofer-Murray, *Genetics*

General Coordinator

DELIA COSGROVE studied English and Environmental and Geographical Science at the University of Cape Town, South Africa, with a third major in Botany which included independent research on the difference in flammability of plants both within and external to the fynbos biome. She graduated from UCT in December 1989. From 1990 until 1999 she lived and worked in Genoa, Italy and Essen, Germany where she gathered experience and expertise in transferring English language business and cross-cultural skills to globally-oriented professionals at various international companies as well as to trainees on educational programmes at state-approved academies. The birth of twins in 1999 led to a career break until 2004 when Delia was appointed as the coordinator of the then newly established GRK 1045: Host-Pathogen Interaction at the University of Duisburg-Essen. In 2009 she moved on to her present position to focus on the detailed formation of BIOME and has been responsible for the daily management and general development of the graduate school since then.

Important to Delia is facilitating to ensure that there is an open and dignified rapport between all members of the graduate school and the faculties. Creativity of vision for BIOME, respect for graduate issues and integrity as well as the symbiotic investment in long-term international interdisciplinary scientific exchange are further aspects to be especially cultivated in the current academic terrain.

Cellular and Molecular Immunology

KARL SEBASTIAN LANG studied Medicine at the University of Innsbruck, University of Tübingen, King's College Hospital, London, and Yale University. He sat his final exam in 2001 in Tübingen where he also did his doctoral thesis at the Department of Immunology and was promoted to medical doctor. Karl held postdoctoral positions at the Department of Physiology in Tübingen and the Department of Pathology in Zürich where, in 2007, he attained his habilitation for Immunology. From 2007 until 2008 this was followed by a stint at the Ontario Cancer Institute Toronto. Since 2008, Karl is a principal investigator at the Department of Gastroenterology, Hepatology and Infectiology in Düsseldorf and, since 2011, a full professor and the Director of the Institute of Immunology at the University of Duisburg-Essen.

Karl's work has been acknowledged by numerous prestigious research foundations and his papers have been prolifically published in highly esteemed journals. Karl's research focuses on the role of T cells in chronic viral infections and autoimmune diseases as well as the influence of type I interferons and T cell responses. He has just recently published a ground-breaking paper on the crucial role of macrophages in lowering type I interferon responses and the activation of adaptive antiviral immune responses in the prevention of the fatal outcome of infection.

CORNELIA HARDT completed her medical doctor's degree in Cellular and Molecular Immunology at the University of Mainz in 1990. She received the *venia legendi* in Molecular Medicine in 1999 at the Ruhr University of Bochum. She became a university professor in Immunolgy in 2002 at the University Hospital Essen. She is a member of the editorial board of the journal "Genes and Immunity". Her research background is cellular and molecular immunology, human genetics and immunogenetics. The main focus of Cornelia's research is on genomics, epigenomics, pharmacogenomics and immunregulation of complex diseases such as multiple sclerosis.

SVEN BRANDAU

Please refer to the "Chairs" section for this biography.

WIEBKE HANSEN studied Biology at the Carolo-Wilhelmina University of Braunschweig. From 1997 until 2000 she did her PhD studies at the German Research Centre for Biotechnology in Braunschweig on the optimisation of retroviral vectors for human gene therapy. In 2000 Wiebke Hansen started to focus her research on molecular immunology as a postdoctoral scientist in the Mucosal Immunology group at the Helmholtz Centre for Infection Research. In 2007 Wiebke moved to the University Hospital Essen, where she has been working as a group leader of Immunregulation at the Institute of Medical Microbiology up until the present. Wiebke Hansen received the *venia legendi* in Medical Microbiology and Immunology in 2010.

Wiebke's main research focus is on regulatory T cells which are well known key players in immunological homeostasis and thought to be involved in the regulation of different immune responses. By molecular and functional analysis she could identify molecules highly expressed by this immunosuppressive T cell subset. In further studies the Immunregulation group is currently developing new approaches to modulate regulatory T cells function *in vivo* for the treatment of dysregulated immune responses during chronic infections and cancer.

Genetics and Cell Biology

VERENA JENDROSSEK studied Pharmacy at the University of Würzburg and Cell Biology at the Ecole Pratique des Hautes Etudes, University of Paris. From 1988 until 1992 she did her PhD studies at the University of Göttingen on the analysis of NADPH-oxidase in immune cells from patients with chronic granulomatosis. She then started focusing her research on the molecular mechanisms of stress-induced apoptosis as a postdoctoral scientist at the Department of Pediatric Oncology, University of Göttingen. In 2000 Verena moved to the University of Tübingen where she continued her work on stress-induced apoptosis at the Departments of Physiology and of Radiation Oncology where she received the *venia legendi* in Physiology in 2003. In 2007 she obtained a full professorship for Cell Biology at the University Hospital Essen, where she established the Molecular Cell Biology group at the Institute for Cell Biology (IFZ). Verena is one of the coordinators of the BIOME core Cell Biology and Genetics and will direct the GRK 1739 "Molecular determinants of the cellular radiation response and their potential for response modulation" as its speaker. The GRK 1739 is a novel German Research Foundation (DFG) funded research training programme at the University of Duisburg-Essen which will be launched in April 2012.

Verena's main research focus is the analysis of molecular mechanisms of the cellular response to chemo- and radiotherapy with a focus on the signalling pathways involved in therapy-induced cell death and associated intrinsic and microenvironment-mediated resistance mechanisms. Moreover, the molecular details of radiation-induced normal tissue damage are explored with a focus on the regulation of radiation-induced tissue inflammation and fibrosis. These studies aim at identifying novel targets for a modulation of the cellular radiation response. The promising approaches to improve treatment outcome are tested *in vitro* as well as in animal models.

HEMMO MEYER studied Human Biology and Medicine at the University of Marburg, Germany, where he also received his PhD working on the molecular cell biology of human cytomegalovirus maturation. He then moved on to work at the Imperial Cancer Research Fund in London and Yale University Medical School, USA, as a postdoctoral fellow. During this time, he became interested in a regulatory system that is governed by the p97 ATPase. His work revealed the basic principles of the system and contributed to establishing p97 as a central element of the ubiquitin-proteasome system. He then moved to ETH Zurich, Switzerland, as an independent group leader. During this time, his group revealed that p97 constitutes a novel regulatory layer in chromatin-associated processes that ensures the genomic stability of proliferating cells. In 2009, Hemmo Meyer accepted a professorial position at the Centre for Medical Biotechnology in Essen.

His goal is to tackle key questions in the molecular biology of cell cycle regulation relevant for cancer development and aging using state-of-the art technology in an international network of collaborations. With regard to young academia, he hopes to transmit his own excitement about basic research in molecular cell biology.

PERIHAN NALBANT studied Chemistry at the TU Dortmund. She completed her PhD studies from 1997 until 2000 at the Max-Planck Institute for Molecular Physiology on the characterisation of sodium/phosphate co-transporters and their regulation by naturally occurring antisense mRNAs. During her postdoctoral studies at the Scripps Research Institute (La Jolla, CA, USA) she focused on the development of live-cell fluorescent biosensors to measure the activities of Rho GTPase proteins most prominently known to control the dynamics of the actin cytoskeleton. In 2007 she visited the Cardiff School of Biosciences as an independent researcher to establish fluorescence-based high-throughput screens for RNAi based pathway analyses. Perihan moved to the University of Duisburg-Essen in 2008 as a junior professor for Molecular Cell Biology up until the present.

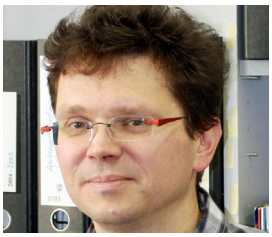
Perihan's research is mainly focused on Rho GTPase signalling pathways controlling the dynamics of the actin cytoskeleton during cell migration and other cellular processes related to cancer cell invasion. Her group is using a multifaceted approach including fluorescent biosensors, RNAi and high-resolution live-cell imaging to dissect how the complex network of upstream regulators affects localised Rho protein signalling and how this is translated into normal as well as aberrant cell migration.

BERND GIEBEL studied Biology at the University of Cologne and received his PhD in 1996 at the Institute for Developmental Biology in Cologne. In his thesis he investigated aspects of the Notch signalling pathway during early neurogenesis of *Drosophila melanogaster*. In 1999 he moved to the Heinrich-Heine-University of Düsseldorf and started to work with human haematopoietic stem and progenitor cells. There, he also established his research group focusing on the mechanisms which control the decision of self-renewal versus differentiation of human somatic stem cells. In 2008 he received the *venia legendi* in Molecular Medicine. In November of the same year he moved with his group to the Institute of Transfusion Medicine, University Hospital Essen where he has continued his studies on human somatic stem cells. Via the identification of two asymmetrically segregating proteins in dividing human haematopoietic stem and progenitor cells, the tetraspanins CD53 and CD63, he became interested in exososomes, small vesicles participating in intercellular communication. Currently, as a second topic, his group is establishing techniques to purify and analyse exosomes for clinical applications.

Ischaemia, Reperfusion and Angiogenesis

HERBERT DE GROOT studied Biology and Medicine at the RWTH Aachen and the University of Düsseldorf. He received his PhD in 1982 and his MD in 1987. From 1982 to 1988 he worked in Düsseldorf at the Institute of Physiological Chemistry I. This work was interrupted in 1986 by a research stay at the University of Rochester Cancer Center, New York, and completed in 1988 by receiving the *venia legendi* in Physiological Chemistry (Biochemistry). From 1989 to 1992 he served as a Heisenberg fellow leading one of the two working groups of the clinical research group "Liver Injury" at the Institute of Physiological Chemistry I and the Department of Gastroenterology, University of Düsseldorf. In this period he also spent a nine-month clinical period at the Department of General Surgery, University of Tübingen. In 1992 Herbert was offered the chair in Physiological Chemistry at the University Hospital Essen.

Herbert de Groot's research focus is on the mechanisms of cell and tissue injury, especially those induced by ischaemia (oxygen deficiency). Current projects include studies on the injury of the intestine, kidney and other organs by ischaemia and subsequent reperfusion, on tissue injury by haemorrhagic and septic shock, and on muscle injury by trauma. In addition, he is developing nano- and microcapsules for the use of artificial oxygen carriers (blood substitutes).



FRANK PETRAT studied Biology at the Ruhr University of Bochum and received his diploma in 1997 (focus: animal physiology). He then joined the Institute of Physiological Chemistry at the University Hospital Essen as a graduate student and received his PhD in 2000 (focus: toxicity/detection of intracellular iron ions). In 2001 Frank Petrat became a group leader and research associate. He started studying the potential of NAD(P)H to act as a direct operating antioxidant in 2003 as a scientific assistant. These studies were completed in 2007 by receiving the *venia legendi* in Physiological Chemistry (Biochemistry). As from 2006 he established various animal models for questions in applied emergency medicine and is still working as a group leader in the field of pathobiochemistry and -physiology.

The main research interests of Frank Petrat are *in vivo* aspects of tissue ischaemia/reperfusion (oxygen deficiency/reoxygenation) injury, e.g. of the small intestine as well as under conditions such as haemorrhagic and septic shock. Based on clinically relevant and well-controlled animal models, the consequences of tissue ischaemia/reperfusion, mechanistic aspects, diagnostic parameters and therapeutic/protective strategies are being studied.

KERSTIN BÖNGLER studied Biology at the Ruhr University of Bochum. From 1999 until 2003 she conducted her PhD studies at the Max Planck Institute for Physiological and Clinical Research in Bad Nauheim. In her thesis, she identified differentially expressed genes in growing collateral arteries. In 2003 Kerstin moved to the Institute of Pathophysiology at the University Hospital Essen. Here, she focused her research on cardioprotective strategies in order to reduce myocardial infarction. In 2009, Kerstin Böngler received the *venia legendi* in Pathophysiology.

Kerstin is interested in the cardioprotective phenomena of ischaemia pre- and post-conditioning which reduce myocardial infarction. Here, her research focuses on the role of mitochondria, which are central for cell survival following ischaemia and reperfusion. Kerstin Böngler characterises the translocation of proteins into mitochondria and studies the importance of specific proteins for mitochondrial function.

Tumour and Signalling

ELKE WINTERHAGER studied Biology at the RWTH Aachen, Germany, and did her PhD studies in the field of vision research at the Research Centre Jülich. From 1980 she started working as a postdoctoral researcher at the Department of Anatomy, RWTH Aachen, where she focused on membrane biology in the field of reproductive biology and received her *venia legendi* in Anatomy in 1986.

In 1986 Elke took over the position of a study director and head of Department of Reproductive Toxicology at the company Grünenthal, Aachen.

In 1990 she moved to the University of Duisburg-Essen's University Hospital as a professor in Anatomy and Embryology and switched to the Institute of Molecular Biology in 2008. Her main research focus is on the cell biology of the endometrium during embryo implantation, embryonic signalling during implantation and hormonal regulation of endometrial genes. Recently she has focused on placental function and development in mice and humans. Besides using several knockout and transgenic mouse models, she also uses trophoblast stem cells as a model for the regulation of trophoblast lineage development. Her main interest is deciphering the role of direct cell-cell communication via connexion channels for this process. In close collaboration with the Department of Gynaecology she works on the pathomechanisms of preeclampsia. Since May 2004 she is a reviewer on the DFG board for the study section "Molecular Biology".

Besides her research work, Elke has held different political positions: from 1996 to 2000 she was Vice President for Research of the University of Duisburg-Essen and from 2001 to 2003 Associate Dean for Research at the Medical Faculty of the University of Duisburg-Essen.

JOACHIM GÖTHERT studied Medicine at the Universities of Bochum, Hamburg, Lübeck, London (University College, UK) and Yale (New Haven, CT, USA). He obtained his medical doctorate by studying the function of immune cells in preterm infants from 1996 until 1998 at the Institute of Immunology and Transfusion Medicine, Medical University of Lübeck. From 1998 until 2000 he started his medical training as a resident physician at the Department of Haematology and Oncology, University Hospital Hamburg. In 2000 he commenced his postdoctoral training as a scholar of the German Research Foundation (DFG) at the Cancer Biology Division (Institute for Child Health Research) in Perth, Australia. The focus of his research was studying normal and malignant haematopoietic development in genetically modified mouse models. From 2002 until 2003 he continued his studies at the same Australian institution as a senior research fellow. In 2004 he returned to Germany and started his own research group at the Department of Haematology, University Hospital Essen. In 2008 he was awarded a junior research group grant from the Stem Cell Network of North Rhine Westphalia. In 2009 he completed his specialised training in internal medicine.

