Second Norwegian Cancer Symposium 2009

Oslo, December 2-4 2009

Frontiers in Cancer Stem Cell Research: From basic science towards a cure

2nd - 4th of December 2009
Oslo, Norway

Holmenkollen Park Hotell
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Welcome!

It is a pleasure to welcome you all to this exciting and cutting-edge meeting. The meeting is the second in an ambitious series of international cancer meetings initiated and generously funded by Norsk Hydro’s Fund for Cancer Research at the Institute for Cancer Research. For us in CAST Cancer Stem Cell Innovation Centre, though, it is our first meeting of this calibre, and it has been a challenge and given us some worries to plan all the details. We hope it will turn out to be a success, which would be mostly due to the fantastic panel of top level speakers selected by our scientific advisory board, and your own active participation. As we had a very good response from the invited speakers, the programme may be a bit busy, but we hope you will have good opportunity for interaction during the breaks and the more social evening events.

Although there are heated discussions on how important and general the rare cancer stem cell concept may be in clinical cases, there is no doubt that stem cell properties are central in this disease. Already important understanding of failing and successful treatment strategies is appearing based on these concepts, and most likely they will lead to new, more successful treatments of currently hard-to-cure cancers.

As we encourage the speakers to present unpublished data, we ask everyone to keep their laptop closed and cameras tucked away during sessions.

Stem cell biology fascinates also the public, and its tremendous impact on the cancer field is an opportunity to demonstrate the importance of basic science for human health. In this perspective we are very pleased that the meeting will by opened by Anne-Grete Strøm-Erichsen, the Norwegian Minister of Health. The Ministry has been active in supporting stem cell research for many years, by funding a national stem cell network, to propose the current act on medical application of biotechnology that allows research on embryonic stem cells, and also by establishing a National Stem Cell Centre at Oslo University Hospital (see www.stemcellnorway.org), which officially was opened last week.

Our topic also receives great interest locally, especially within the Oslo Cancer Cluster (OCC), an association of companies who got the status of Centre of Expertise from the Research Council of Norway. OCC was recently nominated as the most important emerging biotech cluster in Europe, is centered around the Radium Hospital and Cancer research Institute, and several OCC members are partners in our CAST Cancer Stem Cell Innovation Centre. They will build an innovation park around our institute, complete with a high school dedicated to medical topics, science, technology, and business development. But the topic also has the attention of local politicians, including the Mayor, Fabian Stang, as we will experience on Thursday.

Although we have ample funding from Norsk Hydro’s Fund we chose to invite co-sponsoring from a few strategic partners, and are grateful for the unanimous response from the European Association for Cancer Research, the Norwegian Cancer Society, and Oslo Cancer Cluster. We thank all sponsors for their generous support.

The Norwegian Cancer Symposium will be arranged again in 2011. Based on the positive experience of this meeting, we may apply to arrange further meetings on this topic, but the opportunity is allotted on a competitive basis, so keep our URL in mind to see what the next topics may be. I hope anyway to see you again on a future meeting, hopefully with more reports on concrete benefits to patients.

I hope you will all enjoy the meeting!

Ola Myklebost
For the organising committee
NORSK HYDRO’S FUND FOR CANCER RESEARCH

The Fund has the purpose to support cancer research at the Montebello location of the Institute for Cancer Research at the Radium Hospital. The Fund has been operative for more than 30 years and was started by a grant from the Norwegian commercial company Norsk Hydro, who still contribute to the functioning of the Fund. Currently, support from the Fund to the Institute is given in two forms:

1. Travel fellowships for scientists employed at the Institute, for international conferences and courses.
2. Generous support of the “Norwegian Cancer Symposia”, an international, high-quality series of symposia.

Norwegian Cancer Symposia are organised every other year by one (or more) research group active at the Institute. The subject is selected after application to the Fund’s Board of Governors.

The Fund’s Board of Governors consists of scientists from the Institute and externally as well as representatives from Norsk Hydro. The Fund’s assets are invested conservatively in stocks, bonds and bank accounts.

Current members of the Board:
Erik Boye (chair)
Runar Gulhaugen
Per Arne Myklebost
Erik Olai Pettersen
Hanne Guri Belgau (deputy)
Kirsten Skarstad (deputy)
The European Association for Cancer Research (EACR) has always had one guiding aim ‘The advancement of cancer research’. In pursuing this aim, the Association provides services to its members; presents educational, training and scientific meeting opportunities, and supports communication and collaboration between the cancer researchers who make up its membership.

EACR also sets out to raise the profile of cancer research and cancer researchers in Europe and the need for sustained political and economic support. The Association enjoys particularly strong links with other European cancer societies and is a founder member of the European Cancer Organisation (ECCO).

With over 9,000 members, EACR is Europe's largest member society for cancer research. Membership applications can be made on-line at www.eacr.org. The annual fee is just 30EUR and students enjoy free membership for up to four years.

Of particular interest to members at the moment is the outstanding Scientific Programme for EACR-21, the discounted registration rates and the availability of bursaries to support participation. To qualify to apply for these benefits, please join EACR without delay.

**EACR-21:**
The 21st Meeting of the European Association for Cancer Research, 26 - 29 June 2010, Oslo Norway

"This most exciting conference on discovery based translational research will discuss the latest breakthroughs in research and how to speed them effectively into the clinic. New diagnostics, new therapies, new biomarkers and systems biology approaches to drug combinations in the Scandinavian summer." Sir David Lane, Opening Lecturer

Visit [www.ecco-org.eu](http://www.ecco-org.eu) to register and join us in Oslo in 2010

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**About Norwegian Cancer Society**
The Norwegian Cancer Society (NCS) is a nation-wide, non-profit voluntary organization. We work to be the most important private funder of cancer research, to reduce the incidence of cancer and to increase the recovery rate and life quality of cancer sufferers.

Cancer research has been our most important task since 1948. We allocate half of our funds for clinical, experimental, epidemiological and alternative/complementary research. In 2009, we received 261 applications and granted support amounting to 113 million Norwegian kroners.

Research grant applications are considered by 5 specialist groups who submit proposals to a Research Committee. The Committee then makes recommendations to the Society’s board, which
makes the final decision. The NCS also has a Scientific Board, consisting of representatives of the different professional fields from all parts of Norway. Its key task is to identify areas of research that should receive special priority.

To learn more about NCS, apply for funding or to support us, please visit www.kreftforeningen.no

About Oslo Cancer Cluster
Oslo Cancer Cluster is a non-profit member organization committed to improve the lives of cancer patients by accelerating the development of new cancer diagnostics and medicines. We will be one of the world leading cancer clusters by 2013.

Oslo Cancer Cluster was established in 2006, and awarded Norwegian Centres of Expertise (NCE) status by the Norwegian government in June 2007, and provided with long-term funding.

In Norway the cancer research and its application are in world-class in terms of innovation and quality. Norway’s unique infrastructure, from biobanks to extensive patient registries, accelerates translational research which is crucial to convert ideas from the lab into diagnostics and medicines.

Over 70% of all cancer research in Norway is done in the region of Oslo, and the Norwegian member companies in Oslo Cancer Cluster has a large R&D pipeline with over 50 projects in preclinical and clinical phases

Oslo Cancer Cluster already has 60 members, including academic research institutions, industrial companies, health initiatives and support groups in the field of biotechnology with the main focus on cancer. The member companies consist of the Norwegian branches of major pharmaceutical companies, established Norwegian international companies and Norwegian start-up and venture companies.

Oslo Cancer Cluster will accelerate the development of new cancer diagnostics and medicines through:

- Local collaboration and international partnership.
- Increasing our member companies’ ability to attract capital
- Creating an efficient clinical trials network to shorten development timelines
- Oslo Cancer Cluster Innovation Park bridging research, biopharma and education, to help develop the Life Science workforce for tomorrow
Institute for Cancer Research

Institute for Cancer Research has since its foundation in 1954 played a central role within the field of cancer research, both in Norway and internationally. The institute has at present seven research departments and more than 230 employees. More than half of the staff is externally funded. Many of the researches have national and international commissions and ongoing collaborations. The institute is engaged in both basic and translational cancer research involving experimental research. The co-localization with a large cancer hospital fulfills the premises for advanced medical research across sciences, a necessity towards the goals of individualized diagnostics and treatment for cancer patients.

The new research building was officially opened 27th of August 2009

Most of Institute for Cancer Research, Cell Therapy and Center for comparative medicine has its working space in the new building that is close to 18000 m². The building has 6 floors. The idea behind this building is openness and cooperation - "a room with a view". The glass facade is 7000 m².

The symposium will visit the new building for the EACR Key Note Lecture of Hans Clevers and the poster-session on 2nd of December.

The CANCER STEM CELL INNOVATION CENTER (CAST) is an integrated biomedical centre at the Institute for Cancer Research, funded by the Norwegian Research Council, and consisting of established research groups from The National Hospital, Ullevål University Hospital, and University in Oslo, including the industrial partners Affitech, Axellia, PCI- Biotech and Invitrogen Dynal. CAST develops innovative therapeutic and diagnostic approaches that dress specifically stem cell issues in cancer.

See www.cancerstemcell.no.
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**Helpers**

All Post Docs and helpers at  
Institute of Cancer Research  
and  
SFI-CAST; Centre for Research-based Innovation (SFI) - Cancer Stem Cell Innovation Centre  
Inspiration Kommunikasjon & Event
Program Schedule

Wednesday 2nd of December

10.00  
registration
Posters are handed in and mounted by us

13.0  
lunch

Holmenkollen Park Hotel, Saga Hall

Opening

14.00  
Opening by the Norwegian Minister of Health
Welcome by Ola Myklebost

Oslo Cancer Cluster Keynote Lecture

Chair:  
Ola Myklebost

14.10  
Pier Giuseppe Pelicci, Milan, Italy
Biological Properties of Cancer Stem Cells

Session I:  
Stemcellness in tumours I

Chair:  
Rolf Bjerkevig

15.00  
Christopher Heeschen, Madrid, Spain
Pancreatic Cancer Stem Cells – Insights and Perspectives

15.30  
Christiane Bruns, Munich, Germany
Cancer stem cells - target for cancer therapy?

16.0  
coffee

Session II:  
Stemcellness in tumours II

Chair:  
Steinar Aamdal

16.30  
César Cobaleda, Madrid, Spain
The role of cellular plasticity in oncogenic reprogramming

17.00  
Ron McKay, Bethesda, USA
Cell interactions that control stem cells in mammals

18.30  
Bus to new Institute for Cancer Research Building
**Institute for Cancer Research Building**

**EACR Keynote lecture**

19.00  Welcome by Erik Boye, *Hydro’s Fund and Institute for Cancer Research*

*Introduction by Anne-Lise Børresen-Dale, President of EACR*

19.15  **Hans Clevers**, Ulrecht, The Netherlands

Lgr5 Intestinal Stem Cells in self-renewal and cancer

20.00 - 22.00  *Tapas, posters and mingling.*

22.00  *Bus back to hotel*
Thursday 3rd of December

Holmenkollen Park Hotel, Saga Hall

Cancer Society Keynote Lecture

Supported by a grant from Dentist Aase and Wife’s Donation

Chair: Jannikke Ludt, The Norwegian Cancer Society

09.00 William Matsui, Baltimore, USA
The clinical translation of cancer stem cells in B cell malignancies

Session III: Applying stem cell knowledge in today’s treatment

Chair: Gustav Gaudernack

10.00 Hugues de Thé, Paris, France
LIC clearance by treatment-induced oncogene degradation

10.30 Andreas Trumpp, Heidelberg, Germany
Dormancy in Stem Cells

11.00-11.30 Coffee

Session IV: Stem cell programs in cancer

Chair: Stefan Krauss

11.30 Eduard Batlle, Barcelona, Spain
Intestinal stem cell genes in colorectal cancer

12.00 Mahendra Rao, San Diego, USA
Neural stem cells and cancer

12.30 Eric Chi Wai So, London, UK
Dissecting the Molecular Pathways Mediating Self-Renewal of Leukemic Stem Cells

13.00 Poster prize by Bjarte Reve, OCC

13.00 - 1400 Lunch
Session V: Stem cell signalling in tumours

**Chair:** Harald Stenmark

14.15 Ariel Ruiz i Altaba, Geneva, Switzerland
Hedgehog-GLI1 signalling is essential in epithelial tumour cells and drives cancer stem cell expansion