The main focus of his research is on the molecular regulation of normal blood development as well as genetic events leading to the development of leukaemia. Amongst other findings he demonstrated in genetic studies that adult bone marrow stem cells are primarily of foetal origin.

ALEXANDRA GELLHAUS studied Biology at the Carl von Ossietzky University of Oldenburg. From 1999 until 2003 she studied for her PhD at the Institute of Anatomy at the University Hospital Essen, investigating the effect of different gap junction channels on the proliferation and invasion capacity of malignant trophoblast cells. In 2003 Alexandra Gellhaus continued her work as a postdoctoral scientist in the Institute of Anatomy and focused her research on the regulation of CCN family proteins and connexins in human trophoblast proliferation and invasion behaviour. She works in close collaboration with the Department of Gynaecology on the pathomechanisms of preeclampsia, a pregnancy disease which is characterised by intrauterine growth restriction and preterm birth. After two breaks (2007-2008 and 2010-2011) during which she was on maternity leave, she continued her work as a postdoctoral researcher and, in 2008, moved to the Institute of Molecular Biology in Essen until present. In 2011 Alexandra Gellhaus started to prepare her habilitation for Molecular Biology which she will finish in 2012.

Alexandra's main research focus is the regulation of trophoblast proliferation and invasion – processes which are indispensable for proper placenta and embryo development. Furthermore, she is interested in the identification of deregulated placental genes in preeclampsia which is associated with impaired trophoblast invasion and often results in intrauterine growth restriction. Meanwhile, it has become clear that this in turn seems to programme coronary heart disease and hypertension in adults. She could show that CCN3, a matricellular protein which is expressed in the trophoblast and in placental endothelial cells, is deregulated in preeclampsia and might be associated with the deficient trophoblast invasion. In addition, she identified the CCN3 dependent signalling pathways regulating trophoblast proliferation and invasion.

HANNES KLUMP is a principal investigator and physician at the Institute for Transfusion Medicine, University Hospital Essen. He received his diploma (equivalent to an MSc degree) and PhD in Genetics from the University of Vienna for his studies on picornaviral proteinases. From 1998 until 2003 he worked as a postdoctoral fellow at the Heinrich Pette Institute for Experimental Virology and Immunology in Hamburg. His studies focused on the development of a novel, picornaviral 2A-esterase based co-expression system for retroviral gene therapy vectors and the *in vitro* development and expansion of haematopoietic stem and progenitor cells (HSPCs) mediated by ectopic expression of the homeodomain transcription factor HOXB4. From 2004 until 2008, he pursued and extended his work on these topics in the Department of Experimental Haematology at Hannover Medical School (MHH) where he became a group leader within the cluster of excellence "REBIRTH" (JRG Differentiation). After receiving his medical degree at MHH in 2008 he moved to the Institute of Transfusion Medicine in Essen. Since then, his laboratory has continued to work on the *in vitro* development and expansion of HSPCs from pluripotent embryonic stem cells and the molecular mechanisms underlying the stem cell supporting activities of HOXB4. Furthermore, he initiated work on the reprogramming of mouse, marmoset and human cells back to the pluripotent state as a starting point for studies on autologous somatic gene and cell therapy of the haematopoietic system.

Transplantation Medicine

URSULA RAUEN studied Medicine in Düsseldorf and in Aberdeen, Scotland, from 1984 until 1991. She did her internship (AiP) in Tübingen (Department of General Surgery, Eberhard-Karls University) in 1991/92 and received her MD in 1993 (Heinrich-Heine University Düsseldorf) with a thesis on the preservation injury of liver endothelial cells. From 1993 until 2008 she worked as a group leader at the Institute of Physiological Chemistry, University Hospital Essen, where she focused her research on the mechanisms of cold-induced cell injury and the prevention of preservation injury. She received the *venia legendi* in Physiological Chemistry in 2000 with a thesis on cold-induced apoptosis. Since 2008 she is the professor of Physiological Chemistry at the Medical Faculty of the University of Duisburg-Essen and, from 2008-2011, she was a research coordinator of the DFG-funded clinical research unit "Optimisation of Living-Related Liver Transplantation" (KFO 117).

Current research interests focus further on the intracellular mechanisms of cold-induced cell and tissue injury and the mechanisms of preservation and cryopreservation injury. New mechanistical insights have already led to the development of a new cardioplegic/organ preservation solution (currently in its first clinical studies), a vascular preservation solution (approved for clinical use) and a solution for the hypothermic storage of cells for experimental uses. A better understanding of the molecular mechanisms leading to injury or compromising post-storage function is aimed at to provide a basis for the further refinement of the methods for the transport and storage of cells and tissues for clinical (and experimental) purposes.

OLIVER WITZKE studied Medicine at the University Hospital Essen, receiving his medical doctorate in 1996. From 1997 until 1999 he conducted research at the Nuffield Department of Surgery, Transplant Immunology, University of Oxford on tolerance induction after organ transplantation. Back in Germany, from 2000 until 2003, Oliver worked as a researcher at the University Hospital Essen while earning a specialist qualification in internal medicine. In September 2003 he became the senior physician at the Clinic for Renal and Hypertension Diseases in Essen. A further specialist qualification in nephrology was acquired in 2004, followed by the *venia legendi* in Internal Medicine in 2005 with a thesis on the mechanisms of T cell immune tolerance and T cell immune deficiency. In 2006 Oliver was appointed head of the Outpatients' Clinic for Nephrology and Transplantation and, a year later, the deputy medical director of the Clinic for Nephrology, University Hospital Essen. Between 2006 and 2010 Oliver Witzke increased his expertise in the area of infectiology and became increasingly involved in Eurotransplant through the German Transplantation Society (DTG). Since 2011 Oliver is the head of the section for Clinical Infectiology at the University Hospital Essen.

Oliver's main research interests are transplantation immunology and infectiology as well as T cell function under immunosuppression.

MONIKA LINDEMANN studied Medicine at the then University of Essen until 1994. From 1990 until 1992 she did the experimental part of her doctoral thesis at the Institute of Immunology and, from 1994 until 1995, at the Clinic for Internal Medicine (Endocrinology and Nephrology), both at the University Hospital Essen. In 1996 she completed her doctoral thesis on the molecular analysis of tumour necrosis factor gene loci in humans and, thereafter, worked as a research assistant at the Institute of Immunology in Essen. She rotated between Medical Microbiology, Virology and Clinical Chemistry from 1996 until 2001. Since 2001 she is a specialist in Laboratory Medicine. In 1996 she started to focus her research on cellular immunity in immunocompromised patients and, since 1998, she has been working as the group leader of Cellular Immunity. In 2006 Monika Lindemann received the *venia legendi* in Immunology. Since 2009 she has continued her research at the Institute of Transfusion Medicine in Essen where she is currently also a lecturer. In the German Society for Immunogenetics (DGI), Monika Lindemann has been the chairman of the educational committee since 2006 and a board member since 2009. In 2012 she was appointed as a professor. She has received the following specialist training qualifications: Laboratory Medicine (Ärztekammer, 2004), Immunogenentics (DGI, 2006) and Immunology (German Society for Immunology, DGfI, 2011).

Monika Lindemann's main research focus is on transplantation medicine and T cell immunity. She is especially interested in immune transfer via transplantation, cellular vaccination responses in transplant recipients, immune reconstitution after transplantation and *in vitro* markers of rejection.

GRK 1045: Host-Pathogen Interaction

ULF DITTMER

Please refer to the “Chairs” section for this biography.

GRK 1431: Gene Transcription

ANN EHRENHOFER-MURRAY studied Biochemistry at the ETH Zürich, Switzerland. She conducted her graduate research on multidrug resistance proteins in yeast and obtained her PhD in 1994, also from ETH Zürich. From 1994 until 1997 she went to the University of California, Berkeley, USA for her postdoctoral studies on chromatin and DNA replication. She subsequently started an independent research group at the Max-Planck-Institute of Molecular Genetics in Berlin. Ann became an associate professor of Genetics at the Justus Liebig University in 2004 and a full professor of Genetics at the University of Duisburg-Essen in 2005. She has been head of the DFG graduate research training programme “Transcription, chromatin structure and DNA repair in development and differentiation” since 2006.

The research focus of Ann’s group is to understand how cells organise their genetic material in so-called chromatin in the cell nucleus. Differences in chromatin packaging regulate gene expression, which is important for instance for the adaptation of unicellular organisms to changing environmental conditions and for the development of individual cell types within multicellular organisms. Ann’s work has identified key regulators and mechanisms required to establish repressed domains in the eukaryotic genome.

GRK 1739: Radiation Sciences

VERENA JENDROSSEK

Please refer to the “Genetics and Cell Biology” section for this biography.

Selected BIOME Graduate Reports

Molecular mechanisms of radiation-induced normal tissue damage

Federica Cappuccini

Institute of Cell Biology (Cancer Research)
Group: Verena Jendrossek

Radiation-induced pneumonitis and fibrosis constitute dose-limiting side effects of thorax or total body irradiation. *In vivo* investigations suggest that inflammatory responses of the lung tissue involve a complex set of interactions between different resident cell populations, the extracellular matrix (ECM) and infiltrating immune cells. As observed for other fibrotic diseases, an impaired balance between inflammation and repair may be causative for extensive remodelling processes and finally fibrosis. However, it is still controversial whether the inflammatory reaction is a prerequisite for progression to lung fibrosis. Previous studies in our lab showed that the lack of the death receptor CD95 or its ligand prevents radiation-induced lung inflammation (Henzzelmann *et al.*, JNCI 2006). Moreover, it was found that radiation triggers a delayed damage to resident cells, as higher albumin levels and caspase-3/7 activation in the BALF (bronchoalveolar lavage fluid) of irradiated mice were observed at day 21 (Cappuccini, Eldh *et al.*, Radiotherapy and Oncology, 2011).

An improved understanding of the mechanisms linking the initial tissue injury to the inflammatory response and fibrosis development is a prerequisite for developing effective radioprotective strategies. Therefore, considering that the network of pathologic events leading to tissue inflammation and fibrosis is not completely understood, the aim of the present thesis is to define cellular and molecular changes that promote radiation-induced pneumonitis and fibrosis in a murine model of radiation-induced pneumopathy.

For this purpose C57BL/6 or specific genetically modified mice (e.g. RAG2$^{-/-}$) receive 15 Gray (Gy) of thoriac irradiation in a single dose. Lung tissue is collected over 30 weeks after irradiation and used for FACS staining to characterise the (pheno)type of the cell populations or, alternatively, for histological and immunohistochemical analyses of molecular and morphological changes.

Interestingly, as an early result, the analysis of lung sections at day 21 revealed radiation-induced changes in the morphology of resident cells. In particular, irradiation triggered a time-dependent formation of lipid-loaded macrophages and endothelial cells (Figure 1), suggesting a disturbance of lipid metabolism in the

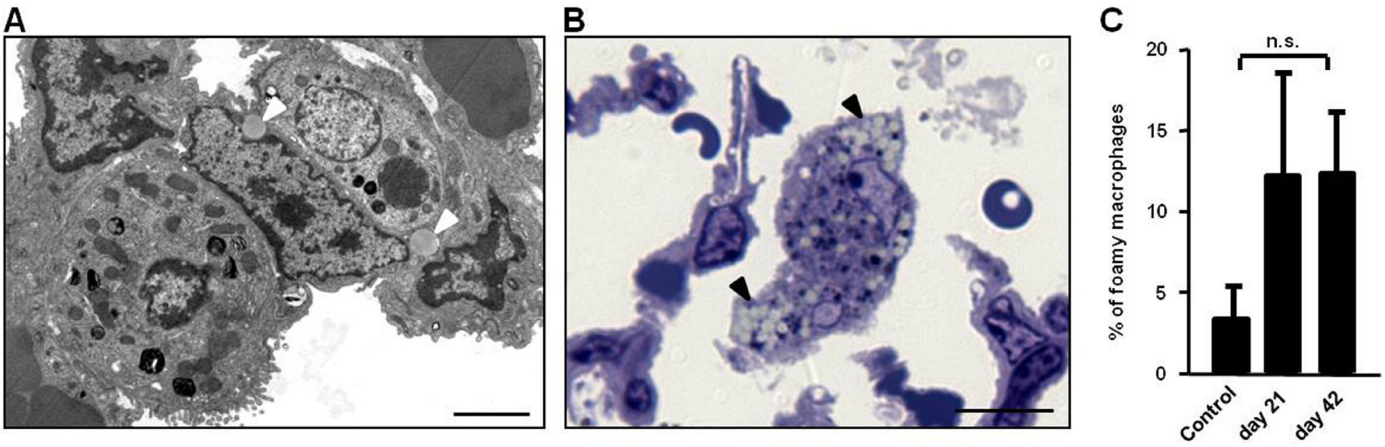


Figure 1: Irradiation triggers the formation of lipid-loaded resident lung cells: WT mice received 0 Gy (sham-control) or 15 Gy hemithorax irradiation. A) Electron microscopy picture of a lipid-loaded endothelial cell at day 21 post-irradiation (white arrows: lipid droplets; scale bar: 2 µm). B) Semi-thin section (0,5 µm) showing a lipid-loaded macrophage at day 42 post-irradiation (black arrows: lipid droplets; scale bar: 10 µm). C) Quantification of lipid-loaded macrophages at day 21 and day 42 in semi-thin slices. Data represent the mean percentage ± SD in lipid-loaded macrophages on the total macrophage number (=100%) obtained in ≥ 3 different sections/value; 2 mice were analysed per condition (n.s., no significant difference; two-tailed unpaired t test). (Adapted from Cappuccini, Eldh *et al.*, Radiotherapy and Oncology, 2011).

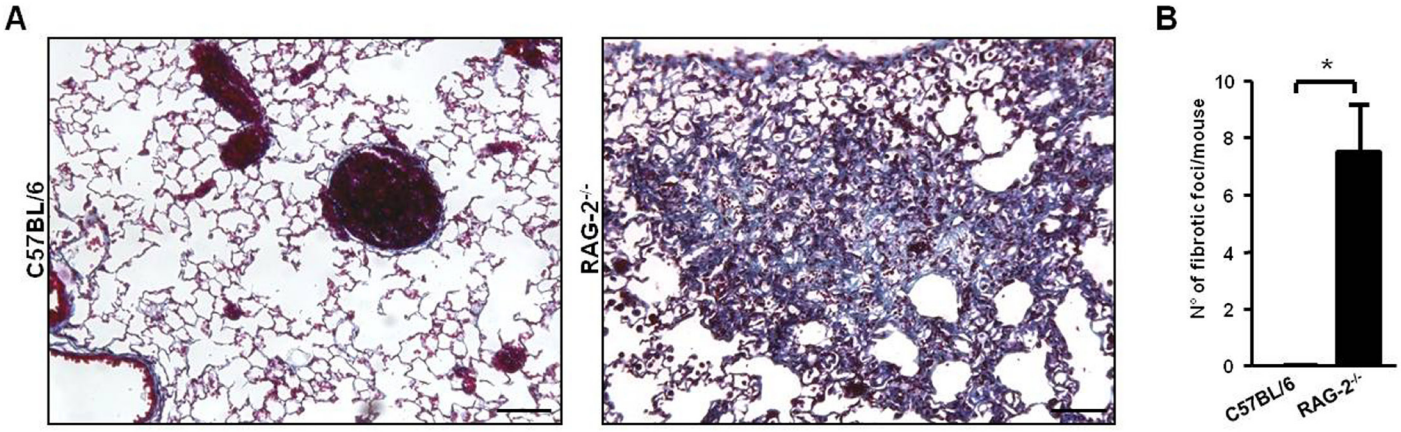


Figure 2: Lack of mature T and B lymphocytes sensitizes to radiation-induced lung fibrosis: RAG-$2^{-/-}$ and C57BL/6 wild type mice received 0 Gy (sham-control) or 15 Gy whole thorax irradiation. Histological analysis was performed in Masson-Goldner Trichrome stained lung sections 24 weeks after irradiation (scale bars: 100 µm). (A) Representative light microscopy pictures of Masson-Goldner Trichrome stained lung sections; (B) Quantification of fibrotic foci in irradiated mice 24 weeks after irradiation. Data represent the mean number of foci counted in ≥ 3 sections (2 mice per condition) (*significant difference $p \leq 0.05$; two-tailed unpaired t test). (Adapted from Cappuccini, Eldh *et al.*, Radiotherapy and Oncology, 2011).

irradiated lung tissue during the pneumonitic phase. At present, the phenotype of the macrophages is being analysed in more detail.

FACS staining of cells obtained from digested lung tissue showed a time-dependent increase in the total number of leukocytes after irradiation. Moreover, in despite of an unaffected percentage of $CD4^+$ T cells at day 21, C57BL/6 irradiated mice presented an higher percentage of activated $CD4^+$ T cells and a significant increase in the percentage of $CD4^+/CD25^+/Foxp3^+$ T cells.

To gain more insight into the role of T lymphocytes, we analysed whether the late effects of thorax irradiation would be altered in RAG-$2^{-/-}$ mice. Due to a total inability to initiate V(D)J rearrangement, RAG-$2^{-/-}$ mice fail to generate mature T and B lymphocytes. Experiments performed on this mouse strain revealed that the loss of mature T and B cells lead to an earlier onset of fibrosis-associated histological changes upon irradiation (24 weeks) compared to the control mice with normal mature T and B cells (Figure 2) (Cappuccini, Eldh *et al.*, Radiotherapy and Oncology, 2011).

Other preliminary data indicate that thorax irradiation triggers changes in the equilibrium between different recruited or resident immune cells, as well as micro-environmental alterations that may promote the change in the phenotype of the immune cells in the lung tissue (unpublished data).

Taken together, the present results from this project demonstrate that radiation-induced tissue damage is associated with a delayed disturbance of tissue integrity and a massive recruitment of inflammatory cells. Importantly, mature lymphocytes are necessary to suppress fibrotic changes.

Current investigations are focusing on the characterisation and on the role of alveolar macrophages, as well as infiltrating leukocytes to shed light on the interactions between the innate and adaptive immunity and their functional relevance for the onset/resolution of inflammation and/or fibrosis in response to ionising radiation.

Expression control of ER aminopeptidases in melanoma cells and its influence on CD8+ T cell epitope generation

Christin Seifert

Clinic of Dermatology
Group: Annette Paschen

Malignant melanoma, the black skin cancer, can be selectively destroyed by autologous CD8+ T lymphocytes. The selectivity is provided by the interaction between the T cell receptor (TCR) and specific surface complexes, consisting of antigenic peptides bound to MHC class I molecules. The antigenic peptides originate from the degradation of tumour proteins, so-called tumour antigens (Figure 1). In general, the multi-catalytic proteasome complex initiates the cleavage of tumour antigens into peptide fragments of different length, thereby defining the C-terminus for the majority of peptides presented by MHC class I molecules. While some of the proteasome products are of the correct size for direct MHC class I binding others are N-terminally elongated peptide precursors that require further trimming by aminopeptidases. Currently two human ER-localised aminopeptidases are known, ERAP1 and ERAP2.

While ERAP1 is known to be of specific relevance for the efficient generation of viral and parasitic antigenic peptides, the importance of the peptidase in processing tumour-associated peptide epitopes has not been determined. Thus, the aim of this thesis was to define the role of ERAP1 in antigen presentation by melanoma cells. As a working model, the autologous tumour-CD8+ T cell system of melanoma patient Ma-Mel-86 was selected. In the cell line Ma-Mel-86a, established from a metastasis of this patient, ERAP1 expression was stably down-regulated by shRNA. Importantly, ERAP1 knockdown did not influence the overall MHC class I surface expression level. To determine the effect of reduced ERAP1 expression in tumour cells on antigen presentation, autologous CD8+ T cells derived from peripheral blood were initially stimulated for several times with Ma-Mel-86a cells. Subsequently, the bulk CD8+ T cells were analysed for their capability to recognise Ma-Mel-86a in comparison to ERAP1 shRNA transfected Ma-Mel-86a melanoma cells. Interestingly, ERAP1 down-regulation impaired the recognition of Ma-Mel-86a melanoma cells by autologous bulk CD8+ T lymphocytes, suggesting that reduced ERAP1 activity led to an alteration of the antigenic epitope repertoire presented by MHC class I molecules. Diminished ERAP1 expression also decreased the recognition of the tumour cells by autologous CD8+ T cell clones of different specificities.

Due to the important role of ERAP1 in epitope presentation by melanoma cells the expression profiles of ERAP1 and ERAP2 as well as their regulation in several cell lines were analysed. Interestingly, the cell lines strongly differed with respect to their ERAP1 and ERAP2 expression levels and with respect to the regulation of ERAPs by Interferon-γ.

The results of this thesis demonstrate that ERAP1 plays an important role in the generation of tumour antigen epitopes in melanoma cells. Thus, it is assumed that a heterogeneous ERAP expression in metastasis of tumour patients is associated with qualitative alterations in the antigenic MHC class I peptide repertoire. This effect could influence the efficiency of epitope-specific tumour therapies such as the adoptive transfer of TCR-transgenic CD8+ T cells and should be taken into consideration for therapy design.

The results of this thesis were selected for oral presentation on following meetings / congresses:

- 8th Cancer Immunotherapy Annual Meeting , 2010, Mainz
- 16th International AEK Cancer Congress, 2011, Düsseldorf
- IRUN Symposium on Immune Recognition of Pathogens and Tumours, 2011, Mühlheim an der Ruhr

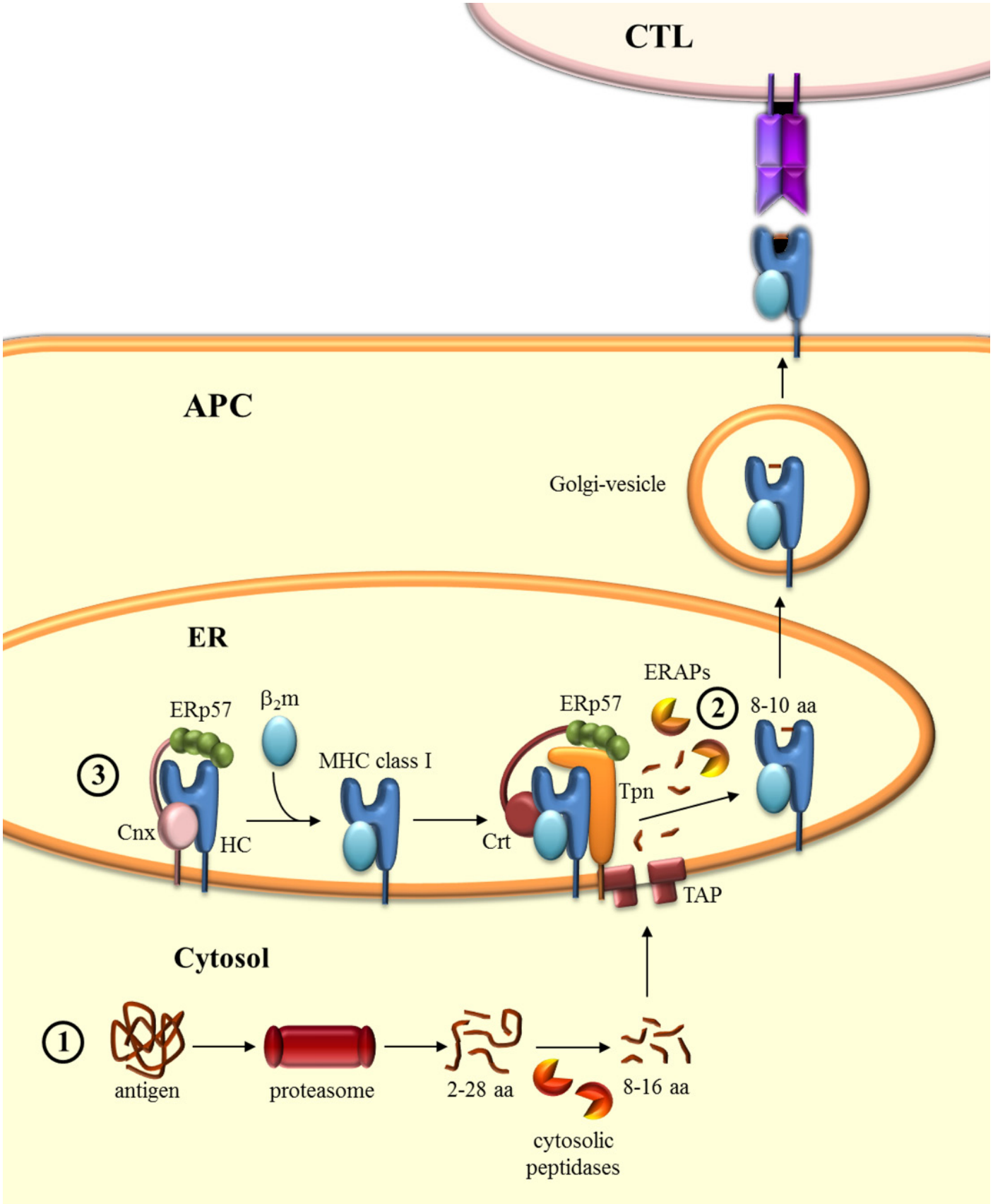


Figure 1: The MHC class I antigen processing und presentation pathway. Antigen processing in the cytosol (1), antigen processing in the ER (2), MHC class I assembling in the ER (3), cell surface presentation of the peptide-MHC class I complex (4). Antigen presenting cell (APC), amino acid (aa), β2-microglobulin (β2m), calnexin (Cnx), calreticulin (Crt), endoplasmic reticulum (ER), ER aminopeptidases (ERAPs), major histocompatibility complex class I (MHC class I), heavy chain of the MHC class I molecules (HC), tapasin (Tpn), transporter associated with antigen processing (TAP), T cell receptor (TCR), cytotoxic T-lymphocyte (CTL).

Functional analyses of haematopoietic CD133^{+}CD34^{+} and CD133$^{low/-}$CD34^{+} cells challenge the existence of human common myeloid progenitor cells

André Görgens

Institute of Transfusion Medicine
Group: Bernd Giebel

Haematopoietic stem cells (HSC) are required to sustain the daily production of hundreds of millions of specialised blood cells fulfilling different functions. Since HSCs have been used for clinical applications for more than 40 years now, they represent the best-studied somatic stem cell entity so far. However, there are many unresolved questions regarding the mechanisms that control their development including their differentiation[1].

According to the classical model of human hematopoiesis, HSCs give rise to multipotent progenitor cells (MPPs), the ancestors of lineage restricted common lymphoid and common myeloid progenitor cells (CLPs and CMPs). CLPs are proposed to further sustain T cell, B cell and Natural Killer (NK) cell differentiation potentials, while CMPs give rise to granulocyte/macrophage progenitors (GMPs) as well as megakaryocyte/erythrocyte progenitors (MEPs) (Figure 1).

Some recent findings, which identified lymphoid-myeloid primed progenitors (LMPPs) in mouse and man, have challenged this model. For example, cells have been identified with partial myeloid and lymphoid potentials, which had lost their potential to form erythroid/megakaryocytic cells[2–4].