14.45 Rune Toftgard, Stockholm, Sweden
Hedgehog signalling in skin cancer and tissue stem cells

15.15 Urban Lendahl, Stockholm, Sweden
Notch signalling in stem cells and cancer

15.45-16.15 Coffee

Session VI: Stem-like cells in breast cancer

**Chair:** Gunhild Mælandsmo

16.15 Marc LaBarge, Berkeley, USA
The role of the microenvironment in organizing and maintaining lineage-specific domains in mammary gland

16.45 John Stingl, Cambridge, UK
Mammary stem and progenitor cells: Understanding the cellular context of breast tumours

17.15 Ole W. Petersen, Copenhagen, Denmark
Routes to breast cancer heterogeneity: Prospective cloning of functionally distinct subpopulations by use of markers from a normal human breast lineage hierarchy

18.15 Buses to the City Hall (Rådhuset)

19.00 Reception by the Mayor of Oslo at the City Hall

*Drinks & light snacks*

*Dinner for speakers / Free evening for participants*
Friday 4th of December

Holmenkollen Park Hotel, Saga Hall

Session VII: Determination of cell fate

Chair: Philippe Collas

09.00 Amanda Fisher, London, UK
Stem Cells and Epigenetic Reprogramming

09.30 Tracy-Ann Read, Durham, USA
Cancer Stem Cells Reflect the Origin of Pediatric Brain Tumours

10.00 Maarten van Lohuizen, Amsterdam, The Netherlands
Role of Polycomb repressors in stem cells, cancer and development

10.30 Peter Dirks, Toronto, Canada
Self renewal and lineage commitment in brain tumour stem cells

11.00 – 11.30 Coffee

Session VIII: Lessons from embryonic systems

Chair: Ragnhild Lothe

11.30 Ralph A. Neumüller, Vienna, Austria
Controlling the balance between self-renewal and differentiation in Drosophila neural stem cells

12.00 Monica Gotta, Geneva, Switzerland
Asymmetric division in C. elegans
EMBO Young Investigator Lecture

12.30 Peter Andrews, Sheffield, UK
Human Embryonic Stem Cells: Commitment, Adaptation and Cancer

13.00 – 14.00 Lunch
Session IX: Epithelial-mesenchymal transition

Chair  

Ola Myklebost

14.15  

Thomas Brabletz, Freiburg, Germany  
Tumour invasion and metastasis: EMT and cancer stem cells

14.45  

Sendurai Mani, Houston, USA  
Generation of Stem-Like Cells via EMT: A New Twist in Tumour Initiation and Progression

15.15  

Christina Scheel, Cambridge, USA  
Formation and Maintenance of Stem Cell Traits induced by EMT

15.45  

Closing remarks

16.0  

End
Abstracts of invited speakers
Recent findings support the concept that cells with the properties of stem cells (SC) are integral to the development and perpetuation of several forms of human cancer, and that eradication of cancer stem cells (CSC) may be essential to achieve cancer cure. However, direct proof of these concepts is still lacking, mainly due the scarcity of appropriate model systems. We are characterizing the biological differences between normal and transformed SCs. SCs are defined by their abilities to generate more SCs (‘self-renewal’) and to produce cells that differentiate. One mechanism by which SCs accomplish these two tasks is asymmetric cell division, whereby each SC divides to generate one daughter with SC fate and one that differentiates. SCs, however, possess the ability to expand in number, as it occurs during development and in adulthood after injury or disease. This increase is not accounted by asymmetric divisions, in which only one daughter cell maintains SC identity. Recent findings in *C.elegans* and *Drosophila* indicate that SCs can also generate daughter cells that are destined to acquire the same fate (symmetric cell division). On the other hand, SC quiescence is critical to maintain tissue homeostasis after injury. We will present our recent findings showing increased symmetric divisions of CSCs in breast tumors (due to inactivation of the p53 tumor suppressor) and dependency of leukemia development on quiescent leukemia SCs (due to transcriptional up-regulation of the cell cycle inhibitor p21 by leukemia-associated fusion proteins). Our findings suggest that that asymmetric divisions of stem cells function as a mechanism of tumor suppression, that SC quiescence is critical to the maintenance of the transformed clone and that symmetric divisions of SCs permits its geometric expansion.
Solid tumours are the most common cancers and represent a major therapeutic challenge. The cancer stem cell hypothesis is an attractive model to explain the functional heterogeneity commonly observed in solid tumours. It proposes a hierarchical organization of tumours, in which a subpopulation of stem cell-like cells sustains tumour growth, metastasis, and resistance to therapy. We will present the most recent advances in the cancer stem cell field, with particular emphasis on pancreatic cancer as one of the deadliest human tumours, and highlight open questions and caveats to be addressed in future studies.

There is increasing evidence that solid tumours including pancreatic cancer are hierarchically organized and sustained by a distinct subpopulation of cancer stem cells. However, direct evidence for the validity of the cancer stem cell hypothesis in human pancreatic cancer remains controversial due to the limitations of xenograft models. However, supportive data are now emerging from mouse models using related or different sets of markers for the identification of murine cancer stem cells.

Therefore, while the clinical relevance of cancer stem cells remains a fundamental issue for this rapidly emerging field, current findings clearly suggest that specific elimination of these cells is possible and therapeutically relevant. Targeting of signalling pathways that are of particular importance for the maintenance and the elimination of cancer stem cell as the proposed root of the tumour may lead to the development of novel treatment regimens for pancreatic cancer. We will present the data for several novel approaches that have been developed in our laboratory over the past 3 years.
ABSTRACTS OF INVITED SPEAKERS

2nd of December at 15.30

S03  Cancer stem cells - target for cancer therapy?

Christiane J. Bruns, Munich, Germany

Advances in the field of stem cell biology have provided renewed hopes that stem cells can be used to treat a wide range of genetic diseases and traumatic injuries. However, advances in the field of cancer cell biology have led to formulation of the cancer stem cell hypothesis, which posits that cancers arise from mutant stem cells. Further, this hypothesis proposes that these stem cells account for cancer recurrence, metastasis, and resistance to conventional treatments. Emerging evidence has shown recently that the capacity of a tumor to grow and propagate is dependent on a small subset of cells, termed “cancer stem cells”. Confirmation of the existence of these rare cells has been accumulated for hematopoietic malignancies, brain cancer, and solid organ malignancies including breast, prostate and colon cancer. The current tumor biology describes a cancer stem cell (CSC) as a cell within a tumor that is able to self-renew and to produce the heterogeneous lineages of cancer cells that comprise the tumor. The implementation of this concept explains the use of alternative terms in literature, such as “tumor-initiating cell” and “tumorigenic cell” to describe putative CSC.

The stem cell hypothesis has recently been explored in pancreatic cancer. In some recent studies pancreatic cancer stem cells showed several features typically seen in stem cells, including the ability to both self-renew and generate differentiated progeny, the ability to differentiate to repeat the phenotype of the tumor from which they were derived, and the activation of developmental signalling pathways such as the Hedgehog, Wnt, and Notch pathway.

However, CSC may not only be associated with tumor initiation and growth but may also play a crucial role in tumor resistance towards standard chemotherapy. Indeed, inherent resistance of stem cells to apoptosis is observed in patients undergoing cytotoxic chemotherapy. Stem cells in the bone marrow are not ablated following chemotherapy and are able to regenerate a normal haematopoietic system after several weeks. Furthermore, studies in CD34+CD38− leukemic stem cells showed that these CSCs were significantly less sensitive to daunorubicin or cytarabine than the bulk population of leukemic blast cells. Similarly, Matsui et al have shown that myeloma CSCs are more resistant to standard therapies to treat myeloma, including chemotherapy and proteosome inhibitors. In addition,
a recent report examining mechanisms of radioresistance in gliomas showed that the fraction of CD133+ CSCs within gliomas is enriched after radiation, because these CSCs have enhanced capacity for DNA repair. Taken together, these studies indicate that treatments targeted specifically to the CSC population will be required to result in an effective cure of cancer.

In the present study, we aimed to identify and to eliminate putative CSC within different human pancreatic cancer cell lines as well as within 5-Fluorouracil (5-FU) –resistant pancreatic cancer cell lines established in our laboratory.

**Fragestellung**
In the present study, we aimed to identify and to eliminate putative cancer stem cells (CSC) within different human pancreatic cancer cell lines as well as within 5-Fluorouracil-resistant pancreatic cancer cell lines established in our laboratory.

**Methoden**
Pancreatic CSC were identified and characterized by flow cytometry, using Hoechst 33342 dye staining. CSC from 5-FU-resistant cell lines were isolated by high-speed flow cell sorting (MoFlo) and the CSC self-renewal pathways were further analyzed by RT-PCR and Western blotting. Isolated CSC within 5-FU-resistant cell lines were exposed to the cancer stem cell targeted therapeutics (RAD001, Cyclopamine) and stained for Hoechst 33342. Furthermore, isolated CSC as well as non-tumorigenic cancer cells within 5-FU-resistant cell lines were orthotopically xenografted in nude mice and the efficacy of stem-cell-targeted therapy in combination with 5-FU was investigated.

**Ergebnisse**
Flow cytometry analysis revealed a significantly high amount of CSC in all chemotherapy-resistant cell lines. Combined 5-Fluorouracil IC50 and cancer stem cell targeted therapy resulted in the reduction of CSC subpopulation independently on 5-FU–chemoresistance status, as detected by staining for Hoechst 33342. In vivo the combination of 5-FU with cancer stem cell targeted therapeutics RAD001 or Cyclopamine significantly decreased the tumorigenic potential of CSC derived from 5-FU-resistant cell lines and dramatically reduced primary pancreatic tumor volume and weight.

**Schlussfolgerung**
Our results demonstrate that chemotherapy-resistant cancer cells contain the increased CSC population (as compared to their parental sensitive cells) that is highly resistant to standard chemotherapy but not towards CSC-targeted therapy in vitro and in vivo. The further characterization of such cells might therefore lead to the development of new molecular and pharmaceutical therapeutics and better anti-cancer strategies.
The role of cellular plasticity in oncogenic reprogramming

César Cobaleda

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It has traditionally been accepted that, during differentiation, developmental options are progressively restricted until commitment to a specific fate is established, and then only terminal differentiation is possible. Although this is usually the case in normal development, the latest experimental evidences indicate that the differentiated state is not always as stable as it was thought to be. A hidden plasticity has been revealed in differentiated cells which allows them to deviate towards other cell types belonging to separate developmental pathways. This plasticity has biological significance since it is necessary for normal development to occur, but it also makes possible the emergence of aberrant lineages when interferences with the normal mechanisms in charge of maintaining cellular identity occur. Of all the possible outcomes of this aberrant reprogramming, the most relevant for human pathology is cancer. If cell fate was unmovable once it has been established, cancer would be impossible. Therefore, the plasticity of the cell suffering the initial oncogenic alteration plays an essential role in cancer development, since only if this cell possesses enough plasticity a tumoral reprogramming will be possible and a tumor will develop. Also, plasticity makes it possible for differentiated cells to acquire cancer stem cell properties in the presence of the appropriate oncogenic insults. Experimental reprogramming of differentiated cells by transient expression of stem cell-specific transcription factors can reset their epigenetic status and (re)open new developmental options. We postulate that oncogenic reprogramming can act in a similar way and that, once the tumoral fate has been established and cancer stem cells have been programmed from normal cells (cancer cells-of-origin), oncogenes might be dispensable for tumour progression. Our experimental results show that this reprogramming is possible, even in the case of neoplasias in which the main tumor population is composed by cells with a mature phenotype.
The work in our group is focused on the biology of stem cells in the laboratory and in the body. One of our primary objectives is to contribute to a comprehensive understanding of the major cellular states and growth signals in mammals. Although at first this might seem an impossibly ambitious goal, there is increasing evidence that we can control cellular transitions from the starting pluripotent cell of the early embryo through fetal development and into late stages of adult life.

We have defined a new class of pluripotent cell in the mouse that shares defining regulatory features with human ES cells. In defined conditions, we have shown that human ES and iPS cells have a standard transcriptome providing a strong basis for any future work using these cells. Data will be presented suggesting that advances in stem cell biology will provide powerful new insight into cancer genetics and tissue regeneration.

Although transplantation of stem cells or their differentiated progeny is a possible therapeutic benefit of stem cell biology, our in vivo studies suggest an alternate strategy. We have shown that increasing the numbers of stem cells either by transplantation or by boosting the endogenous stem cells confers improvement in models of ischemic stroke, Parkinson’s disease and liver injury. This work suggests that endogenous stem cells can be controlled through the signaling pathways central to cancer biology. The idea that stem cells and differentiated cells are intimately linked may provide general strategies to promote recovery from degenerative disease and regulate inappropriate cell growth.