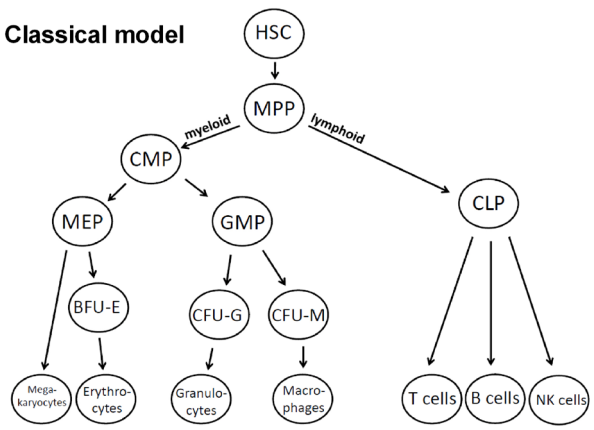


Figure 1: The classical model of human hematopoiesis

Previous results of our group showed that upon cultivation, umbilical cord blood derived CD133^{+}CD34^{+} cells segregate into a CD133^{+}CD34^{+} (CD133^{+}) and a newly arising CD133$^{low/-}$CD34^{+} (CD133^{low}) cell population (Figure 2A). Apart from identifying differentially expressed cell surface antigens which we could associate with asymmetric cell divisions, our work showed that primitive haematopoietic cells revealing long-term myeloid potentials exclusively resided within CD133^{+} cell populations[5].

Since we could associate CD133 expression with asymmetric cell divisions, we wondered whether CD133 also reflects lineage decisions during human HSPC development. Thus, we decided to analyse the developmental potential of both CD133 subpopulations in more detail.

We first tested for long-term lymphoid potentials (using the NK-initiating cell *in vitro* assay) and tested for cells which upon transplantation are able to reconstitute the haematopoietic system of immune-compromised NOD/SCID-mice (SCID-repopulating cells, SRCs). Similar to HSPCs with long term myeloid potentials, HSPCs with long-term lymphoid potentials (NK-ICs) as well as SRCs exclusively derived from CD133^{+} cell fractions as well (Figure 2A).

Next, we tested both CD133 subpopulations in clonogenic myeloid differentiation assays, the so-called colony-forming-cell (CFC) assays. The results obtained (examples of different colonies are depicted in Figure 2B) revealed that erythro-myeloid progenitors, which create CFU-MIX colonies, are concentrated within the CD133^{low} cell population. Furthermore, CD133^{+} cell

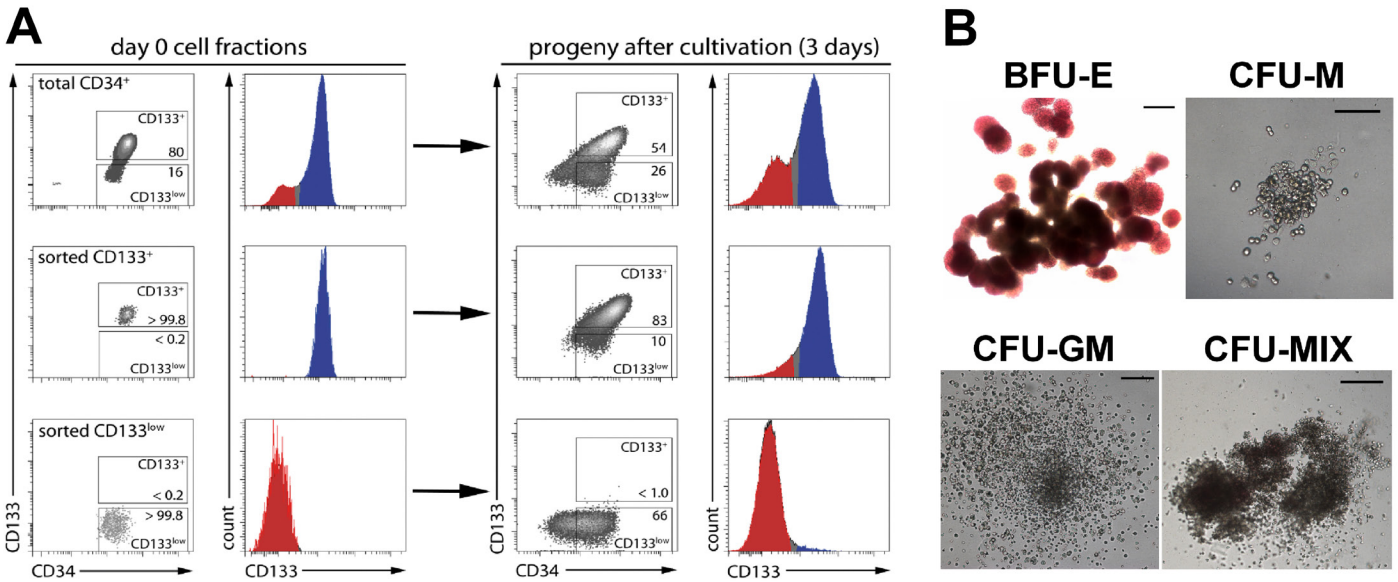


Figure 2: (A) Definition of CD133 subpopulations of fresh and cultured CD34+ cells which were purified by flow-cytometrical sorting. **(B)** Examples of different types of haematopoietic colonies obtained in colony-forming-cell (CFC) assays.

fractions completely lost CFU-MIX potentials upon cultivation. Strikingly, we found the CFU-GM colonies within the CD133+ cell population. Since we never observed CD133low cells regaining their CD133 expression, our data strongly supports that, in contrast to the classical model, CFU-MIXs, the proposed CMP analogs, and CFU-GMs arise from independent branches of the haematopoietic tree.

Since CFU-GM as well as CFU-MIX colonies contained granulocytes, we next characterised them in more detail. The resulting data revealed that CFU-GM colonies exclusively contain neutrophils and CFU-MIX colonies exclusively basophils and eosinophils.

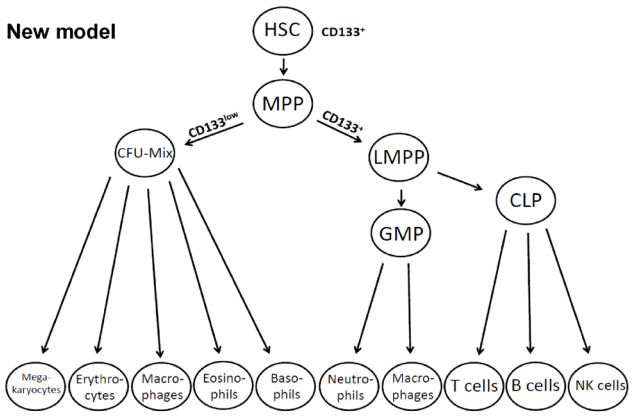


Figure 3: New model of human hematopoiesis

In summary, our data are not in line with the classical model of hematopoiesis and rather support a myelo-based model of human hematopoiesis, which predicts that lympho-myeloid and erythro-myeloid progenitors derive from multipotent HSCs/MPPs and challenges the existence of proposed human CMPs (Figure 3).

References

1. Görgens, A. & Giebel, B. Self Renewal of Primitive Hematopoietic Cells: A Focus on Asymmetric Cell Division - Chapter 3 in Umbilical Cord Blood: A Future for Regenerative Medicine, edited by S. Kadereit and G. Udolph. 51-75 (2010).
2. Adolfsson, J. *et al.* Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* **121**, 295-306 (2005).
3. Giebel, B. & Punzel, M. Lineage development of hematopoietic stem and progenitor cells. *Biol Chem* **389**, 813-24 (2008).
4. Kawamoto, H. *et al.* A map for lineage restriction of progenitors during hematopoiesis: the essence of the myeloid-based model. *Immunol. Rev.* **238**, 23-36 (2010).
5. Beckmann, J. *et al.* Asymmetric cell division within the human hematopoietic stem and progenitor cell compartment: identification of asymmetrically segregating proteins. *Blood* **109**, 5494-501 (2007).

Rho GTPase signalling in U2OS osteosarcoma cell migration

Nina Schulze

Department of Molecular Cell Biology, ZMB
Group: Perihan Nalbant

Cell migration is essential for many fundamental biological processes such as embryonic development, tissue regeneration and an effective immune response. However, in pathological situations such as chronic inflammatory diseases and tumour metastasis many regulatory processes are defective and lead to aberrant migratory behaviour. Rho family GTPases are pivotal modulators of cell migration by regulating the actin cytoskeleton as well as adhesion organisation (Raftopoulou and Hall, 2004). Rho GTPases cycle between an inactive GDP-bound and an active GTP-bound state. The activating nucleotide exchange leads to a conformational change which enables the binding of downstream effector proteins. The activity of Rho proteins is tightly controlled by several types of regulators: the guanine nucleotide exchange factors (GEFs) facilitate the activating nucleotide exchange, whereas GTPase activating proteins (GAPs) inactivate the protein by catalysing the hydrolysis of the bound GTP. Today it is known that aberrant regulation of Rho GTPases contributes to cancer progression and many GEFs and GAPs are described as proto-oncogenes (Vigil *et al.*, 2010).

One of our major goals is to identify RhoGEFs involved in cancer cell migration. A database analysis showed that so far 83 human proteins are thought to act as

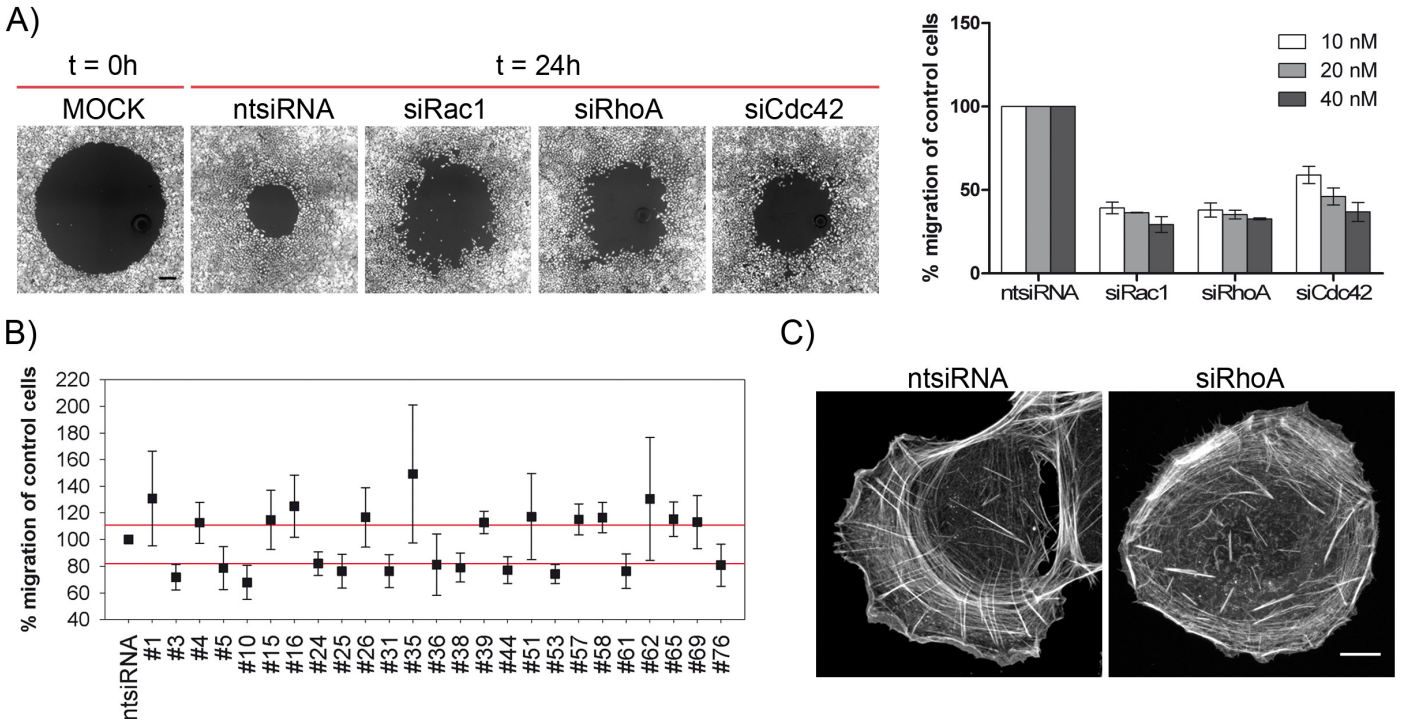


Figure 1: (A) Establishment of the *in vitro* wound healing assay using siRNAs targeting Rac1, RhoA and Cdc42. Left: Representative images are shown depicting a newly generated wound (t=0) and siRNA treated cells 24 h after wounding. Right: Different concentrations of siRNAs were tested and migration efficiency was calculated relative to ntsiRNA. **(B)** In the initial screen 25 GEFs were identified as hits. The migration efficiency (mean ± SD) of GEF-depleted relative to ntsiRNA treated cells of four independent experiments is shown. Red lines indicate upper and lower thresholds for identification of a hit which was calculated by the plate ± SD method. **(C)** Representative confocal images showing the actin cytoskeleton of U2OS osteosarcoma cells treated with ntsiRNA or RhoA siRNA. Scale bar, 10 µm.

GEFs for the 20 known Rho GTPases. To characterise their contribution to cell migration in tumour cells each individual known RhoGEF was depleted using RNAi in U2OS osteosarcoma cells and investigated in an *in vitro* wound healing assay. In order to have a reproducible assay with defined conditions such as wound size, we used the 96-well plate Oris™ cell migration assay (Platypus Technologies). To quantify the migration efficiency of the cells we utilised automated microscopic image acquisition and quantitative image analysis with ImageJ. The conditions for the screen such as siRNA amount, incubation time and migration duration were optimised by use of siRNAs targeting the three best characterised Rho GTPases Rac1, RhoA and Cdc42 (Figure 1A). The primary screen was performed using a mix of four different oligonucleotides per target gene and was repeated four times. The migration efficiency of the GEF-depleted cells was compared relative to ntsiRNA treated cells. GEFs were listed as hits when the migration efficiency was above or below the set threshold (calculated by using the plate mean ± SD method). In the initial screen we were able to identify 25 proteins as hits. Twelve of these showed an inhibition of cell migration, whereas the depletion of 13 GEFs resulted in an accelerated migration (Figure 1B). Currently, a secondary screen using the individual oligonucleotides from the initial siRNA mixtures is being performed in order to exclude unspecific off-target effects. Detailed analysis of the actin cytoskeleton in GEF depleted cells will help to understand the underlying reason for the migration defect. This is shown exemplary for the depletion of RhoA in Figure 1C. In particular, we will focus on analysis of actin dynamics as it is known that continuous and coordinated actin remodeling is essential for proper cell migration.

In order to understand how the spatio-temporal activity patterns of Rho GTPases are translated into distinct cellular behaviours, we are focusing on effector proteins of the formin family. Most formins are well known modulators of the actin cytoskeleton and thought to act as effector proteins downstream of Rho GTPases (Chesarone *et al.*, 2010). The mammalian formin FHOD1 (formin homology 2 domain containing 1) shares a similar domain organisation with other formins suggesting a role in cellular actin dynamics as well. Indeed, activated mutants of FHOD1 strongly associate with actin filaments and enhance stress fibre formation (Gasteier *et al.*, 2005). However, knowledge of FHOD1 function as well as its regulation by Rho GTPase proteins in cellular context is still fragmented. We have established RNAi-mediated depletion of the protein in U2OS cells to gain a more detailed understanding of the cellular role of FHOD1 and found a significant decrease of cell migration in cells lacking the protein. Currently, underlying mechanisms leading to this migration defect are being investigated.

References

1. Chesarone, M.A., DuPage, A.G., and Goode, B.L. Unleashing formins to remodel the actin and microtubule cytoskeletons. *Nat. Rev. Mol. Cell Biol.* **11**, 62-74 (2010).
2. Gasteier, J.E., Schroeder, S., Muranyi, W., Madrid, R., Benichou, S., and Fackler, O.T. FHOD1 coordinates actin filament and microtubule alignment to mediate cell elongation. *Exp. Cell Res.* **306**, 192-202 (2005).
3. Raftopoulou, M. and Hall, A. Cell migration: Rho GTPases lead the way. *Dev. Biol.* **265**, 23-32 (2004).
4. Vigil, D., Cherfils, J., Rossman, K.L., and Der, C.J. Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? *Nat. Rev. Cancer* **10**, 842-857 (2010).

Aberrant mural cell recruitment to lymphatic vessels and impaired lymphatic drainage in a murine model of pulmonary fibrosis

Anna-Katharina Meinecke

Institute of Physiology,
Group: Christian Stockmann

Idiopathic Pulmonary Fibrosis (IPF) is a chronic and progressive lung disease with increasing mortality that ultimately leads to ventilatory restriction and respiratory failure within 3-5 years from the time of diagnosis (Olson *et al.*, 2007). Pulmonary fibrosis is characterised by formation of fibrotic foci and extensive remodeling of the lung parenchyma (Selman and Pardo, 2006). However, the events that trigger this process remain enigmatic and there has been little progress in the development of therapeutic strategies for this disease, owing to the fact that its pathogenesis is poorly understood. Therefore, elucidating the pathogenic pathways that underlie the development of pulmonary fibrosis will help to identify novel therapeutic approaches with an improved clinical outcome.

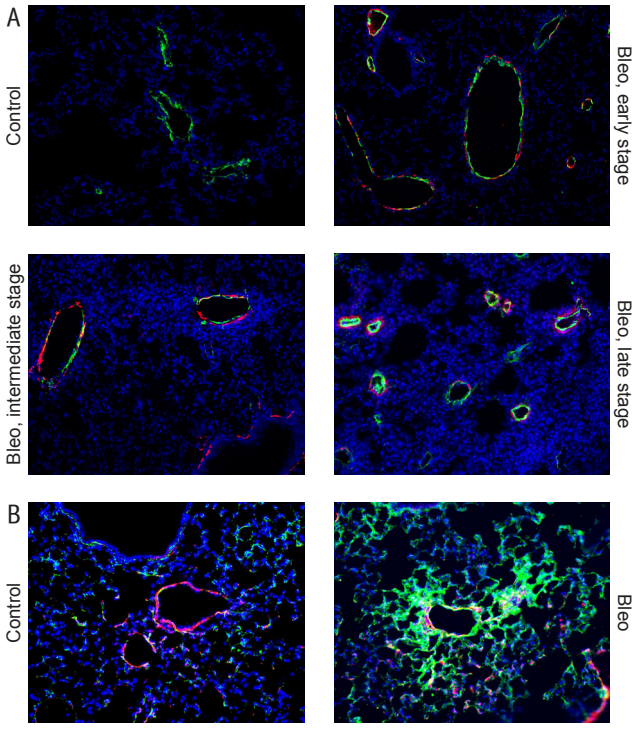


Figure 1: (A) Immunodetection of mural cells (SMA) and Lyve-1-positive vessels in fibrotic lungs of mice treated with bleomycin (Bleo) (10 mg/kg body weight twice a week i.p.) and in healthy controls of mice treated with PBS (100 µl twice a week i.p.) at day 28. Fibrosis is shown at early, intermediate and late stage. **(B)** Immunodetection of basement membrane (Laminin) and lymphatic vessels (VEGFR3) on lung sections of bleomycin-treated and PBS-treated mice at day 28. Scale bars equal 100 µm.

Remodeling of the pulmonary vasculature is a hallmark observation during pulmonary fibrosis (El-Chemaly *et al.*, 2009). It is well established that the lymphatic vasculature is essential for the removal of interstitial fluid and the maintenance of tissue homeostasis and recent studies highlight that pulmonary lymphatics undergo remodeling during pulmonary fibrosis (Petrova *et al.*, 2004, Aukland and Reed, 1993). However, the mechanisms that underly this remodeling process, its consequences on lymphatic vessel function as well as the impact on fibrotic changes in the lung remain to be elucidated. Therefore, we analysed the remodeling of pulmonary lymphatics, its effect on lymphatic drainage and lung tissue homeostasis in a murine model of bleomycin-induced pulmonary fibrosis. In our study we conducted a structural comparison of lymphatic vessels in healthy control lungs and fibrotic lungs in a murine model of pulmonary fibrosis. Here, we show that lymphatics exhibit ectopic mural cell coverage and excess basement membrane deposition (Figures 1A and 1B).

Aberrant recruitment of mural cells occurs early during the development of pulmonary fibrosis and the abnormal lymphatic vascular patterning in fibrotic lungs is driven by ectopic expression of platelet-derived growth factor B (PDGF-B) in lymphatic endothelial cells and signalling through platelet-derived growth factor receptor-β (PDGFR-β) in associated mural cells. The aberrant mural cell coverage of lymphatic vessels fostered the accumulation of both, macromolecular protein and hyaluronic acid as well as fibroblasts within the perilymphatic space, indicating a decrease in permeability and lymphatic transport. In a functional assay we were able to confirm impaired lymphatic drainage that was restored by inhibition of PDGF-B/PDGFR-β signalling. Taken together, our results indicate that aberrant mural cell recruitment to lymphatic vessels and impaired lymphatic drainage are critical events in the onset of pulmonary fibrosis and that targeting these processes has the potential to ameliorate pulmonary fibrosis.

The role of tissue-plasminogen activator in regulating ATP-binding cassette transporters at the ischaemic blood-brain barrier

Hripsime Snkhchyan

Department of Neurology, Vascular Neurology and Dementia Group: Dirk M. Hermann

The blood-brain barrier (BBB) plays a crucial role in brain function and protection by narrowly regulating brain homeostasis and microenvironment (Rubin, 1999). The BBB is formed by endothelial cells that tightly interact with pericytes, astrocytes, neurons and the extracellular matrix (ECM), forming the neurovascular unit (NVU) (Abbott *et al.*, 2006). The BBB constitutes a physical barrier due to the presence of specialised tight junctions between endothelial limiting the passage of blood-prone molecules into the brain (Hawkins and Davis, 2005). In parallel, the BBB is complemented by several transport and exchange systems, among which drug transporters belonging to the ATP-binding cassette (ABC) transporter family (Miller, 2010).

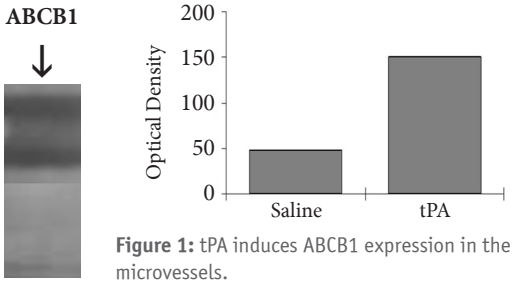


Figure 1: tPA induces ABCB1 expression in the cerebral microvessels.

Ischaemic stroke induces BBB breakdown by deregulating tight junctions between brain endothelial cells, leading to brain edema formation and neuronal injury exacerbation (Hawkins and Davis, 2005). Moreover, the BBB represents a big challenge in stroke pharmacotherapies by preventing the entry of neuroprotective compounds into the ischaemic brain (Hermann and Bassetti, 2007). Our group showed that ischaemic stroke differentially induced ABC transporter regulation at the BBB (Spudich *et al.*, 2006; Kilic *et al.*, 2008; ElAli and Hermann, 2010). Upon stroke the expression of ABCB1, which impedes drugs entry into the brain, is enhanced at the luminal side (i.e. blood facing side) of the BBB (Spudich *et al.*, 2006), whereas the expression of ABCC1, which facilitates drugs entry into the brain, is reduced at the abluminal side (i.e. brain facing side) (Kilic *et al.*, 2008). The low density lipoprotein (LDL) related protein 8 (LRP8, i.e. ApoER2) has been shown to be involved in this differential regulation (ElAli and Hermann, 2010).

The systemic administration of tissue-type plasminogen activator (tPA) within a restricted time window is the only Food and Drug Administration (FDA) approved treatment for stroke. Recently, it has been shown that tPA contributes in BBB breakdown and neuronal injury after stroke (Yepes *et al.*, 2009) via mechanisms involving LRP1 that acts as a receptor for tPA at the BBB. Many neuroprotective compounds promoting stroke recovery were tested in human patients in the past, but unfortunately without breakthroughs. Strikingly, the role of systemic tPA administration on ABC transporter regulation at the BBB upon stroke have never been investigated. Therefore, in our project we are interested in verifying whether and how tPA would influence ABC transporter expression at the BBB upon stroke, namely ABCB1 and ABCC1, which could have a deep impact on brain drug pharmacotherapies.

To elucidate this question, we performed preliminary screening experiments by using C57BL/6 adult mice which were subjected to a 30 min middle cerebral artery (MCA) occlusion using an intraluminal filament technique (ElAli and Hermann, 2010). These animals were treated with either 200 µL vehicle (0.9% normal saline), or tPA (10 mg/kg; Actilyse, Boehringer- Ingelheim), which was administered immediately after reperfusion via the common carotid artery (Zechariah *et al.*, 2010). Microvessels were isolated using dextran gradient separation technique (ElAli and Hermann, 2010), lysated and subjected to 7% SDS-PAGE electrophoresis and Western blot analysis for ABCB1.

Our preliminary screening experiments showed that tPA indeed induces ABCB1 expression in brain microvessels. The next step would be exploring the molecular mechanisms involved in tPA-induced ABCB1 transporters regulation at the BBB after stroke by deciphering LRP1 signalling and processing at the BBB. Therefore, by understanding the role of tPA in regulating ABC transporters at the BBB, we hope to provide new approaches that aim to enhance stroke therapy strategies.

The local T cell response in tumour formation: biological role of cytotoxic T cells and regulatory T cells

Ilseyar Akhmetzyanova

Institute of Virology
Group: Ulf Dittmer

Despite a considerable proportion of medical research devoted to understanding cancer and its causes, the outcome of cancer is still characterised by uncontrolled tumour growth and dissemination of tumour cells to different organs. Approximately 12% of all human cancers worldwide are associated with oncogenic viruses. The incidence of virus-related malignancies, particularly lymphomas, is influenced by environmental and host co-factors. For example, human hepatitis B virus and human hepatitis C virus are associated with 80% of carcinomas of the liver, Epstein-Barr Virus is associated with 30% of Hodgkin's lymphomas and human papillomavirus is positive in >95% of carcinomas of the cervix of the uterus. During the last three decades significant research progress has been made toward understanding the role of these pathogenic viruses in cancer which are now considered in some cases as the driving forces of tumour growth and proliferation. Nowadays it is quite clear that the immune system in some patients with oncoviral infection cannot completely control tumour development and protect against its progression and metastasis. However, the host immune system in tumour control remains the main native protection, especially with a well-defined etiological factor (viral).

Therefore, the aim of our study is to analyse the T cell immune responses that are essential in the local tumour microenvironment. We focused our study on the biological role of cytotoxic T cells that control tumour growth and regulatory T cells (Tregs) that counteract anti-tumour immune response. FBL-3 cells are a Friend retrovirus (FV)-induced tumour cell line of mouse origin. FBL-3 cells do not produce infectious FV, but represent a highly immunogenic virus-transformed tumour cell line that expresses immunogenic FV antigens. FBL-3 tumours grow locally before being rejected due to the $CD8^+$ T cell response and the depletion of $CD8^+$ T cells leading to progressive tumour growth and the death of mice. Tumour-specific $CD4^+$ T cells seem to be less important for tumour rejection in this model. The local interaction of tumour cells and different populations of immune cells during tumour formation and rejection was studied in this model by exploiting the draining lymph nodes compared to non-draining lymph nodes. We show here that there is an expansion of FV-specific $CD4^+$ T cells producing cytokines and cytotoxic molecules in the early phase of tumour growth. Importantly, although $CD4^+$ T cells cannot control tumour development in the absence of $CD8^+$ T cells, additional ablation of Tregs enabled the mice to again reject the tumour. This dual treatment also augments production of the cytokines and cytotoxic molecules by $CD4^+$ T cells and increases FV-specific $CD4^+$ T cell and cytotoxic responses. Therefore, Tregs play a pivotal role in modulating $CD4^+$ T cell responses during tumour formation. The capacity to reject tumours acquired by tumour-reactive $CD4^+$ T cells largely depends on the direct suppressive activity of regulatory T cells. Collectively, these data suggest that $CD4^+$ T cells can gain cytotoxic activity against tumour cells when $CD8^+$ T cells are inactive, but this activity is tightly controlled by Tregs during tumour rejection. This study addresses a matter of urgent need by providing deeper insights into immune mechanisms of T cell interaction during tumour formation.