RON MCKAY received a B.Sc. in 1971 and a Ph.D. in 1974 from University of Edinburgh, where he studied under Edwin Southern examining DNA organization and chromosome structure. He received postdoctoral training at University of Oxford working with Walter Bodmer examining the first restriction-fragment-length polymorphism (RFLPs) in the human genome. In 1978, he became a senior staff investigator at Cold Spring Harbor Laboratory concentrating on two areas: developing the first immunoassay for DNA-protein complexes and establishing the field of molecular neuroscience. Joining the MIT faculty in 1984, Dr. McKay identified neural stem cells as a tool to study brain development and function. In 1993 he joined the NIH as Chief of the Laboratory of Molecular Biology at NINDS. In 2003 he was appointed to direct the NIH Stem Cell Facility. His laboratory studies pluripotent and somatic stem cells with a particular focus on the mechanisms of regeneration and cancer.


The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. Current models state that 4-6 crypt stem cells reside at the +4 position immediately above the Paneth cells in the small intestine; colon stem cells remain undefined. Lgr5/Gpr49 was selected from a panel of intestinal Wnt target genes for its restricted crypt expression. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. In addition, Lgr5 was expressed in rare cells in several other tissues. Using an inducible Cre knock-in allele and the Rosa26-LacZ reporter strain, lineage tracing experiments were performed in adult mice. The Lgr5+ve crypt base columnar cell (CBC) generated all epithelial lineages over a 14 month period, implying that it represents the stem cell of the small intestine and colon. The expression pattern of Lgr5 suggests that it marks stem cells in multiple adult tissues and cancers.

We have now established long-term culture conditions under which single crypts undergo multiple crypt fission events, whilst simultaneously generating villus-like epithelial domains in which all differentiated cell types are present. Single sorted Lgr5+ve stem cells can also initiate these crypt-villus organoids. Tracing experiments indicate that the Lgr5+ve stem cell hierarchy is maintained in organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche.

Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in in Lgr5+ve stem cells leads to their transformation within days. Transformed stem cells remain located at crypt bottoms, while fueling a growing microadenoma. These microadenomas display unimpeded growth and develop into macroscopic adenomas within 4-6 weeks. When APC is deleted in short-lived Transit Amplifying (TA) cells using a different Cre mouse, the growth of the induced microadenomas rapidly stalls. Even after 30 weeks, large adenomas are very rare in these mice. We conclude that stem cell-specific loss of APC results in progressively growing neoplasia. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the “cancer stem cell”-concept.
S07 The clinical translation of cancer stem cells in B cell malignancies

William Matsui

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

Emerging evidence suggest that many human cancers consist of phenotypically and functionally heterogeneous cell types. Moreover, only a minority cells within some tumors is capable of clonogenic growth. We have studied multiple myeloma and found that the mature plasma cells that form the majority of tumors cells are incapable of long-term proliferation. Instead, these malignant plasma cells arise from highly clonogenic and self-renewing cells that resemble normal memory B cells. We will discuss the role of multiple myeloma cancer stem cells in therapeutic resistance as well as the development of novel targeting and biomarker strategies as we move these findings into the clinic.
Acute promyelocytic Leukemia (APL) is characterized by a specific t(15;17) translocation, generating a PML/RARA fusion protein. Expression of PML/RARA, a potent transcriptional repressor induces APL in mice. Retinoic acid (RA) and arsenic trioxide, both directly target PML/RARA-mediated transcriptional repression and protein stability, inducing rapid differentiation of the promyelocytes and clinical remission of APL patients.

We have demonstrated that RA, in addition to induction of granulocytic differentiation, triggers growth arrest and progressive clearance of Leukemia Initiating Cells (LIC), both ex vivo and in vivo. Suboptimal RA concentrations or the expression of PLZF/RARA allow complete RA-induced differentiation, but neither LIC clearance nor disease remission. Thus RA-induced differentiation and LIC clearance are two uncoupled events. The RA/arsenic trioxide association, which dramatically synergises for PML/RARA degradation but not for differentiation, rapidly clears LIC in a proteasome-dependent manner, resulting in APL eradication in murine models and patients. Cyclic AMP signalling also accelerates RA-induced LIC clearance and PML/RARA degradation, which is dependent on a cAMP-regulated phosphorylation site of PML/RARA. Thus, activation of cAMP signalling could be a novel clinically achievable oncogene-targeted therapy.

Collectively, these results demonstrate that LIC clearance, that mirrors PML/RARA degradation, rather than differentiation, is the primary basis for APL cure by the RA/arsenic trioxide association. In contrast to differentiation therapy, which has never extended out of the APL model, there are several indications that oncogene degradation could be a generally applicable therapeutic strategy to clear LICs in several types of tumours.
S09  Dormancy in normal and malignant stem cells

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Adult stem are required to maintain highly regenerative tissues such as the skin, the intestinal epithelium and the hematopoietic system. Mouse hematopoietic stem cells (HSCs) are the most well characterized somatic stem cell to date, and serve as a model for understanding other adult stem cells present in the mammalian body. Using two types of label-retaining assays we have identified a long-term dormant population within the most immature HSCs (Lin-Sca1+cKit+CD150+CD48-CD34-). Computational modeling suggests that dormant HSCs (d-HSCs) divide about every 145 days, or 5 times per lifetime. d-HSCs harbor the vast majority of multi-lineage long-term self-renewal activity. While they form a silent reservoir of the most potent HSCs during homeostasis, they are efficiently activated to self-renew in response to bone marrow injury or G-CSF stimulation. After re-establishment of homeostasis activated HSCs return to dormancy, suggesting that HSCs are not stochastically entering the cell cycle, but reversibly switch from dormancy to self-renewal under conditions of hematopoietic stress¹,². One of the reasons cancer stem cells are thought to escape anti-proliferative chemotherapy is their relative dormancy³. We now have shown that treatment of mice with Interferon-alpha family leads to the activation and proliferation of dormant HSCs in vivo, which sensitizes them to chemotherapy drugs. HSCs lacking either the interferon-a/b receptor, STAT1 or Sca-1 are insensitive to IFNa stimulation, demonstrating that STAT1 and Sca-1 mediate IFNa induced HSC proliferation⁴. The implications of these results for the design of strategies to target dormant CML stem cells not targetable by imatinib alone will be discussed.

The inner layer of the intestinal tube, the intestinal epithelium, is in a constant process of renewal. Hundreds of millions of terminally differentiated intestinal cells are replaced by new cells every day during the life of an adult organism. This tremendous regenerative power is ultimately sustained by a small population of stem cells. Compelling evidence has demonstrated that intestinal stem cells (ISCs) are also the origin of colorectal cancer (CRC). At the onset of CRC, mutational activation of the Wnt signalling pathway lock ISCs into an immature phenotype that unbalances self-renewal in the epithelium. As a result, mutant ISCs give rise to adenomas, a benign type of tumor that precedes CRC. We have recently established a protocol for the isolation and profiling of different cell populations within the mammalian intestinal epithelium. These experiments have allowed us to define a subset of genes that show an ISC-restricted expression domain. Here I will discuss recent data regarding the role of ISC genes in the initiation and progression of CRC.
S11  Neural stem cells and cancer

Mahendra Rao

Life Technologies, 7335 Executive Way, Frederick, MD 21704 USA

Neural cells are present in limiting quantities in the adult brain and the phenotype of tumors seen in the CNS are distinct from those seen in early development. Two possible explanations are feasible with respect to the cell of origin for these tumors. Either a stem cell population generates tumors at all stages and changes over time or different dividing progenitors generate tumors at different stages of development. I will present our results and those of our collaborators Dr. Rich and Dr. Steindler in trying to isolate the tumor propagating cell from glioblastoma tissue and the comparison of gene expression profiles with normal stem and progenitor cells.
3rd of December at 12.30

S12  Dissecting the molecular pathways mediating self renewal of leukemic stem cells

Chi Wai Eric So

Institute of Cancer Research/King’s College London

In acute myeloid leukemia (AML) where leukemic stem cells (LSCs) have been functionally identified, the most prevalent chimeric leukemia associated transcription factors (LATFs) arise from mutations of the retinoic acid receptor (RARα), the core-binding factors (AML1 or CBFβ), the mixed lineage leukemia protein (MLL) and its downstream targets, Hox. LATFs are believed to act as the initiating events in converting normal cells into pre-LSCs with enhanced self-renewal property, which will eventually acquire additional genetic and/or epigenetic events to become LSCs. To gain further insights into the underlying transformation mechanisms, we have recently dissected the essential components associated with a number of LATFs. We demonstrated that 1) aberrant self-association is a prevalent mechanism for oncogenic activation of LATFs including MLL, RARα and AML1 fusions (Kwok et al., 2006; Kwok et al., 2009; So and Cleary, 2004); 2) recruitment of DNA binding cofactor such as RXR is essential and a potential therapeutic target for RARα-mediated transformation in acute leukemia (Zeisig et al., 2007); 3) protein arginine methyltransferase 1 (PRMT1) is a novel and indispensable epigenetic component for an oncogenic MLL fusion complex, which also provides the first direct evidence linking PRMTs to human cancer (Cheung et al., 2007), and proposes epigenetic therapies as an alternative and promising avenue for targeting LATFs in LSCs (Zeisig et al., 2008). In this talk, we will also discuss 1) critical functional crosstalk between Bmi-1 and Hox pathways in mediating normal and leukemic self-renewal; and 2) molecular pathways potentially responsible for oncogenic conversion of pre-LSCs to LSCs.

HEDGEHOG-GLI signaling in cancer and stem cells

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Hedgehog (HH)-GLI signaling modulates precursor proliferation in the late embryonic and postnatal brain and controls brain stem cell behavior in stem cell niches. This activity on precursors/stem cells in development and homeostasis seems conserved in other organs. Inappropriate HH-GLI signaling is essential for the growth of a large variety of human cancers, including those of brain, skin, prostate, lung and pancreas. Interestingly, active HH-GLI signaling is also required by glioblastoma stem cells for their self-renewal and survival. Recent data on the role of HH-GLI signaling in colon cancer and its cancer stem cells will be presented.

A framework for the integration of oncogenic inputs and loss of tumor suppressor function by the Gli code, acting as an information nexus regulating cancer stem cell behavior, will also be discussed.


Varnat, F., et al. (2009). Human colon cancer epithelial cells harbor active HEDGEHOG-GLI signaling that is essential for tumor growth, recurrence, metastasis and stem cell survival and expansion. EMBO Molecular Medicine In press.

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S14  Hedgehog signalling in skin cancer and tissue stem cells

Rune Toftgard

Center for Biosciences, Karolinska Institutet, Stockholm, Sweden.

The Hedgehog (Hh) signalling pathway ending with activation of the GLI transcription factors is of central importance during embryo development, tissue maintenance and regeneration and is implicated in control of stem cell proliferation and renewal. When aberrantly activated the Hh pathway contributes to cancer development in several tissues including the skin, brain, pancreas, breast and the prostate. Basal Cell Carcinomas (BCC) showing similarities to early hair follicles are the most common skin cancers and are dependent on deregulated Hh signalling. However, the cell of origin for BCC has remained unknown. Using mouse models conditionally expressing GLI1 or with homozygous inactivation of the \textit{Ptc1} gene we reveal that stem cells marked by Lgr5 expression and residing in the non-permanent part of the growing hair follicle represent a cell of origin. This stem cell population also contribute to wound healing and moreover, the wound environment accelerates BCC development. We find that wounding allows repopulation of the permanent part of the hair follicle and the interfollicular epidermis by progeny of Lgr5 expressing stem cells. The results provide insights into the mechanisms by which tissue injury increases the risk for cancer development.
3rd of December at 15.15

S15  Notch and hypoxia cross-talk in stem cells and cancer

Urban Lendahl

Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

We have previously shown that the Notch signaling pathway intersects with the cellular response to hypoxia (low oxygen) at multiple levels. One node of intersection is the interaction between the Notch intracellular domain (ICD) and the transcriptional regulator HIF1-alpha; a second intersection point is represented by the fact that the asparagine hydroxylase FIH-1 can hydroxylate not only HIF1-alpha but also Notch ICDs. Functional Notch signaling is required for certain aspects of the cellular response to hypoxia, including control of neural and myogenic progenitor cell differentiation and epithelial-to-mesenchymal transition (EMT) in tumor cells. With these data as a platform we have now begun to explore the consequences at the transcriptome level of the Notch-hypoxia cross-talk. In ES cells, we have identified novel downstream genes in both the hypoxia and Notch pathways, and genes that are in various manners co-regulated by the two signaling mechanisms. We also explore the role of Notch signaling in tumor formation and promotion in a xenograft mouse model for mammary tumors. Our data indicate that xenografted MCF7 cells engineered to express a high level of Notch signaling grow faster, have an increased propensity to form mammospheres and are more prone to metastasize. MCF cells with high Notch signaling also exhibit a shift from oxidative phosphorylation to glycolytic metabolism. The underpinning mechanisms for the increased tumor formation capacity and the metabolic switch are being investigated, and may, at least in part, be related to novel links between Notch signaling and the cytokine response.
S16 The role of the microenvironment in organizing and maintaining lineage-specific domains in mammary gland.

Mark LaBarge
Berkeley, USA

In adult tissues, multi-potent progenitor cells are some of the most primitive members of the developmental hierarchies that help maintain homeostasis. That progenitors and their more mature progeny share identical genomes yet reside in distinct locations, suggests that fate decisions are directed by interactions with extrinsic soluble factors, ECM, and other cells, as well as physical properties of the ECM. Utilizing a highly-parallel combinatorial microenvironment functional screening platform in combination with 3D organotypic culture systems we demonstrated that multipotent human mammary progenitor cells are surprisingly flexible in their cell fate decisions in response to different microenvironments. A number of the putative cell-extrinsic determinants of cell fate that were identified in our screens were juxtaposed to the stem cell-enriched zones of the terminal ducts in vivo, but were not necessarily inside of them. This suggests that there is a means by which progenitors can experience different microenvironments in vivo. Utilizing a bioengineered microwell culture platform we have quantified self-organization of myoepithelial and luminal epithelial cells over time, with and without perturbing influences. We propose a general mechanism by which different lineages of mammary epithelial cells sort and self-organize in vivo to maintain tissue structure and homeostasis.
The characterization of the cells that make up the mammary epithelium is essential for understanding the origins and the clinical presentation of breast tumours. To identify the cells that constitute this hierarchy, we used fluorescence-activated cell sorting (FACS) in combination with in vitro colony forming cell (CFC) assays and in vivo transplantation assays to examine the growth and differentiation properties of phenotypically distinct subsets of mammary epithelial cells. Our results indicate that in the mouse, mammary stem cells reside within the basal cell compartment and have a CD45<sup>-</sup>Ter119<sup>-</sup>CD31<sup>-</sup>(Lin<sup>-</sup>)EpCAM<sup>med</sup>CD49<sup>fhigh</sup> phenotype, whereas the transit amplifying cells appear to reside within the luminal cell compartment since they have a Lin<sup>-</sup>EpCAM<sup>high</sup> phenotype. Further analysis of the luminal cell compartment of the virgin mammary epithelium reveals that this population can be further resolved into 3 phenotypically distinct subsets of cells based on the differential expression of Sca1 and CD49b. Two of these subsets of cells are progenitors, one of which has a CD49b<sup>+</sup>Sca1<sup>+</sup> phenotype and expresses the estrogen receptor (ER) and the other has a CD49b<sup>-</sup>Sca1<sup>-</sup> phenotype and is ER<sup>-</sup>. The third and largest subset of cells is a population of ER<sup>+</sup> cells that lacks clonogenic activity and have a CD49b<sup>-</sup>Sca1<sup>-</sup> phenotype. Studies with human mammary epithelial cells reveals a similar hierarchical structure with mammary stem cells having a basal EpCAM<sup>low</sup>CD49<sup>fhigh</sup> phenotype and a large population of progenitor cells that residing within the luminal cell compartment. These studies will provide a cellular framework for understanding the origins of breast tumours.
3rd of December at 17.15

S18 Routes to breast cancer heterogeneity: Prospective cloning of functionally distinct subpopulations by use of markers from a normal human breast lineage hierarchy

Ole William Petersen

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Tumor heterogeneity is a hallmark of cancer and it is responsible for tumor progression and resistance to therapy. According to Nowell’s classical theory of clonal evolution tumor heterogeneity is caused by genetic instability and phenotypic drifting. Thus, tumors arise from a single “mutated” cell which upon subsequent additional alterations gives rise to more aggressive subpopulations within the original neoplastic clone. These cells may leave a large number of offspring by chance, or new mutations may provide a growth advantage over the other tumor cells. Waves of such clonal expansion and selection drive the process. Therefore, any cancer cell can potentially become invasive and cause metastasis. This stochastic model predicts that the evolution of cancer cells is influenced by intrinsic (e.g. signaling pathways) or extrinsic (e.g. microenvironment) factors. These influences are unpredictable or random and result in heterogeneity in the cell phenotype or in the tumor initiating capacity. A key tenet of this model is that all cells of the tumor are equally sensitive to such stochastic influences. Moreover, tumor initiating cells cannot be identified prospectively or enriched for by for sorting cells based on intrinsic characteristics.

Recently, our understanding of tumor heterogeneity has been expanded through “the hierarchy model” which predicts that cancers contain a minority population of tumor initiating cells or cancer stem cells (CSC) that resist treatment and give rise to the bulk of the more differentiated tumor cells. Thus, a tumor can be considered a hierarchy defined by a maturation process analogous to normal tissue homeostasis. Therefore heterogeneity arises as a consequence of the presence of biological distinct classes of cells with differing functional abilities and behavior within the hierarchy. As opposed to the stochastic model the hierarchy model predicts that tumor-initiating cells can be identified prospectively and purified from the bulk of non-tumorigenic population based on intrinsic characteristics. The fact that most epithelial cancers are composed of cells that retain at least some level of differentiation suggests that the cancer stem cell generates a lineage restricted progeny with a finite life span which nevertheless constitute the majority of the tumor. It follows that the bulk of the tumor would die out without being replenished from the cancer stem cells. Other than that little is known about the function of differentiated cancer cells.
Evidence will be presented here for the existence of a stem cell hierarchy in the normal breast and in breast cancer.
4\textsuperscript{th} of December at 09.00

**S19  Stem Cells and Epigenetic Reprogramming**

Felipe Pereira, Francesco Piccolo, Tomomi Tsubouchi, Stephan Sauer, Matthias Merkenschlager and Amanda Fisher

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One of the most pressing objectives of medical research today is to try and develop approaches that will restore the function of tissues damaged by accident or disease. An important goal for this work is the isolation of stem cell populations to replace missing or non-functioning cells. However unless the recipient and donor stem cells are very closely matched problems of immune rejection are likely to occur.

An alternative strategy is to convert differentiated cells (such as white blood cells) from patients into immature ‘tailored’ stem cell populations by reprogramming. Here we have fused human white blood cells and mouse embryonic stem cells to create transient experimental heterokaryons in which the human nucleus is reprogrammed to become stem cell-like. This kind of ‘differentiation reversal’ is rapid, stable and re-sets multi-lineage potential. Reprogramming requires the stem cell specific factor Oct4, but does not require the pluripotency-associated factor Sox2. Using this approach in combination with genetically engineered mouse ES cells (conditional mutants or RNAi targeted) we are currently interrogating the factors that are required to successfully directly reprogram human blood cells. In the longer-term these studies aim to define a cocktail of proteins that can be used to efficiently generate patient-specific stem cells from lymphocytes.
Medulloblastoma is the most common malignant pediatric brain tumor for which the prognosis for younger children remains poor. Improved therapies may come from a better understanding of the cellular and molecular basis of medulloblastoma. Identifying the cell of origin for a tumor is important because it allows knowledge of the normal cell to provide insight into the abnormal behavior of the tumor so that key differences and vulnerabilities can be identified and possibly exploited in developing new therapies. Furthermore, recent studies suggest that cells resembling the cell of origin may persist in mature tumors and that these so called “cancer stem cells” may be critical for propagating these tumors. If so, identifying the cell of origin and the cancer stem cell may facilitate development of more effective therapies.

We have identified the cell of origin and the cancer stem cell for medulloblastomas with mutations in the Sonic hedgehog-Patched signaling pathway. Humans with such mutations have an increased susceptibility to medulloblastoma. Moreover, patched mutant mice develop tumors that resemble human medulloblastoma, and hence represent a valuable model for the disease. We used conventional and conditional patched knock-out mice and have shown that medulloblastomas may be initiated in either granule neuron precursor (GNPs) or stem cells (NSCs), but they ultimately adopt a GNP phenotype rather than NSC phenotype. Moreover, these tumors are propagated by cells that express the GNP marker Math1 and CD15 and resemble neural progenitors. Our data challenges the notion that all brain tumors are propagated by stem-like cells and raises the possibility that CD15 may be used to identify and target cancer stem cells in brain tumors. Therefore, we conclude that the cell of origin in some patched-associated tumors is a Math1+ granule neuron precursor, and that this cell type is required not only for tumor initiation but also for tumor propagation.
Role of Polycomb repressors in stem cells, cancer and development

Maarten van Lohuizen

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Repressive Polycomb-group (Pc-G) protein complexes and the counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in the dynamic maintenance of proper gene expression patterns during development, acting at the level of chromatin structure. As such, they are important controllers of cell fate. When deregulated, these master switches of gene expression are strongly implicated in formation of a diverse set of cancers. An example is the Pc-G gene Bmi1 which is overexpressed in medulloblastoma, Non small cell lung cancer, hepatocellular carcinoma and breast cancer and Glioma and is causally implicated in leukemia. We and others have recently implicated Bmi1/Pc-G as a critical regulator of stem cell fate in hemapoietic stem cells, neural stem cells, mammary epithelial precursor cells and ES cells. In addition, we have shown that Bmi1 is regulated by the Shh pathway and that the Ink4a/ARF tumors suppressors are critical Bmi1 target genes in stem cells and in cancer formation. However our recent work on brain cancer (Glioma) points to important ink4a/ARF-independent Bmi1 targets involved in adhesion and motility. Comprehensive profiling of Polycomb target genes in Drosophila revealed its crucial conserved role in repressing lineage differentiation pathways and morphogens, including Wg, Hh, Delta and Notch. Furthermore, we have characterized in detail an essential E3-ubiquitin ligase activity in the PRC1 Polycomb complex that consists of a functional Ring1B-Bmi1 heterodimer. This E3 ligase activity is required for maintenance of Polycomb repression in normal- and cancer stem cells and hence offers potential novel ways to target cancer stem cells or tumor re-initiating cells in which the activity of this E3 ligase is hyperactivated. This is further substantiated by a novel way by which the activity of the Ring1B.Bmi1 E3 ligase is controlled. The implications of these findings for stem cell biology, development and cancer will be discussed.
4\textsuperscript{th} of December at 10.30

S22  Self renewal and lineage commitment in brain tumour stem cells

Peter Dirks

Hospital for Sick Children, University of Toronto, Toronto, Canada

Our laboratory is interested in which cells of an established brain tumor maintain the tumor’s growth. Previously, we have shown that human brain tumors contain subpopulations of cells, enriched for by sorting of cell surface markers, which demonstrate stem cell properties in vivo, as well as in vitro. Recently, we have shown that further modifications of serum free culture supports improved derivation and propagation of cancer precursor cells in vitro. These brain cancer stem cell lines maintain tumorigenic ability in vivo, allowing for chemical and genetic drug discovery research to be conducted in vitro and then confirmed after testing in vivo. In this presentation, we will present recent data on Notch and Wnt signaling in regulation of brain tumor stem cell proliferation and differentiation. Our data supports that these developmental signaling pathways are predominantly involved in lineage choice and modification of tumor phenotype.
Stem cells are characterized by their ability to create two kinds of daughter cells. On one hand, they can self-renew and create identical copies of themselves. On the other hand, they give rise to more lineage-restricted cells, which ultimately undergo terminal differentiation. We use Drosophila neuroblasts as a model system to understand, how the balance between those different types of progeny is controlled. Drosophila neuroblasts divide asymmetrically: One daughter cell retains neuroblast characteristics while the other cell divides only once more to create two terminally differentiating neurons. During each neuroblast division, the proteins Numb, Prospero and Brat segregate into one of the two daughter cells where they cooperate to prevent self-renewal and induce terminal differentiation. Mutations in these segregating determinants lead to over proliferation of the neuroblast pool and the formation of a stem cell derived tumor. To understand the biological processes and regulatory networks controlled by these factors, we have carried out a genome-wide RNAi screen for defects in neuroblast self-renewal. We use a library of transgenic RNAi lines targeting over 80% of the genes in the Drosophila genome. By expressing each of the over 20,000 RNAi constructs contained within this library in neuroblasts, we have identified over 600 genes involved in lineage specification in the Drosophila brain. We have quantified the resulting phenotypes to generate a phenotypic barcode expressing the degree of abnormality in neuroblast size, proliferative capacity and daughter cell number. Hierarchical clustering of the resulting phenotypes can classify genes by biological function and identifies, for example, 5 new genes involved in cytokinesis, 110 new genes regulating cell growth and self-renewal and 10 new tumor suppressors. How genome-wide analysis in vivo opens new avenues to understand biological processes will be the key topic of my talk.
Asymmetric cell division is a fundamental process essential for the generation of cell diversity during development and for the self-renewal of stem cells. Four steps are required to successfully achieve an asymmetric cell division: 1) a polarity cue breaks the symmetry of the mother cell; 2) cortical domains are established which define the polarity of the cell; 3) cell fate determinants are segregated along the axis of polarity; 4) the mitotic spindle is aligned along the axis of polarity such that at division cell fate determinants are partitioned in the two daughter cells (1). While we know some of the players involved in these processes, the regulation and interconnection of the signaling cascades underlying these events remain elusive.