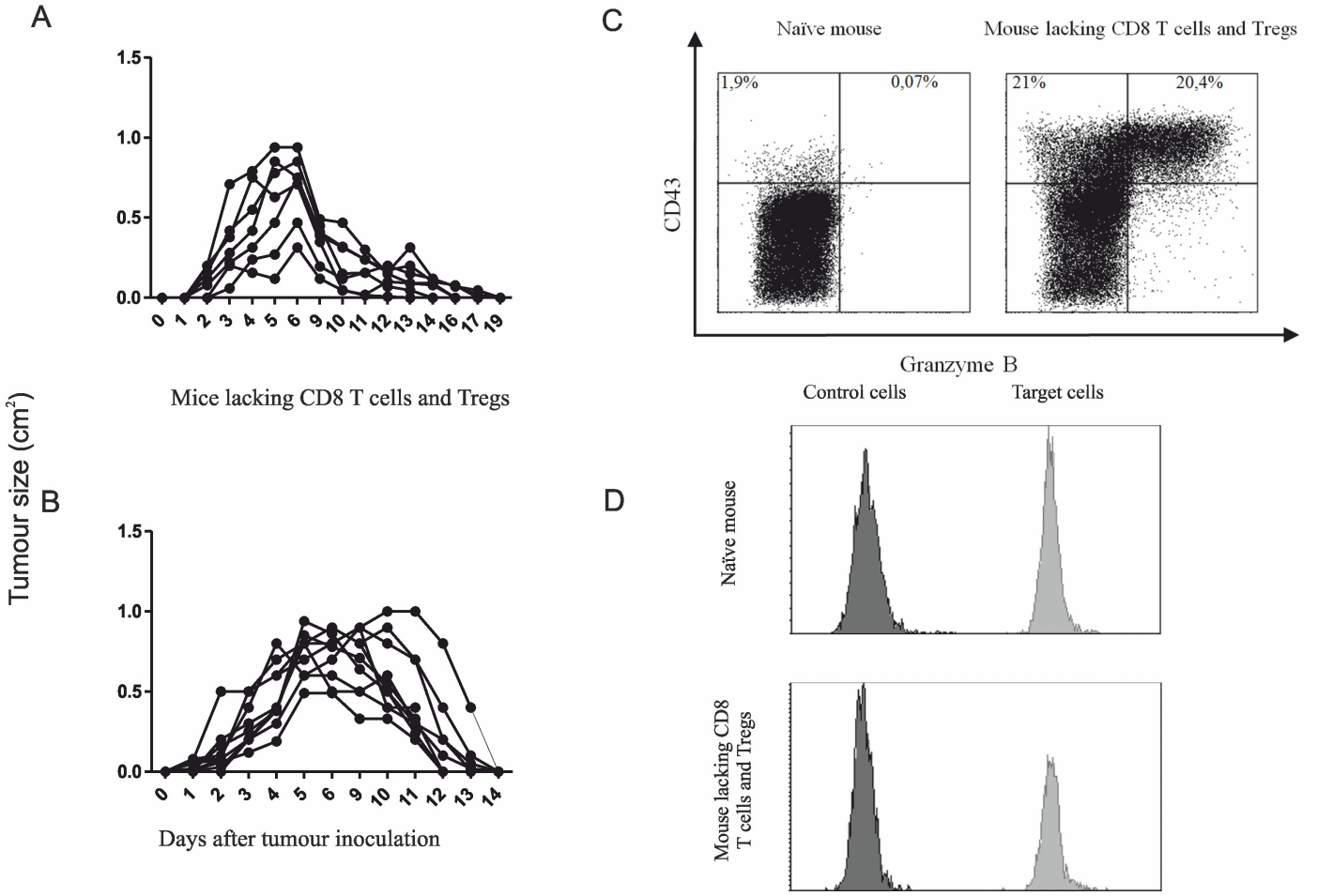


Figure 1: A, B: Kinetics of tumour growth in non-depleted mice (A) and mice depleted of $CD8^+$ and regulatory T cells (B). **C, D:** Lymphocytes from draining lymph nodes analysed by flow cytometry C: A representative dot plot of acvtivated (positive for the activation–induced isoform CD43) $CD4^+$ T cells producing the cytotoxic molecule, granzyme B, in different treatment of mice. **D:** Representative histograms of *in vivo* cytotoxic T-lymphocytes assay. Control and target cells (cells that are supposed to be eliminated by $CD4^+$ T cells) were transferred into tumour-bearing mice. Two hours later, lymphocytes were isolated from the draining lymph nodes and analysed by flow cytometry to determine the percentage of remaining target cells.

Regulation of melanoma cell ligands binding to the activating immune receptor NKG2D

Anja Heinemann

Department of Dermatology
Group: Annette Paschen

Malignant melanoma is the most serious form of skin cancer which arises from pigmented cells (melanocytes) mostly located in the skin. With an incidence increasing at a rate of 2-5% per year, cutaneous melanoma is an international scourge that disproportionately targets young individuals. Melanocytes are the cells that make melanin, which gives skin its color. Melanin also protects the deeper layers of the skin from the sun's harmful ultraviolet (UV) rays. Upon high doses of UV light, the melanocytes may begin to grow abnormally, become cancerous and transform into a melanoma. Despite much research, the treatment of the advanced disease is still quite challenging and immunotherapy is one of the strategies.

In contrast to normal cells, melanoma cells express a variety of different surface molecules. Among these molecules are the ligands of the immunoreceptor NKG2D (Natural Killer Group 2, member D). NKG2D is an important activating NK cell receptor, which also functions as a co-stimulatory receptor on cytotoxic T cells (CTLs). Ligands of NKG2D are surface molecules, structurally related to classical MHC class I molecules that in humans either belong to the MIC (MICA, MICB) or the ULBP (ULBP1-6) molecule family. NKG2DL have been detected on tumour cells from different entities, sensitizing them to killing by NK cells and CTLs. However the mechanisms that control their expression in cancer cells are largely unknown.

Recently, the NKG2DL ULBP2 was identified as a prognostic marker for malignant melanoma, whereby the question about its regulation arises. The fact that mRNA expression of NKG2DL has been detected in several normal tissues that lack NKG2DL proteins points towards a tight regulation of mRNA translation. miRNAs are small noncoding RNAs, which recently emerged as central regulators of mRNA degradation and/or translation, due to their capability to bind to complementary sequences within the 3´-untranslated region (3´-UTR) of specific mRNAs.

Focusing on post-transcriptional mechanisms, this study provides evidence that the tumour-suppressive microRNAs (miRNAs) miR-34a and miR-34c control ULBP2 expression. Both miRNAs directly target the 3'-untranslated region of ULBP2 mRNA as demonstrated by reporter gene expression analyses. In melanoma cells expression of ULBP2 surface molecules and miR-34a inversely correlate. Accordingly, ULBP2 was upregulated in the presence of specific miRNA inhibitors, while transfection of melanoma cells with miR-34 mimics down-regulated ULBP2 which in turn diminished tumour cell recognition and specific tumour cell lysis by NK cells. ULBP2 levels decreased also upon treatment of tumour cells with the small molecule inhibitor Nutlin-3a that by blockade of MDM2 activates the tumour-suppressor p53. Molecular analysis revealed that the negative effect of Nutlin-3a on ULBP2 expression was due to the p53 mediated increase of cellular miR-34 levels. These findings are summarised in the model presented in Figure 1.

Taken together, this study demonstrates that tumour-suppressive miR-34a and miR-34c act as ULBP2 repressors and point to an involvement of p53 in ULBP2 regulation, which strengthens the role of the specific NKG2DL in tumour immune surveillance.

Publication

Heinemann, A., Zhao, F., Pechlivanis, S., Eberle, J., Steinle, A., Diederichs, S., Schadendorf, D., Paschen, A. Tumor Suppressive MicroRNAs miR-34a/c Control Cancer Cell Expression of ULBP2, a Stress-Induced Ligand of the Natural Killer Cell Receptor NKG2D. *Cancer Res.* (2012) [Epub ahead of print].

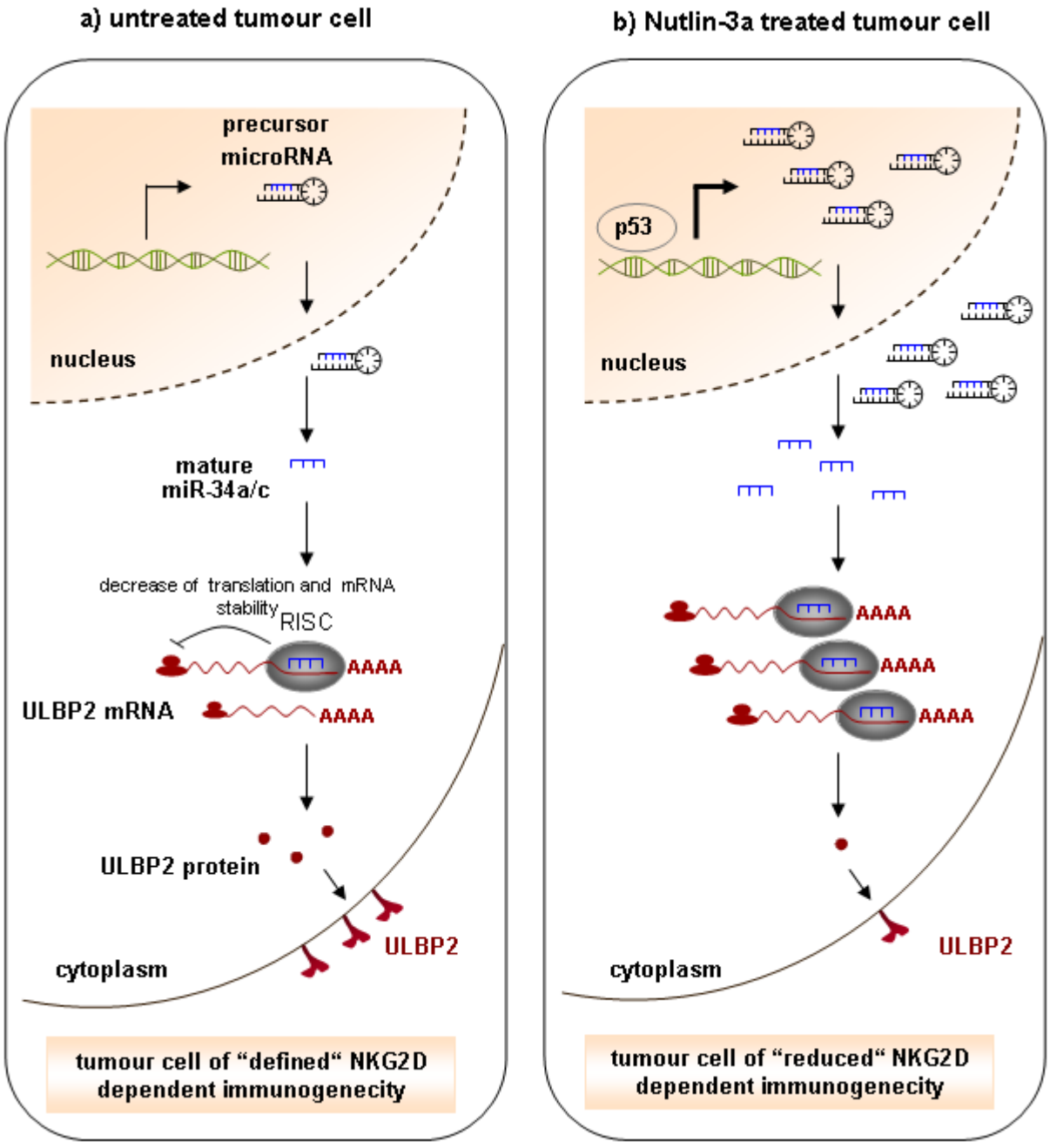


Figure 1: The tumour-suppressive miR-34a and miR-34c and p53 play an important role in the regulation of the NKG2DL ULBP2. A) The members of tumour-suppressive miRNA family miR-34 influence the stability and/or translation of ULBP2 mRNA by binding to the 3´-untranslated region, thereby determining a defined NKG2D dependent immunogenicity of the cell. B) p53 is described as transcriptional regulator of miR-34 family members. Nutlin-3a, a p53 stabilisator reduces ULBP2 cell surface expression and thereby the NKG2D dependent immunogenicity of the cell. Down-regulation of ULBP2 is due to the cellular increase of miR-34.

TLR signal mediation towards oxidative burst

Anna Sigmund

Institute of Medical Microbiology
Group: Carsten Kirschning

The vertebrate immune system is composed of two different branches: the evolutionary older innate immunity and the specific adaptive immunity. The temporally delayed acquired immunity is characterised by the production of specific antibodies and the creation of the immunological memory. By contrast, the fast innate immune system builds the first line of defense against invading pathogens. The innate immunity is mediated by a number of germline encoded pattern recognition receptors (PRR). These receptors specifically recognise conserved microbiological components like cell-wall components, flagellin or nucleic acids, so called pathogen associated molecular patterns (PAMPs). One famous family of the PRRs that transmits the signal "specific infection" into the cytoplasm is the membranous Toll-like receptor (TLR)-family. Up until now there are 13 known family members in mice and 10 in humans. According to a common view, myeloid differentiation primary response protein 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF) largely exclusively mediate TLR-downstream signal transduction leading to e.g. the activation of the transcription factor NF-κB and release of inflammatory mediators such as cytokines and nitrogen monoxide (NO) (Figure 1).