We use the embryo of *C. elegans* as a model system to study this process.

The first division of the embryo is asymmetric, resulting in two cells that are different in size and fate (2). This asymmetry depends on the function of the conserved PAR proteins, which control localization of cell fate determinants and positioning of the mitotic spindle (3). Downstream of PAR proteins, heterotrimeric G proteins play a key role in positioning and orienting the mitotic spindle. PAR and heterotrimeric G proteins and their role in asymmetric cell division are highly conserved (4). By performing enhancer and suppressor screens we have identified new genes that control polarity and spindle positioning. I will present our current understanding on how these genes regulate such processes in the early *C. elegans* embryo.

A key feature of pluripotent stem cells is that when they divide, they must choose between self renewal and commitment to differentiation. Further, if they commit to differentiate they must choose between different lineages. Some degree of spontaneous differentiation is common in cultures of human ES cells. This can confuse studies of human ES cell behavior if assays are based on assessment of the population as a whole, without taking account of the consequent heterogeneity of such cultures. Further, a propensity for differentiation provides a basis for selective pressures that may lead to the appearance of variant ES cells that exhibit an increased probability of self renewal over differentiation, or cell death through apoptosis. Indeed human ES cell lines do accumulate non-random genetic changes on prolonged culture. These genetic changes include amplifications of chromosomes 12, 17 and X similar to those seen in embryonal carcinoma (EC) cells, the stem cells of teratocarcinomas and the malignant counterparts of ES cells. Thus the progressive culture adaptation of human ES cells in culture provides a unique model that may be pertinent to the progression of stem cell based cancers. Accumulating evidence suggests that the ‘stem cell compartment’ in both ES and other stem cells, including cancer stem cells, may be composed of distinct substates. Another aspect of culture adaption of human ES cells is that it alters the population dynamics of ES cultures, particularly affecting the behavior of substates within the stem cell compartment. Understanding the nature of these substates and their interactions may provide insights into the mechanisms that control self renewal, commitment to differentiation and lineage selection of ES and, ultimately iPS cells. Inevitably these same mechanisms may also play a role in cancer progression.
Most colorectal adenocarcinomas (CRC) have mutations in the APC gene leading to overexpression of the Wnt-pathway effector beta-catenin. By acting as a transcriptional activator, nuclear beta-catenin is involved in two fundamental processes in embryonic development: epithelial to mesenchymal transition (EMT) and generation of stem cells.

We have previously shown, that in particular tumor cells at the invasive front of CRCs accumulate nuclear beta-catenin, undergo an EMT and aberrantly express EMT-associated transcriptional repressors, like ZEB1. The amount of such cells strongly correlates with clinical outcome and metastasis formation.

We further showed that ZEB1 represses target genes involved in basement membrane formation and epithelial cell polarity. Moreover we report that ZEB1 directly suppresses transcription of members of the miRNA-200 family, which strongly activate epithelial differentiation in pancreatic, colorectal and breast cancer cells. Notably, the EMT-activator ZEB1 itself is the predominant target downregulated by these miRNAs. These results indicate that ZEB1 triggers a microRNA-mediated positive feedback-loop, which stabilizes EMT and promotes invasion of cancer cells. Strikingly, metastases show again a differentiated phenotype and lack nuclear beta-catenin, indicating a mesenchymal-epithelial re-transition (MET) and a regulatory role of the tumor environment during malignant tumor progression.

Based on the developmental functions of beta-catenin and our data we propose, that the EMT-associated tumor cells at the invasive front act as "migrating cancer stem cells" which can re-differentiate and, depending on the range of dissemination, give raise to the primary carcinoma metastases. We suggest that both primary tumors and metastases are derived from a pool of EMT-associated "migrating cancer stem cells" at the tumor host interface. They are defined by strong nuclear beta-catenin accumulation and EMT-phenotype, which gives these epithelial tumor cells a feature driving malignant tumor progression including metastasis: the unusual combination of a migratory and stem cell phenotype.
The majority of deaths from carcinoma result from the development of metastatic disease. In order to metastasize, carcinoma cells must complete a complex multistep process including invasion, entering into and distribution via the circulation, excavasation, and expansion of microscopic metastases into complete macroscopic metastases. Studies of metastases have found that select carcinoma cells that have reactivated a latent embryonic program —the epithelial-mesenchymal transition (EMT)— have increased overall metastatic potential. EMT is a complex series of cellular reprogramming events through which epithelial (i.e. early carcinoma) cells lose their epithelial characteristics and acquire mesenchymal-like characteristics. Since these mesenchymal characteristics include increased migration and invasion to epithelial cells, this aberrant activation of EMT during carcinoma progression, was typically associated with increased cancer cell dissemination (i.e. initial steps of the metastatic process). However, we recently found that EMT also endows carcinoma cells with properties similar to tumor-initiating, cancer stem cells (CSCs), and these properties may enable the expansion of disseminate cancer cells into full-fledged metastatic nodules. Thus, it now seems plausible that EMT confers carcinoma cells with most, if not all, the traits needed for metastasis. The acquisition of stem cell characteristics has demonstrated an increased importance of EMT in not only metastatic disease but also for development of resistant and refractory tumors, which have been associated with CSCs. Therefore, a better understanding of the EMT process at the molecular level is likely to yield new insights into the mechanisms of breast cancer progression and ultimately suggest new avenues for therapeutic intervention.
S28 Formation and Maintenance of Stem Cell Traits induced by EMT

Christina Scheel, Christine Chaffer, Leonardo Rodrigues, Kong-Jie Kah, Wenjun Guo, Zuzana Keckesova, Haihui Lu and Robert A. Weinberg

Epithelial-Mesenchymal Transitions (EMTs) are transdifferentiation programs that effect critical morphogenetic steps during embryonic development. We have shown that, when re-activated in the adult organism, passage through an EMT confers on transformed and non-transformed human mammary epithelial cells (HMEC) traits of cell populations enriched in stem cells. Our focus is to understand the molecular underpinnings of the link between EMT and stem cell programs in normal and cancer cells.

We have established several ways to induce HMEC into an EMT: a) through ectopic expression of EMT-Transcription Factors (TF) such as Twist, Snail and Zeb1, b) through induction of EMT by paracrine factors and c) by isolating a spontaneously arising mesenchymal/stem cell-like sub-population within HMEC. These different mesenchymal/stem cell-like HMEC lines have allowed us to survey the induction and maintenance of EMT programs within the normal tissue hierarchy and during transformation. We have derived an “EMT core gene expression signature” pinpointing pathways and a transcriptional circuitry shared in common by HMEC cells induced into EMT by alternate means: this analysis revealed the TF Zeb1 as a master regulator of the EMT program in HMEC. We are currently studying the epistatic relationships of Zeb1 with other EMT-TF active in HMEC.

Further, we are focussed on the induction of EMT programs through contextual signals. We discovered that HMEC maintain the resultant mesenchymal/stem cell-like state through mutually reinforcing autocrine loops involving the same factors that previously triggered entrance into EMT. This induction and subsequent maintenance of the EMT program is dependent on activation of Wnt and TGF-beta pathways. Finally, we have discovered that HMEC spontaneously generate mesenchymal/stem cell-like cells in culture. In conclusion, we begin to understand that HMEC retain a remarkable plasticity in culture; they break out of the hierarchical model of differentiation by moving backwards into a less differentiated state through EMT.
Poster abstracts
Breast carcinomas have previously been shown to harbor subpopulations of tumor initiating cells (TIC), with stem cell like properties (Al-Hajj, 2003). Identification and characterization of these subpopulations are of utmost importance for development and validation of new therapeutic targets in breast cancer. However, the study of such cells in solid tumors is challenging due to scarcity of fresh tumor material and technical difficulties related to tumor dissociation. Isolation of the small fraction of live TICs for downstream characterization is therefore a complex task. To identify and study TIC in breast carcinomas, we aimed to use reproducible tumor models representative for the clinical situation, and exploit the tumor heterogeneity to define subpopulations enriched for TIC. From freshly obtained human primary mammary adenocarcinoma specimens (MAS), two continuously growing xenograft models were established in immunodeficient mice; MAS 9806 and MAS9812. Molecular characterization revealed MAS9806 model as luminal-type whereas the MAS9812 model was defined as a basal-like subtype of breast cancer. Xenografts were passed orthotopically in mammary fat pads (MFP) of nude mice and the passed xenografts resembled the histopathology of the original primary tumor (Bergamashi 2009). To isolate subpopulations enriched for TIC, single cell suspensions from both xenografts were stained with antibodies towards cell surface antigens EpCam (ESA), CD49f and CD24. Cell populations were identified using FACS sorting, and subsequently in vitro functional characterization, i.e. mammosphere initiation assay, and 3D on top matrigel assay. In both xenografts three phenotypically distinct subpopulations were identified. From the luminal type xenograft, we found: EpCamlowCD49fneg, EpCamnegCD49fpos and EpCamposCD49fneg and from the basal type xenograft: EpCamnegCD49fneg, EpCamnegCD49fpos and EpCammposCD49fneg. Staining with anti-CD24 or -CD44 antibodies gave no additional definition of tumor cell subpopulations. The three different cell populations were subjected to in vivo tumorigenicity experiments in NOD SCID mice, expression analysis, and further molecular characterizations. In our study, we have, from orthotopically growing mammary adenocarcinoma xenografts, identified subpopulations of tumor cells with differential tumorigenic potential, supporting a hypothesis of TIC populations within mammary adenocarcinomas.

P32 Tumor stem cells in SV40-induced mouse mammary carcinomas: molecular pathways and practical applications

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We constructed transgenic mice expressing the SV40 early gene region under control of the WAP-promoter, which induces transgene expression specifically in the mature mammary gland (WAP-T mice). SV40 early gene expression mimics a variety of genetic lesions observed in human mammary carcinogenesis, including loss of wtp53 function. SV40 large T (LT) mediated loss of wtp53 function led to activation of c-Met expression due to abrogation of c-met repression by wtp53. c-Met not only is essential for initiation of mammary carcinogenesis, but also important for tumor progression, as the c-met gene is amplified and strongly expressed in nearly all undifferentiated tumors. To assess possible additional effects of p53 mutations, we crossed WAP-T mice with WAP-mutp53 mice (mice transgenic for mutant p53 minigens, also under control of the WAP-promoter). Co-expression of mutp53R270H resulted in increased tumor incidence, an aggravated tumor phenotype, and strongly enhanced metastasis. We identified multipotent mammary epithelial stem/progenitor cells expressing the endogenous wap-gene (and thus also the transgene) as the tumor initiating cells in our transgenic mice. Tumor initiating cells can be recovered from tumors and, after orthotopic transplantation, less than 10^2 tumor cells form tumors in syngeneic mice with a phenotype indistinguishable from endogenously growing mammary carcinomas. The data indicate that WAP-T tumors contain a high proportion of tumor stem cells. From a bi-transgenic WAP-T/WAP-mutp53 tumor, we established an SV40-transformed mammary epithelial cell line (G-2 cells) with tumor stem cell characteristics (clonality, self renewal, potency, and tumor initiation). Upon orthotopic transplantation into syngeneic mice 10^3 G-2 cells form mammary carcinomas that closely resemble endogenous tumors. G-2 cells thus provide a convenient tool for the analysis of molecular and biological properties of tumor stem cells, of tumor cell dissemination and metastasis, and are suitable for preclinical testing of drugs intended to discriminate between effects on tumor cell growth and metastatic behavior.