Another crucial defense mechanism triggered by TLRs is reactive oxygen species (ROS) production by a multi-enzyme complex, the phagocyte NADPH oxidase (NOX2). ROS, such as superoxide anion, hydrogen peroxide and hydroxyl radicals are highly reactive compounds that not only play a crucial role in

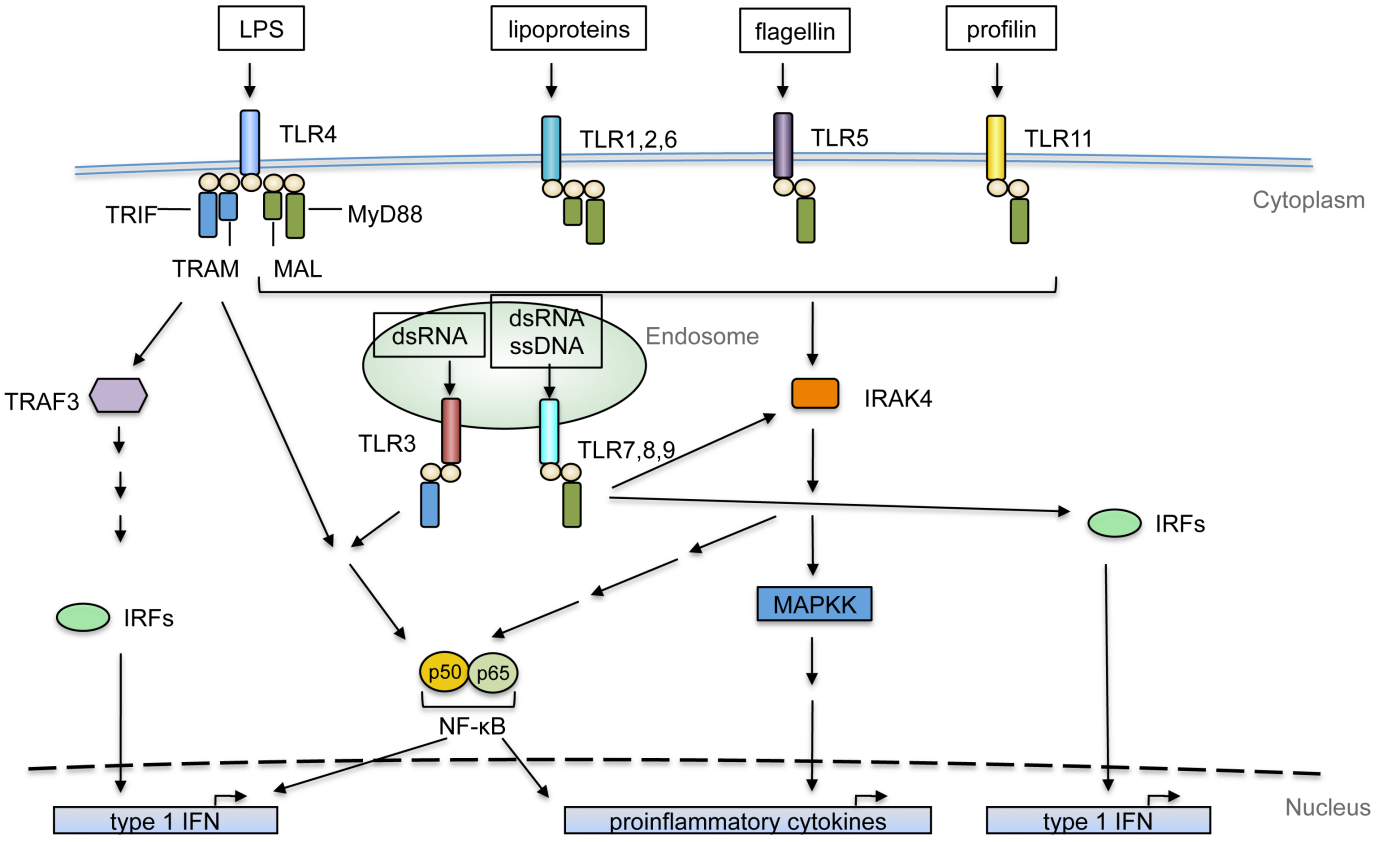


Figure 1: Toll like receptor signalling. TLRs get activated by for example components of the bacterial cell wall (LPS, lipoproteins), flagellin or nucleic acids. The two adaptor-molecules myeloid differentiation primary response protein 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF) mediate the signalling of all here shown TLRs. The transcription of proinflammatory cytokines and type I inteferones are typical TLR downstream events.

killing invading microorganisms but also in activating diverse signalling pathways, chemotaxis, antigen cross-presentation and in immune modulation. A lack of it is associated with a strongly impaired immune system effectiveness as exemplified by the genetic disorder chronic granulomatous disease (CGD), which is caused by defects of NOX2. CGD patients suffer from recurrent and often life-threatening bacterial and fungal infections. On the other hand, uncontrolled superoxide production contributes to inflammatory disorders such as rheumatoid arthritis (RA) and atherosclerosis in the pathologies of which TLRs might be involved. Understanding the undergoing activation mechanisms could help to find a therapy, such as the possible pharmacological inhibition of the massive ROS production.

We screened a leukocyte expression library in yeast and identified a subunit of NOX2 as a TLR7 intracellular domain (icd) and not MyD88 interaction partner. Upon over-expression in human embryonic kidney (HEK293) cells, we observed ligand-dependent complex formation of NOX2 component with TLR2 and TLR4. Challenge of macrophages with heat-inactivated bacteria but not with single TLR agonists induced phosphorylation of the NOX2 subunit, indicating synchronous multiple PRR activation as a requirement for the activation of the responsible kinase. Oxidative bursting itself, however, upon stimulation with single TLR ligands, was as strong as upon bacterial challenge. It depended on expression of respective TLRs. By contrast, neither MyD88 nor TRIF were involved in ROS production upon whole bacterial and single TLR agonist challenge supporting a model in which the NOX2 subunit interacts with TLR ICDs autonomously to possibly become phosphorylated at the receptor. Our results indicate that the pathway from the cell surface that ends in TLR-driven assembly of the active NOX2 complex might involve only one prior protein-protein interaction (Figure 2).

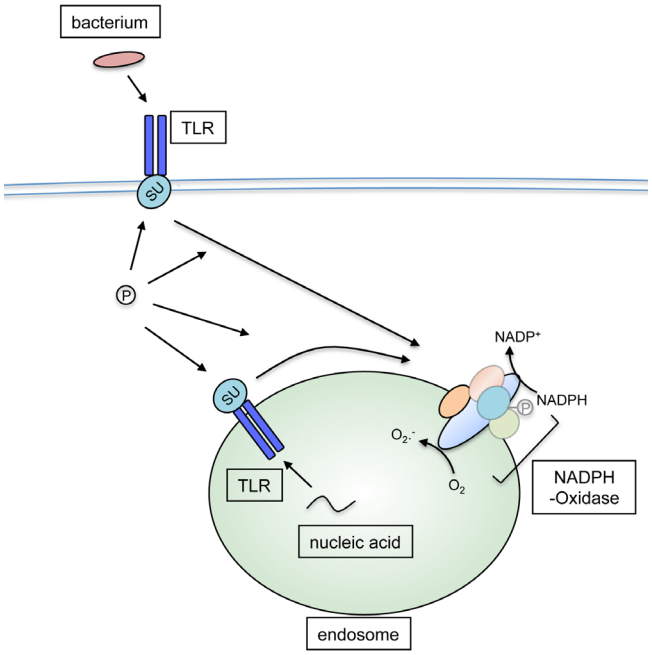


Figure 2: Model of TLR mediated ROS production. Upon TLR activation the NADPH subunit (SU) binds directly to the TLR and gets phophorylated. One or some of these phosphorylations might dissociate the subunit from the TLR and support directing to and association with rest of the NADPH oxidase complex to induce reactive oxygene production.

Analysis of the CD8⁺ T cell response against hepatitis C virus in injection drug users

Kathrin Skibbe

Institute of Virology
Group: Jörg Timm

Worldwide, approximately 180 million people are infected with hepatitis C virus (HCV). The transmission of HCV is closely linked to blood-blood contact, so that especially in developed countries, HCV is mainly acquired through injection drug use. While acute HCV infection is often associated with only mild or no symptoms, up to 80% of patients develop a persistent infection. Chronic hepatitis C in turn can lead to liver cirrhosis and cancer thereby representing a major health burden.

HCV-specific antiviral CD8⁺ T cells, which can kill virus-infected cells and secrete antiviral cytokines, play a major role in the immune response against HCV. Initially both patients who go on to spontaneously resolve HCV infection and those who develop chronic infection have similar CD8⁺ T cell responses, but secondary failure of the immune response occurs in patients with persistent infection.

Several reasons for this failure have been proposed. One is that HCV as an RNA virus has a high mutation rate. Consequently, even though HCV-specific CD8⁺ T cells are still present, they can no longer recognise the virus once mutations in the targeted epitopes have been acquired. A second factor responsible for the failure of the CD8⁺ T cell response in chronic HCV patients is T cell exhaustion, which is characterised by the loss of effector functions in the presence of persisting high amounts of antigen. Furthermore, the comparison between chronic HCV patients and patients with resolved infection has shown that HCV-specific CD8⁺ T cells not only lose their function during viral persistence, but their absolute number wanes as well. This deletion of virus-specific cells might be due to apoptosis, as a similar apoptotic effect has been described for CD8⁺ T cells that were activated by hepatocytes in a mouse model, which might be relevant for HCV as a hepatotropic virus.

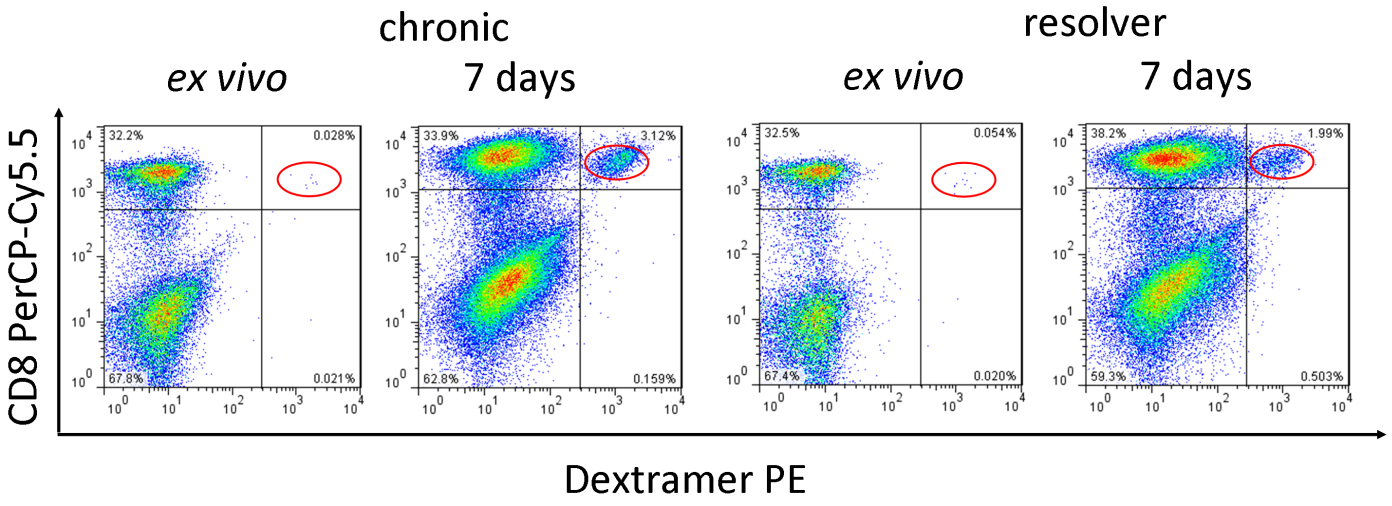


Figure 1: HCV-specific CD8⁺ T cells: HCV-specific CD8⁺ T cells (red circles) can be identified and analysed in both chronic and resolved HCV patients via flow cytometry after staining with anti-CD8 antibody and multimers specific

The aim of this study is therefore to further investigate the mechanisms behind the failure of the $CD8^+$ T cell response in HCV infection, especially with regard to apoptotic markers.

As a first step the apoptotic BH3-only protein Bim (Bcl2-interacting mediator) was investigated. This molecule has been previously found to be upregulated in $CD8^+$ T cells from patients chronically infected with HBV as well as in cells activated by hepatocytes. To test the hypothesis that the continuous activation of HCV-specific $CD8^+$ T cells in the liver is associated with Bim-mediated premature apoptosis during HCV persistence, HCV-specific $CD8^+$ T cells from patients with spontaneously resolved and chronic HCV infection (12 patients each) were stained with HLA I/peptide-multimers and the expression of Bim and the activation marker CD38 was analysed by flow cytometry *ex vivo* and after antigen-specific stimulation.

It was found that Bim was not differentially regulated in $CD8^+$ T cells from resolved or chronic HCV infection *ex vivo* indicating that Bim expression does not depend on the infection outcome *per se*. Upon antigen-specific stimulation Bim and CD38 were both upregulated irrespective of the disease status, supporting the hypothesis that activated $CD8^+$ T cells are more prone to apoptosis.

In future we would like to do further phenotypic analyses of HCV-specific $CD8^+$ T cells. A broader and more unbiased approach might be achieved by flow cytrometrically sorting HCV-specific cells to perform microarrays.

Pathogenesis of Hodgkin lymphoma

Markus Schneider / Annette Schmidt

Institute of Cell Biology (Tumour Research)
Group: Ralf Küppers

Hodgkin lymphoma (HL) is one of the most common lymphomas in the Western world. A hallmark of the disease is the rare occurrence of the malignant mono- or multinucleated Hodgkin Reed-Sternberg (HRS) cells, which usually account for only 1% of the tumour mass and are embedded in a micromilieu of infiltrating immune cells. The scarcity of malignant cells within the tumour hampers the analysis of the HRS cells. Although HRS cells are derived from pre-apoptotic germinal centre (GC) B cells, they have lost their 'B cell identity', meaning that multiple B cell-specific genes are down-regulated.