P31 Identification of Tumor Initiating Cell Populations from Human Mammary Adenocarcinoma Xenografts

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Breast carcinomas have previously been shown to harbor subpopulations of tumor initiating cells (TIC), with stem cell like properties (Al-Hajj, 2003). Identification and characterization of these subpopulations are of utmost importance for development and validation of new therapeutic targets in breast cancer. However, the study of such cells in solid tumors is challenging due to scarcity of fresh tumor material and technical difficulties related to tumor dissociation. Isolation of the small fraction of live TICs for downstream characterization is therefore a complex task. To identify and study TIC in breast carcinomas, we aimed to use reproducible tumor models representative for the clinical situation, and exploit the tumor heterogeneity to define subpopulations enriched for TIC. From freshly obtained human primary mammary adenocarcinoma specimens (MAS), two continuously growing xenograft models were established in immunodeficient mice; MAS 9806 and MAS9812. Molecular characterization revealed MAS9806 model as luminal-type whereas the MAS9812 model was defined as a basal-like subtype of breast cancer. Xenografts were passed orthotopically in mammary fat pads (MFP) of nude mice and the passed xenografts resembled the histopathology of the original primary tumor (Bergamashi 2009). To isolate subpopulations enriched for TIC, single cell suspensions from both xenografts were stained with antibodies towards cell surface antigens EpCam (ESA), CD49f and CD24. Cell populations were identified using FACS sorting, and subsequently in vitro functional characterization, i.e. mammosphere initiation assay, and 3D on top matrigel assay. In both xenografts three phenotypically distinct subpopulations were identified. From the luminal type xenograft, we found: EpCamlowCD49fneg, EpCamnegCD49fpos and EpCamposCD49fneg and from the basal type xenograft: EpCamnegCD49fneg, EpCamnegCD49fpos and EpCammposCD49fneg. Staining with anti-CD24 or -CD44 antibodies gave no additional definition of tumor cell subpopulations. The three different cell populations were subjected to in vivo tumorigenicity experiments in NOD SCID mice, expression analysis, and further molecular characterizations. In our study, we have, from orthotopically growing mammary adenocarcinoma xenografts, identified subpopulations of tumor cells with differential tumorigenic potential, supporting a hypothesis of TIC populations within mammary adenocarcinomas.
P33 Canine mammary carcinosarcomas express the embryonic transcription factors Sox2 and Oct4

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Our research is focused on the different phenotypes of cancer present in the breast, although carcinomas that originate from the epithelial cells dominate, sarcomas and carcinosarcomas also appear and the origin of the latter is poorly understood. Mammary carcinosarcomas are biphasic tumors with a malignant epithelial and a malignant mesenchymal component. They only appear in women and in female dogs (Misdorp et al 1999). Three different hypotheses on their origin have been described (Zhuang et al 1997): i) the collision theory stating that two independent tumors are located adjacent to each other; ii) the combination theory where stem cells/multipotent progenitor cells are the origin; iii) the conversion or metaplastic theory where the basal/myoepithelial cells by metaplasia or transdifferentiation change their phenotype.

We investigated four primary canine mammary carcinosarcomas and their corresponding cell lines. As expected, the epithelial cells in all four tumours strongly expressed keratins, Interestingly, all primary tumours contained cells that expressed neurofilaments. Similar results were obtained when spheroids from the established cell lines were investigated. These results lead us to study the presence of the transcription factors Sox2 and Oct4, which are expressed in embryonic stem cells. All primary tumours expressed Sox2, studied by immunohistochemistry, whereas fewer tumour cells were positive against Oct4. Tumour cells in all spheroids expressed Sox2, studied by Western blots and immunohistochemistry and Oct4, studied by immunohistochemistry, but to a lesser degree.

In conclusion, our results are in favour of stem cells present in mammary carcinosarcomas. However, their role in the tumorigenesis has not been delineated yet.

References

P34 The role of HMGA2 in invasiveness and stemness in breast cancer

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Recent studies elucidated the presence of cancer stem cells (CSC) that have the ability to regenerate tumors. These CSCs share some characteristics with normal stem cells, including self-renewal and differentiation capacity. In breast cancer, it has been reported that a subpopulation of CD44+/CD24low contains CSCs. HMGA2 is a member of the high-mobility group A (HMGA) family that encodes a small chromatin-associated protein. HMGA2 is widely expressed in undifferentiated cells during embryonic development whereas it is rarely detected in normal adult tissues. HMGA2 is abundantly expressed in some tumors, and high expression of HMGA2 is correlated with occurrence of metastasis and poor prognoses in some human cancers. Several mechanisms linking HMGA2 with oncogenic activity are reported. We have reported that HMGA2 is required for the induction of epithelial-mesenchymal transition in cooperation with TGF-β.

To assess the role of HMGA2 in invasiveness and stemness in breast cancer, we stably transfected short hairpin RNA against HMGA2 into MDA-MB-231 cells. The abilities of migration and invasion are reduced in shHMGA2 transfected cells, which suggests that high tumor HMGA2 levels correlate with enhanced malignant properties in breast cancer cells. More than 85% of MDA-MB-231 cells exhibit the CD44+/CD24- phenotype, which is not affected by knockdown of HMGA2. However, shHMGA2 transfected cells form significantly less primary and secondary mammospheres. As clonogenic mammosphere assays measure the self-renewal capacity of CSCs, our results suggest that HMGA2 may control specifically the self-renewal ability of breast CSCs.
P35 Analysis of NANOG, OCT-4 and SOX-2 function in human breast stem/progenitor cells

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Estrogen is required for the normal physiology of the mammary gland and is also implicated in tumorigenesis. We have characterized human breast/progenitor cells and investigated the role of estrogen in their growth and differentiation. To date, the relevance of the embryonic stem cell markers NANOG, OCT-4 and SOX-2 in the human mammary gland has not been addressed. Therefore, we determined their expression in breast stem/progenitor cells and their possible involvement in the regulation of self-renewal and/or differentiation. To address these questions we investigated the expression of NANOG, OCT-4 and SOX-2 in double positive (DP) cells co-expressing the luminal and myoepithelial markers EMA and CALLA, respectively, and in double negative (DN) cells not expressing these markers, which we previously reported to be candidate stem/progenitor cell populations (Clayton et al, 2004). In parallel, we assessed the expression of these markers in suspension cultures (mammospheres) using normal and cancer cells from patients. We found that expression of stem cell markers was elevated in breast stem/progenitor cells compared to in differentiated cells and their expression was reduced upon induction of differentiation. In addition, overexpression of Nanog and Sox-2 in MCF-7 breast cancer cells, in particular, increased the percentage of stem/progenitor cell populations. Finally, estrogen reduced the pool of stem/progenitor cells in primary normal and cancer cells and inhibited self-renewal in mammosphere assays, while tamoxifen had opposite effects. This work should contribute to deepen our understanding of mammary stem cell biology.

P36 Axl is an essential epithelial-to-mesenchymal transition-induced regulator of breast cancer metastasis and patient survival

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Abstract: Metastasis underlies the majority of cancer-related deaths. Hence, furthering our understanding of the molecular mechanisms that enable tumor cell dissemination is a vital health issue. Epithelial-tomesenchymal transitions (EMT) endow carcinoma cells with enhanced migratory and survival attributes that facilitate malignant progression. Characterization of EMT effectors is likely to yield new insights into metastasis and novel avenues for treatment. We show that the presence of the receptor tyrosine kinase Axl in mammography-detected primary breast cancers independently predicts strongly reduced overall patient survival, and matched patient metastatic lesions show enhanced Axl expression. We demonstrate that Axl is strongly induced by epithelial-to-mesenchymal transition in pre-malignant mammary epithelial cells that establishes an autocrine signaling loop with its ligand, Gas6. Using epi-allelic RNA interference analysis in metastatic breast cancer cells we delineated a distinct threshold of Axl expression for mesenchymal-like in vitro cell invasiveness, and to form tumors in foreign and tissue engineered microenvironments in vivo. Importantly, Axl knockdown completely prevented the spread of highly metastatic breast carcinoma cells from the mammary gland to lymph nodes and several major organs, and increased overall survival, in two different optical imaging-based experimental breast cancer models. Thus, Axl represents a novel downstream effector of tumor cell EMT that is required for breast cancer metastasis. The detection and targeted treatment of Axl-expressing tumors represents an important new therapeutic strategy for breast cancer.
P37 The contribution of drug resistant cancer stem cells to paediatric brain tumours

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A significant proportion of paediatric brain tumours do not respond to chemotherapy and are categorized as high-risk, others respond only to therapy only to later relapse. Drug resistance is therefore a major confounding factor in the treatment of paediatric brain tumours. We have laid the foundation for proving the hypothesis only a sub-population of cells is responsible for driving paediatric brain tumour progression and relapse. Seven newly derived paediatric brain tumour cell lines, representative of a broad range of tumour types, were fully characterised. These cells lines have retained genomic changes present in the original tumour; they contain a cancer stem cell component (defined by membraneous CD133 expression and nuclear Sox2 expression); they are able to undergo limited multi-lineage differentiation; and they can readily form tumours in vivo. Our ability to continuously derive CSC enriched neurospheres from standard monolayer cultures means that we can assay therapeutic response in a robust system that is true to the tumour of origin, but also representative of that tumour type. To investigate the drug resistance potential of our cell lines, the clonogenic survival of monolayers and neurospheres to the topoisomerase II poison etoposide was tested. Apart from bOLIG1, neurosphere derived cells were significantly more resistant to etoposide compared to monolayers (p<0.001). We have demonstrated endogenous expression of ABCB1 and ABCC1 in all 7 lines using western blotting, flow cytometry and immunofluorescence. We then treated cells with clinically employed drugs that are known to be substrates of ABC drug transporters (etoposide, irinotecan, cisplatin and methotrexate). Drug resistant clones expressed CD133 and show increased expression and function of ABCB1 and in some cases ABCG2 (although only after several rounds of treatment). This data indicates that ABCB1 plays a key role in intrinsic drug resistance and that ABCG2 mediated resistance may be acquired upon tumour progression.

P38 Cancer stem cells in Glioblastoma, what are they?

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Glioblastoma multiforme (GBM) is one of the most heterogeneous tumours, both at the genetic and the cell morphology level. It has been proposed that only a subset of cancer cells display stem cell properties and are tumourigenic in vivo (cancer stem cells, CSCs). However there is now growing evidence that expression of a putative stem cell marker, such as CD133, cannot define the only GBM subpopulation with tumour initiating capability. A number of studies have also shown that tumour initiation depends on the microenvironment and the animal model used, rather than being an intrinsic property of a subpopulation of tumour cells. In this project we aim to characterize subpopulations of tumour cells with stem cell characteristics from GBM xenografts and determine whether these cells are a subpopulation of the tumour (bonafide CSCs) or represent a changing entity adapting to the signals from the microenvironment. To address this question we apply flow cytometry to identify and characterize small subpopulations of cells within a highly heterogeneous tumour population, according to cell surface and internal markers and according to their drug efflux properties (side population). We have set up an immunodeficient GFP expressing mouse xenograft model, that recapitulates the invasive and angiogenic features of human GBM (Niclou, 2008). The use of a GFP mouse allows to distinguish between tumour and host cells, an important aspect since both populations could include cells with stem cell properties. The presence of several putative CSC markers as well as lineage specific epitopes is examined within the tumour and host cell populations.
P39  BMI-1 is a marker for poor prognosis in oligodendroglioma

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BMI-1 is a component of the polycomb repressive complex 1. This protein complex is involved in the epigenetic regulation of gene expression. In neural stem cells, the effects of BMI-1 maintain pluripotency. During the last decade, the theory of cancer stem cells has emerged, suggesting that in brain tumors, including gliomas, there exists a subpopulation of tumor cells with stem-cell like characteristics. Applying BMI-1 as a potential marker for tumor stem cells, we investigated the protein expression of BMI-1 and the BMI1 gene locus in a series of 305 clinical glioma samples.

We found that in grade 2 and 3 oligodendroglioma and oligoastrocytoma, a high frequency of BMI-1 expression is clearly associated with poor outcome. We show that the median survival time was 191 months if the expression of BMI-1 was low, whereas the median survival was only 68 months if BMI-1 expression was high. To control for confounding factors, BMI-1 protein expression was compared with clinical features. The independent nature of BMI-1 expression as a prognostic marker was established using a multivariate Cox regression analysis. We also found that BMI1 deletions were associated with poor outcome in grade II-IV astrocytomas and glioblastomas.

We conclude that the polycomb factor BMI-1 seems essential for the malignant progression of brain tumors, suggesting a potential molecular target in the treatment of gliomas, especially by inducing differentiation pathways in cancer stem cells.