Project 1: Molecular mechanisms of deregulated gene expression in the pathogenesis of Hodgkin lymphoma (Markus Schneider)

Usually B cell lymphomas retain the key phenotypic and functional features of the differentiation stage from which they derive. However, HRS cells of HL are the exception to this rule because they have almost completely lost their B cell phenotype and express markers of various other haematopoietic cell types such as T cells, dendritic cells and macrophages. Up until now only some underlying mechanisms responsible for

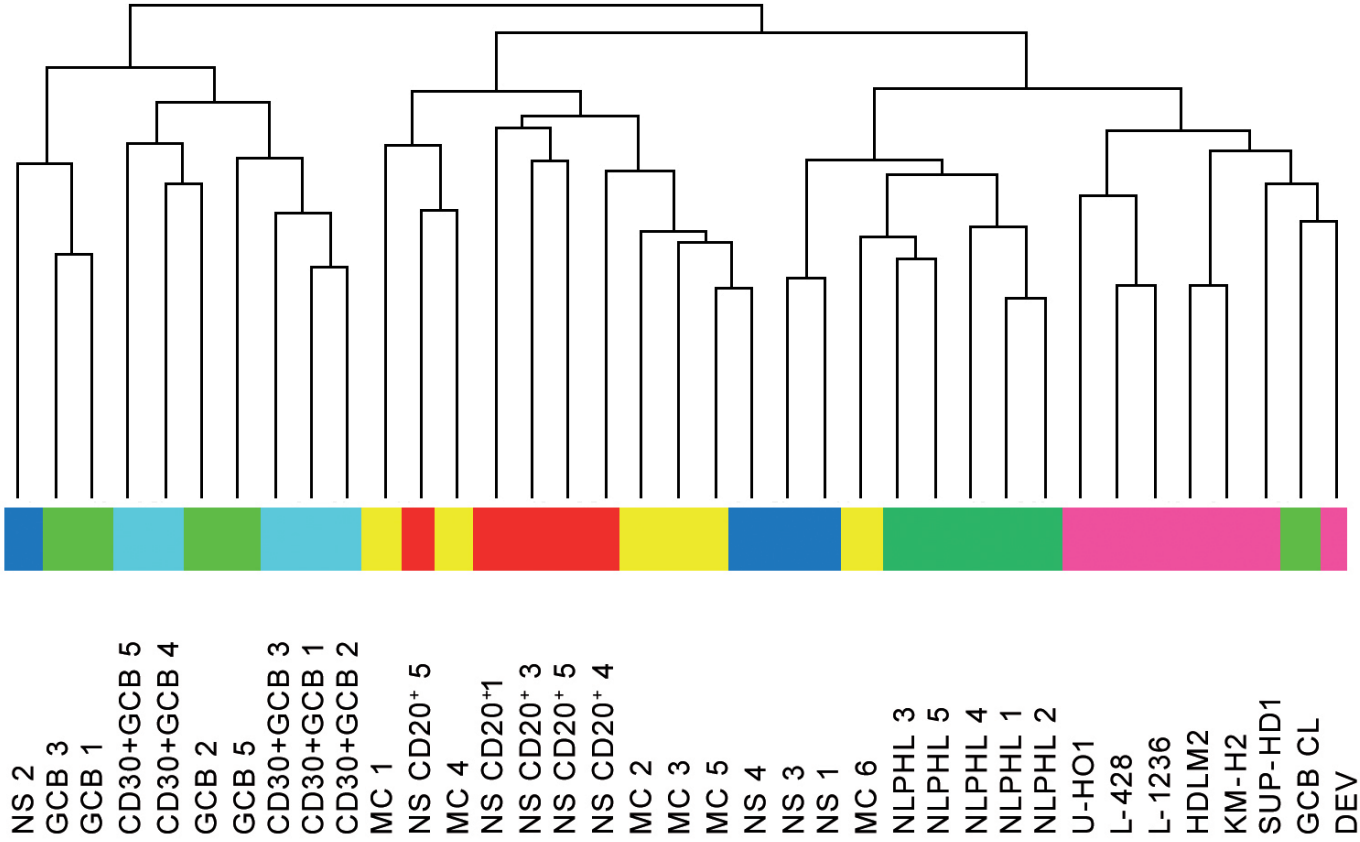


Figure 1: Unsupervised hierarchical clustering analysis of miRNA expression in GC B and HRS cells. The clustering is based on the 200 miRNAs showing the most variable expression. Different cell types are indicated by the colour code: light green: GC B cells; turquoise: CD30+ GCB cells; yellow: Mixed cellularity HL; blue: nodular sclerosis HL; dark green: nodular lymphocyte predominant HL; pink: HL cell lines.

this dramatic "dedifferentiation" or "reprogramming" have been clarified and it is suggested that factors responsible for the "dedifferentiation" are crucial for the malignant transformation process. For example, it was shown that HRS cells express Notch1, a transcription factor responsible for T cell differentiation and the suppression of B cell development. In addition, the down-regulation of many B cell-specific genes is caused by epigenetic mechanisms.

Comparison of the gene expression profiles of HRS cells with normal B cells and other B cell lymphomas revealed the expression of transcription factors in HRS cells which are typically expressed in stem and precursor cells, for example MYC and CEBPβ. The expression of CEBPβ, the main transcription factor for the myeloid differentiation, was detected by immunohistochemistry and/or by Western blot analysis in HRS cells in the majority of cases and in HL cell lines.

To better understand the underlying mechanisms of the "dedifferentiation" of HRS cells and the possible contribution of the stem and precursor factors MYC and CEBPβ, we plan to down-regulate these transcription factors via lentivirally encoded shRNA in HL cell lines. Subsequently, the effect of the down-regulation of the genes on cell survival, proliferation and cell phenotype will be studied. First transduction experiments have been performed which are currently being evaluated. To gain further insight into the function of the over-expressed transcription factor in HRS cells, these factors will also be over-expressed in normal B cell via lentiviral transduction.

A further aspect in this study is that the generation of miRNA expression profiles of HRS and normal B cells (see below) might lead to the identification of miRNAs whose deregulated expression in HRS cells is involved in the "dedifferentiation" process. The potential role of miRNAs in the "dedifferentiation" of HRS cells will be studied accordant to their aberrant expression in HRS cells either by re-expression or inhibition via antagomirs. The consequences of this manipulation on the phenotype of HRS cells survival and proliferation will be studied.

Project 2: The role of microRNA in the pathogenesis of Hodgkin lymphoma (Annette Schmidt)

MicroRNAs (miRNA) are short (18-25 nt), single-stranded molecules that control gene expression on the post-transcriptional level. They are involved in vital processes like cell proliferation, differentiation or stress response. Dysregulated miRNA expression has been found in various types of cancer. Our aim is to study the role of microRNAs in the pathogenesis of HL.

The above–mentioned scarcity of HRS cells impedes the molecular analyses of HL. In this study, we established an RT-qPCR based protocol for the miRNA profiling of microdissected HRS cells obtained from paraffin-embedded lymph node biopsies. To allow the analysis of very low HRS cell numbers, a miRNA cDNA pre-amplification protocol was successfully developed.

We investigated different HL subtypes, among them $CD20^-$ nodular sclerosis (NS), $CD20^+$ nodular sclerosis ($CD20^+$), mixed cellularity (MC) and lymphocyte predominant (LP) HL. Expression of 360 miRNAs was determined in 22 patients and compared to that of tonsillar GC B cells. Additionally, miRNA expression profiling for 7 HL cell lines was performed. Unsupervised clustering analyses revealed significantly different miRNA expression patterns between GC B and HRS cells as well as among different HL subtypes and cell lines. Our data suggest that HL subtypes might be classified by specific miRNA expression patterns. We are currently performing functional studies with HL cell lines in which the expression of specific miRNAs with deregulated expression in HRS cells is down-regulated to reveal the role of the over-expression of these miRNAs for the pathophysiology of HL.

Characterisation of post-translational modifications on the centromeric histone variant CenH3/CENP-A

Juliane Pfrötzschner

Centre for Medical Biotechnology
Group: Ann Ehrenhofer-Murray

The organisation of DNA in the nucleus of every eukaryotic cell in the form of chromosomes and chromatin is essential for the accurate propagation of the genome during the mitotic cell cycle. Generally, the DNA is packaged with histone proteins that build nucleosomes and facilitate tight packaging as well as chromosome segregation. Differences in the packaging determine gene expression and gene silencing. The canonical nucleosome consists of four different histone proteins, which are post-translationally modified by certain enzymes, altering the binding properties of superior chromatin-factors.

Chromosome segregation is mediated by a defined region at each chromosome, the centromere, which forms a platform for the formation of the kinetochore, a large protein assembly that connects centromeric segments to the microtubules for chromosome segregation during mitosis. The centromere of the yeast *Saccharomyces cerevisiae* consists of a single nucleosome that carries CenH3/CENP-A (termed Cse4) instead of canonical histone H3. A 125 bp DNA-fragment is wrapped around this nucleosome and is subdivided into three elements termed CDEI, CDEII and CDEIII. CDEI recruits a dimer of Cbf1, CDEII is an AT-rich segment that folds around the core nucleosome and CDEIII binds the Cbf3 complex of the inner kinetochore (Figure 1).

In this project, we have asked whether Cse4 is regulated by post-translational modifications (PTMs), as is the case for canonical histones. For this purpose, Cse4 was purified from yeast cells and PTMs were identified by mass spectrometry. Indeed, we found that Cse4 carries PTMs on several lysine, arginine and serine residues, and that these modification states are required for centromere function and chromosome segregation.

An arginine methylation site was identified, and the absence of methylation on this residue causes growth defects and chromosome segregation defects in cells lacking other kinetochore factors. Here, we will further characterise these PTMs on Cse4 in order to increase our understanding of how epigenetic modifications regulate the assembly of the kinetochore and other processes during chromosome segregation. We will seek to identify the enzymes responsible for these modifications, and we will determine how they regulate centromere function. Furthermore, we will attempt to identify interaction partners of Cse4 whose association is regulated by PTMs on Cse4. Since some of these PTMs lie in the N-terminus of Cse4, we are attempting to determine the three-dimensional structure of this part of Cse4 in order to identify essential structural properties that are required for the physical interaction to other, non-histone proteins.

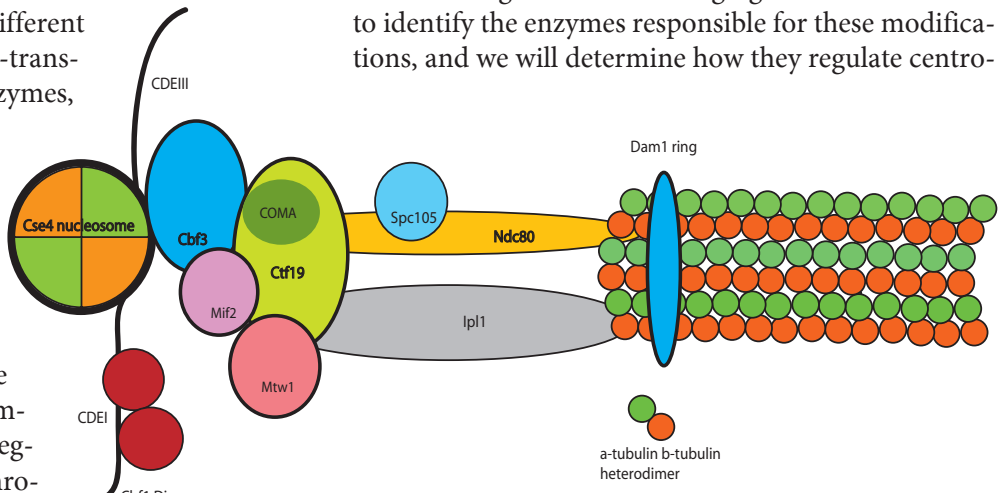


Figure 1: Model of the kinetochore and point centromere of *Saccharomyces cerevisiae* containing the histone H3 variant Cse4. In this organism a single microtubule attaches to the outer layer of the kinetochore. The DNA-kinetochore interface is mediated by the centromeric DNA elements and proteins of the inner layer of the kinetochore. The four-subunit Cbf3 complex binds the CDEIII segment. One important component of the kinetochore linker layer is the Ctf19 complex comprising the COMA sub-complex (*C*tf19, *O*kp1, *M*cm21 and *A*me1) that connects the DNA-kinetochore interface to the microtubule binding components of the kinetochore. Protein complexes of the outer layer, such as the Ndc80 and Dam1complexes, bind the plus-end of the microtubule.

Acknowledgements

We are deeply indebted to the following bodies for their financial support of the Graduate School of Biomedical Science without whom this whole undertaking would have been impossible to realise:

- Faculty of Medicine, University Hospital Essen, University of Duisburg-Essen
- Faculty of Biology, University of Duisburg-Essen
- Centre for Medical Biotechnology (ZMB), University of Duisburg-Essen
- Rectorate and Science Support Centre (SSC), University of Duisburg-Essen
- Stiftung Universitätsmedizin Essen, University Hospital Essen, University of Duisburg-Essen
- International Research Universities Network (IRUN)

Additionally we are highly grateful to the following internal centres at our university for their provision of soft skill workshops:

- ProFor
- West German Tumour Centre (WTZ)
- Mediment Programme

We would also like to express our sincere thanks to all those various colleagues at the University of Duisburg-Essen and the IRUN partner universities who have collaborated with us with refreshing willingness and drive to make shared visions and projects work.

Credits

Editing: Ulf Dittmer, Sven Brandau, Delia Cosgrove

Layout: Martin Sowa

Photographs: The following material was kindly provided by: Jörg Timm (Robert–Koch–Haus, cover); Beate Fraß (Spine, p. 9); Ulf Dittmer/Kim Hasenkrug (Treg cell, p. 10); Bernd Giebel/Peter Horn (Endothel m Actin rb Myosin IIa MeOH, p. 10); Frank Petrat (Artificial oxygen carriers, p. 11) and Nicholas Wagner (WTZ, p. 12 & p. 16).

Printed by: UniversitätsDruckZentrum (UDZ), University of Duisburg-Essen

biome [bī-ōm] *n.* a collective term used to describe a distinctive regional biotic community; an acronym for the Graduate School of Biomedical Science at the University of Duisburg-Essen established in 2010, an academic association offering state of the art doctoral training to young scientists, the heritage of a recent innovative research renaissance in the Ruhr region.

Graduate School of Biomedical Science

Robert-Koch-Haus
Virchowstr. 179
Universitätsklinikum Essen
University of Duisburg-Essen
45147 Essen
GERMANY

Tel.: +49 (0)201 723 4257
Fax: +49 (0)201 723 5472

biome@uni-due.de
http://www.uni-due.de/biome