P40  CD95 triggers invasion of glioblastoma cells

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Invasion of surrounding brain tissue by isolated tumour cells represents one of the main obstacles to a curative therapy of glioblastoma multiforme (GBM). We have recently shown that tumour interaction with the surrounding brain tissue induces CD95 Ligand expression. Binding of CD95 Ligand to CD95 on glioblastoma cells recruits the Src-family member Yes and the p85 subunit of phosphatidylinositol 3-kinase (PI3-K) to CD95, thereby leading to inhibition of glycogen synthase kinase 3-β pathway. Subsequent activation of matrix metalloproteinases finally results in increased invasion of the tumoral cells into the surrounding brain parenchyma. In a murine syngenic model of intracranial GBM, neutralization of CD95 activity dramatically reduced the number of invading cells in the non tumour-bearing hemisphere. To further confirm the clinical relevance of these results, we have now isolated tumour initiating cells (TICs) from GBM patients. In a xenotransplant mouse model, these cells developed invasive tumours after 1 to 16 weeks. Secondary derived tumours showed a more aggressive phenotype which included a dramatic increase in proliferation and stemness and subsequently of the time to give rise to a tumour – time was always halved. Triggering CD95 in TICs induced recruitment of the PI3-K subunit p85 and inhibition of glycogen synthase kinase 3-β. These signalling events resulted in higher migration potential of TICs. These results underline the clinical relevance of CD95 in GBM invasion and strengthen the need of anti-CD95 agents as front therapy for GBM.
**P41**  
Generation of an Ependymoma Cell Line With Stem Cell-Like Properties

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Nonresectable ependymomas are associated with poor prognosis despite intensive radio- and chemotherapy. The molecular pathogenesis of ependymoma initiation and progression is largely unknown, and novel treatments are difficult to develop due to the lack of appropriate pre-clinical models. We here report on the generation of a first ependymoma cell line (termed DKFZ-EP1NS) with long term self-renewal capacity. Using serum-free neurosphere media conditions, we were able to isolate ependymoma cells growing in spheres, which were then characterized for genetic aberrations, marker expression and tumorigenicity in an orthotopic xenotransplant model.

The DKFZ-EP1NS cells were kept in neurosphere culture for up to 9 months, displaying long term self-renewal. Freezing for storage and thawing was routinely possible as with other cell lines. Genetic aberrations (loss at 1p36, chromosome 9 and 14q) found in the DKFZ-EP1NS corresponded to the aberrations discovered in the primary ependymoma as well as in subsequent recurrent tumors of the patient. DKFZ-EP1NS cells display several markers associated with normal as well as cancer stem cells. Orthotopically transplanted mice displayed first tumors after 9 months in the striatum of the brain, and tumors phenotypically recapitulated the original tumor. Serial transplantation yielded secondary tumors in half the time. Interestingly, the orthotopic niche seems to be required for the induction of a heterogeneous tumor histology, since subcutaneous and intraperitoneal transplantation did not recapitulate the original intracranial ependymoma phenotype. In conclusion, we were able to establish a first ependymoma cell line with stem-cell like properties recapitulating human disease in an orthotopic xenograft model, paving the way to pre-clinical evaluation of drugs targeting the cancer stem cell compartment in ependymoma.

**P42**  
NEIL3: A Repair Enzyme Homologue Involved in Proliferation and Differentiation of Neural Stem Cells

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The major cell types comprising the mammalian central nervous system (CNS) are derived from neural stem cells (NSCs). Here we characterize a novel gene function, Neil3, which seems to be involved in neural stem cell maintenance. Although Neil3 is a homologue to the DNA glycosylases Neil1 and Neil2, which are involved in the base excision repair pathway, no DNA glycosylase activity has been found for Neil3 and its molecular function is unknown. We show here that during embryogenesis in mice, Neil3 is highly expressed in the subventricular zone (SVZ), known to harbour neural stem/progenitor cells. The expression peaks at the same time as neurogenesis starts (12-13 days after fertilization) and decreases during development. We further demonstrate that neurospheres derived from newborn Neil3⁻/⁻ mice show impaired ability to proliferate and that overexpression of Neil3 in wild-type neurospheres blocks gliogenesis. Taken together our results suggest that Neil3 is not a classical glycosylase but play an important role in maintenance and differentiation of stem/progenitor cells in the brain.
P43  ALDH1 Positive Glioblastoma Cells Show Brain Tumor Stem Cell Capacity  

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Glioblastoma (GBM) is the most aggressive primary brain tumor and resistant to all therapeutic regimens. Relapse occurs regularly and might be caused by a poorly characterized tumor stem cell subpopulation escaping therapy. We suggest aldehyde dehydrogenase 1 (ALDH1) as a novel stem cell marker in human GBM. Using the neurosphere formation assay as a functional method to identify brain tumor stem cells we show that high levels of ALDH1 in established glioblastoma cell lines facilitate neurosphere formation. In contrast cell lines without expression of ALDH1 are not capable of forming tumor spheroids. Inhibition of ALDH1 in vitro decreases both, the number of neurospheres and their size. High levels of ALDH1 keep tumor cells in an undifferentiated, stem cell-like state indicated by low expression of Beta-III-Tubulin. In addition we found ALDH1 expression to a varying degree in primary cultures obtained from fresh tumor samples and by immunohistochemistry in GBM tissue specimens.

P44  Regulation of glioblastoma tumorigenicity by TGFβ and BMP  

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Glioblastoma (GBM) is the most malignant brain tumour for which all therapies are ineffective. Glioma initiating cells (GICs) appear as new promising therapeutic targets. Still, very little is known about the regulatory mechanisms that control their biology. Transforming growth factor beta (TGFβ) is associated with a poor prognosis in this disease, favoring tumor growth, invasiveness and sustaining the renewal of the GICs whereas bone morphogenic proteins (BMP) show opposite effects, inducing GICs to differentiate and therefore to loose their tumorigenicity.  

We characterized a glioblastoma cell line that contains GICs and responds to both TGFβ and BMP stimulations. We investigate the molecular pathways by which TGFβ and BMP exert their specific effects on GICs self-renewal and differentiation, respectively. We want to identify specific targets downstream of TGFβ and BMP that could provide new strategies to deplete GBM from their tumorigenic cells. Major regulators of the epithelial to mesenchymal transition (EMT) induced by TGFβ have been recently linked to stem cell biology. We propose to establish whether EMT mediators are involved in the regulation of GICs and in the invasive phenotype characteristic of GBM. Finally, TGFβ induces important changes in the extracellular matrix (ECM) that contributes to GBM progression. We propose to investigate whether the ECM participates to maintain GICs and therefore favor their spreading within the host environment. We expect that this work will help in the understanding of the regulatory mechanisms involved in GICs biology.
P45 Highly infiltrative brain tumours show reduced chemosensitivity associated with a stem cell-like phenotype

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Aims: Cancer stem-like cells might have important functions in the development of chemoresistance. We have developed a model where highly infiltrative brain tumours with a stem-like phenotype were established by orthotopic transplantation of human glioblastomas to immunodeficient rats. Serial passaging in the animals gradually transformed the tumours into a less invasive and more angiogenic phenotype (high-generation tumours). The invasive phenotype (low-generation tumours) were characterized by an increase in stem cell markers and increased phosphorylation of kinases in the phosphatidylinositol 3-kinase (PI3K)/AKT pathway. These markers were reduced in the serially passaged vascular tumours. The present study was aimed at investigating how the two phenotypes responded in vitro to doxorubicin, a clinically potent cytotoxic drug for solid tumours. Methods: Biopsy spheroids were implanted and passaged intracranially in nude rats. Gene expression and protein analyses were performed, and drug sensitivity was assessed. Results: Microarray analysis revealed gene ontology categories connected to developmental aspects and negative regulators of differentiation, especially in the infiltrative stem cell-like tumours. The highly invasive low-generation stem-like phenotype was chemoresistant compared to the more angiogenic and aggressive high-generation phenotype. Moreover, by interfering with the PI3K/AKT signalling pathway it was possible to sensitize tumour spheroids to drug treatment. Finally, real-time quantitative PCR showed down-regulation of the stem cell markers Nestin and Musashi-1 in low-generation biopsy spheroids following PI3K inhibition. Conclusions: Highly invasive tumours with a stem-like phenotype are more chemoresistant than angiogenic tumours derived from the same patients. We suggest that treatment resistance in glioblastomas can be related to PI3K/AKT activity in stem-like tumour cells, and that a targeted interference with the PI3K/AKT pathway might differentiate and sensitize this subpopulation to chemotherapy.

P46 ADAM (A Disintegrin And Metalloprotease) expression in the human brain tumour stem cell niche

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The brain tumour stem cells are less dependent on their niche than neural stem cells and this may be through differential cell adhesion properties. Thus, adhesion molecules may be key elements in stem cell proliferation and tumour development. Our research analyzes the adhesion molecules expression in neural and brain tumour stem cell niches. We are particularly interested in the ADAM genes, which have been suggested to play important roles in the development of the nervous system, regulating proliferation, migration, differentiation and survival of various cells. ADAMs are known to be expressed in the brain and some ADAMs seem interesting to study in the brain tumour stem cell niche: ADAM2 is expressed in the hippocampus and ependyma(1); ADAM8-10-12-15-17-19 are enriched in glioblastomas and ependymomas(2,3); ADAM10 is essential for Notch signalling(4); ADAM17 is expressed in neuroblastas(5) and activates epidermal growth factor receptor(6,7); ADAM21 is expressed in glioblastomas(3); ADAM22-23 are present in the brain(8-10). These proteins have an adhesion domain receptor for integrins, and disrupt cell-matrix interactions. Some ADAM proteins also have a protease domain.

We analyzed ADAM expression by qRT-PCR on mouse stem cells samples and showed that ADAM10&17 are enriched in neuroepithelial and tumour spheres, as well as control samples. We also investigate which cells in tumour express ADAMs before exploring an eventual role of these molecules in the proliferation of brain tumour stem cells.

P47 Astrocytes stimulated by thyroid hormone promote hippocampal stem cell growth and neuronal differentiation

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Postnatal thyroid hormone deficiency leads to selective damage of the hippocampal stem cell niche in vivo, while the subventricular niche is unaffected. These neurogenic niches are strongly influenced by local astrocytes. The aim of the present study was to determine whether tri-iodothyronine (T3) stimulation of hippocampal and subventricular zone (SVZ) astrocytes would influence neural stem cells (NSCs) from the corresponding regions through secreted soluble factors. Astrocytes were derived from postnatal day 1 (P1) rat brains and NSCs from P21 brains. Hippocampal and SVZ astrocyte-conditioned media were collected after T3 (50nM)-treatment and used for culturing NSCs from the same region. As control, conditioned medium (CM) collected from untreated astrocytes was supplemented with the same amount of residual T3 found in the T3-treated CM. Hippocampal astrocyte-derived CM after T3-treatment resulted in increased NSC expansion due to increased proliferation and decreased cell death, whereas no effect of SVZ conditioned medium was observed. However, migration of neurosphere cells was increased in both regions by T3-treated CM. Quantitative PCR of T3-treated astrocytes displayed increased mRNA levels of Bdnf, Noggin, Wnt3a and thyroid hormone receptor alpha (Tr-alpha) in the hippocampal cell system, but not in the SVZ system, whereas Pedf was increased only in the SVZ cell system. mRNA levels of Vegfa and thyroid hormone receptor beta (Thrβ) were increased in both systems. In addition, VEGFA protein levels were increased in the CM from both regions. These results suggest that a lack of soluble factors from astrocytes could contribute to the selective damage of hippocampal neurogenesis under conditions of thyroid hormone deficiency.

P48 TNF-RELATED APOPTOSIS-INDUCING LIGAND AND HEAT SHOCK PROTEIN 90 INHIBITION EXERT A SYNERGISTIC EFFECT ON COLON CANCER STEM CELL DEATH INDUCTION

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We recently demonstrated the existence of a small population of tumorigenic stem like cells in colon cancer, which is included in the high-density CD133-positive cell population, accounting for about 2.5% of the tumor cells. These CD133+ cells can be unlimitedly expanded in serum free culture conditions in the presence of specific growth factors. Both freshly-isolated and cultured CD133+ cells are able to reproduce the original tumor upon injection into immuno-compromized mice and appear as an optimal preclinical model for colon cancer.

TNF-Related Apoptosis-Inducing Ligand (TRAIL) has attracted great attention in recent years as a promising anti-cancer reagent because its recombinant soluble derivatives are able to induce apoptosis in a broad range of tumor cells, with a very limited toxicity in normal cells. Heat Shock Protein 90 (HSP90) is a molecular chaperon implicated in the cell cycle regulation and cell survival promotion. In particular, HSP90 seems to promote cell survival by stabilizing anti-apoptotic proteins. Thus, the inhibition of HSP90 would down-regulate the function of anti-apoptotic proteins and promote apoptosis. HSP90 is efficiently inhibited by 17-(Allylamino)-17-demethoxygeldanamycin (17AAG), which binds with a high affinity to the ATP binding pocket of Hsp90.

On the basis of our in vitro high-throughput drug screening, which point to HSP90 as a potential therapeutic target in colon cancer stem cells, we decided to examine the possibility to sensitize these cells to TRAIL induced apoptosis via HSP90 inhibition. Colon cancer stem were treated with TRAIL either as a single agent or in combination with 17AAG. The results revealed a remarkable synergistic effect of these two compounds on cell death induction, as assessed by in vitro viability assays. Such synergistic effects were confirmed in cancer stem cell-based xenografts where the combination of TRAIL and 17AAG resulted in a significant reduction of tumor size, considerably higher than those observed with standard chemotherapy. These findings may provide useful information for the development of novel therapeutic approaches aimed at directly targeting the tumorigenic population of colon cancer.
P49 Identification of Modifiers of Intestinal Tumourigenesis in the APC\textsuperscript{Min} Mouse using Mouse Chromosome Substitution Strains

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Genetic differences in human individuals are known to confer a major effect on disease susceptibility, such as developing cancer. However, due to the complex polygenic nature of cancer susceptibility, means of assessing the influence of the individual genetic background in humans on the lifetime risk of developing cancer, and/or prognosis, are severely limited for want of further knowledge of tumour initiation processes. Insight into the genetic requirements for cancer initiation and development has been gained from studies in mice, in particular, the mouse model of human colon carcinogenesis, the APC\textsuperscript{Min} mouse. As differing genetic backgrounds of mouse strains are known to influence disease phenotypes, such as tumor multiplicity, we have set up a screen to identify novel global modifiers of the APC\textsuperscript{Min} induced tumor phenotype in the mouse intestine using Chromosome Substitution Strains (CSSs). These mice carry single chromosomes of the PWD/Ph (PWD) strain on a C57BL/6 (B6) background. Modifiers of tumour size and incidence following the introduction of PWD derived chromosomes in APC\textsuperscript{Min} mice (F1) are clearly evident from our first analyses, thus demonstrating the suitability of this system for dissecting the genetic foundations of intestinal tumourgenesis. Furthermore, via massive parallel studies of the genome-wide changes in the transcriptome and epigenetic regulators of gene control - such as DNA methylation and histone modifications, from ‘normal’ and ‘tumour’ material derived from mice and humans, we also aim to isolate key epigenetic modifiers involved in colon cancer initiation, progression and stem cell function. We anticipate the identification of multiple genetic traits affecting cancer initiation and progression. Ultimately this work will provide a means to define low- and high-risk groups among patients, and accordingly tailor treatment and follow-up regimes.

P50 Eradication of colon cancer stem cells by EpCAM/CD3-bispecific BiTE antibody MT110

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Keywords: EpCAM, CD3, cancer stem cell, T cell-engaging, antibody, BiTE, MT110, immunotherapy

With their resistance to genotoxic and anti-proliferative drugs and potential to grow tumors and metastases from very few cells, cancer stem or tumor-initiating cells (CSCs) are a severe limitation for the treatment of cancer by conventional therapies. Here, we explored whether human T cells that are redirected via an EpCAM/CD3-bispecific antibody called MT110 can lyse colorectal CSCs and prevent tumor growth from CSCs. MT110 recognizes EpCAM, a cell adhesion molecule expressed on CSCs from diverse human carcinoma, which was recently shown to promote tumor growth through engagement of elements of the \textit{wnt} pathway.

We used primary human colon cancer stem cells (pCSC) expressing Oct 4, nestin, telomerase, SSEA 3/4, AP, CA199, and were also found to express cancer stem cell surface markers CD133, CD44, CD166 and EpCAM. Subcutaneous injection of as few as 100 pCSC cells into NOD/SCID mice led to tumor formation in 4/4 injections. PBMC or CD\textsuperscript{8} T cells were used as effector cells for redirected lysis via MT110. To determine the degree of lysis of pCSCs, we plated lysis reactions on soft agar and observed outgrowth of colonies, a hallmark of pCSCs. MT110 can lyse colorectal CSCs and prevent tumor growth from CSCs. MT110 recognizes EpCAM, a cell adhesion molecule expressed on CSCs from diverse human carcinoma, which was recently shown to promote tumor growth through engagement of elements of the \textit{wnt} pathway.

Eradication of colon cancer stem cells by EpCAM/CD3-bispecific BiTE antibody MT110 appears to have high potential for eradication of EpCAM-expressing CSCs.
P51  Impact of FGF Signals on Gastrointestinal Tumor Progression

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Abstract

Epithelial-to-mesenchymal transition (EMT) is an essential transdifferentiation process in development and EMT-like phenomena are associated with tumor metastasis. Recent studies have demonstrated that the induction of EMT in neoplastic cells results not only in the acquisition of mesenchymal traits but coincidently in the expression of stem cell markers.

In an RNAi-based loss of function phenotypic screen we tested approx. 400 metastasis candidate genes in human colon cancer SW480 cells. We isolated 24 genes, including Fgf9, whose expression was essential for the maintenance of a mesenchymal, potentially metastatic phenotype. In line with the RNAi screen, we demonstrated that blockade of the FGF receptors resulted in re-epithelialization and blocked cell motility. We delineated signaling cascades and transcriptional networks downstream of the Fgf receptor in colon cancer cells. Interestingly, we found that Fgf receptor signals also impinge on the regulation of stemness of intestinal cells.

Taken together, our results demonstrate multiple roles for Fgf signals in the intestine. We will discuss implications for tumor progression.

P52  The kinase TNIK is an essential activator of Wnt target genes

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Wnt signalling maintains the undifferentiated state of intestinal crypt/progenitor cells through the TCF4/β-catenin activating transcriptional complex. In colorectal cancer activating mutations in Wnt pathway components lead to inappropriate activation of the TCF4/β-catenin transcriptional program and tumourigenesis. The mechanisms by which TCF4/β-catenin activate key target genes are not well understood. Using a proteomics approach, we identified Tnik, a member of the germinal centre kinase family as a Tcf4 interactor in the proliferative crypts of mouse small intestine. Tnik is recruited to promoters of Wnt target genes in mouse crypts and in Ls174T colorectal cancer cells in a β-catenin-dependent manner. Depletion of TNIK and expression of TNIK kinase mutants abrogated TCF-LEF transcription, highlighting the essential role of the kinase activity in Wnt target gene activation. In vitro binding and kinase assays demonstrate that TNIK directly binds both TCF4 and β-catenin and phosphorylates TCF4. siRNA depletion of TNIK followed by expression array analysis demonstrated that TNIK is an essential, specific activator of Wnt transcriptional program. This kinase may present an attractive candidate for drug targeting in colorectal cancer.
P53  Identification and localization of human intestinal stem cells (hISCs) and expression studies of key regulatory members in homeostasis maintenance
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The small intestine is composed of contiguous villi and crypts. Villi consist of three types of mature epithelial cells; the enterocytes, the goblet cells and enteroendocrine cells. In addition to defensin-producing Paneth cells, the crypts are mainly occupied by undifferentiated cells, and have long been known to contain a functional stem cell compartment. Still, the exact location of the intestinal stem cells (ISCs) has remained controversial. This has primarily been due to the lack of unique molecular markers. Recent studies have identified a single marker, Lgr5/GPR49, a leucine-rich orphan G-protein-coupled receptor, that specifically labels stem cells in the mouse small intestine as well as other adult tissues (Barker et al., 2007). In this study we have identified Lgr5+ cells in human small intestine and colon, and the results suggest a possible difference in localization of hISCs in the small intestine and colon. In other cellular systems prostaglandin E2 (PGE2) are reported to have regulatory effects on stem cell proliferation (Kleiveland et al., 2008). PGE2 is also known to affect epithelial cell proliferation and regulation in response to injury. Constitutive cyclooxygenase 2 (Cox-2) expression was demonstrated by immunohistochemistry in colonic epithelium, suggesting that PGE2 are continuously being produced and secreted in the vicinity of Lgr5+ cells and may thus influence proliferation and differentiation processes of putative colon hISCs. Surprisingly, when performing laser microdissection (LMD) and qRT-PCR on pure crypt cell populations (and cells of the lamina propria) both from normal and inflammatory tissue, we detect a different expression of prostaglandin EP receptors in the small intestine versus colon. This is most likely reflecting significant differences in EP receptor signaling and function in these tissues. Our observations add new information to the understanding of the interplay between Cox-2, PGE2 and the EP receptors in the intestinal epithelium.

P54  Polycomb repressive complex 1 maintains compact chromatin structure independent of histone ubiquitination
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Mechanisms controlling higher-order chromatin structure are poorly understood. Here we show that polycomb repressive complexes are required to maintain a compact chromatin state at Hox loci in embryonic stem (ES) cells. In contrast with global chromatin decompaction in cells deficient in linker histones, there is specific decompaction of polycomb target loci in the absence of PRC2 or PRC1 activity. This is due to PRC1, since decompaction occurs in Ring1B null cells that still have PRC2-mediated H3K27 methylation. Moreover, the ability of PRC1 to maintain a compact chromatin state is independent of the histone ubiquitination activity of Ring1B. Consistent with this, we observe a dosage dependent effect in Ring1B heterozygous cells with an intermediate opening of Hox higher-order chromatin structure. The fact that this does not simply lead to an activation of Hox gene expression in ES cells, leads us to suggest that PRC-mediated chromatin compaction acts to co-ordinate the correct response of target loci to differentiation signals.
P55 Utilising culture adaptation of human embryonic stem cells as a model for oncogenesis
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Human embryonic stem (hES) cells have been shown to acquire genetic changes during prolonged maintenance in vitro. These changes can increase their growth capacity in culture, and as such these mutant cells are considered culture adapted. Culture adaptation must result from an increase in propensity for self-renewal, and it is of note that the karyotypic changes commonly observed in culture adapted hES cells are also frequently seen in embryonal carcinoma (EC) cells, the stem cells of germ cell tumours, and the malignant counterparts of ES cells. Assuming that such changes provide stem cells with an intrinsic growth advantage, we have focussed on a particular genetic change (amplification of 17p11.2) common to two culture adapted hES cell lines, and identified candidate genes which may be implicated in stem cell fate.

P56 DNMT3B plays a role in RA-induced neuronal differentiation of human embryonal carcinoma cells
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Dynamic change of global DNA methylation has been identified to be crucial for early embryogenesis especially before preimplantation stage, during which embryonic stem cells are derived. However, the role of de novo DNA methylation during ES cell self-renewal and differentiation mostly remains unknown. In this study, we used a doxycycline-inducible shRNA system to investigate the function of DNMT3B, one of the de novo methyltransferases, in undifferentiated and retinoic acid (RA)-induced differentiating human embryonal carcinoma cells NTERA2/D1. We have found that knockdown of DNMT3B significantly affects the expression of stage-specific embryonic antigens including increasing of SSEA1 and decreasing of SSEA3 and TRA-1-60. In addition, cell cloning efficiency is remarkably reduced upon DNMT3B knockdown. However, expression of the two key pluripotency specific transcription factors OCT4 and NANOG have not been changed by DNMT3B knockdown. These evidences indicate that DNMT3B might contribute to the stability of stem cell state. NTERA2/D1 cells are capable of differentiating into a post-mitotic phenotype with features of mature neurons upon exposure to 10⁻⁸ M RA. In order to investigate the role of DNMT3B during differentiation, we induced the cells with 10⁻⁸ M RA for three weeks after DNMT3B was knocked down. Interestingly, knockdown of DNMT3B dramatically enhances the yield of TUJ1 positive neurons. By knocking down DNMT3B at different time points during RA induced differentiation, we have also identified that neuronal differentiation has only been enhanced when the knockdown was carried out at the early stage of differentiation, indicating that DNMT3B might involved in the initiation of neuronal lineage commitment.
P57 Epigenetics of human embryonic and embryonal carcinoma stem cells

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Teratocarcinoma is a sub-type of non-seminoma testicular germ cell tumor. They comprise of embryonal carcinoma cells and teratoma, the stem cells and their differentiated derivatives, respectively. Ex vivo culture of embryonal carcinoma cells shows a low tendency of the stem cell differentiation. Most embryonal carcinoma cell lines derived from teratocarcinoma possess this phenotype in vitro, and they have been defined as nullipotent stem cells. On the other hand, an embryonal carcinoma cell line namely NT2/D1 can be induced to differentiate considering them as pluripotent stem cells. To date the mechanism by which most embryonal carcinoma cell lines acquire after in vitro culture to maintain their nullipotency, the inability to differentiate, has not been reported. To test whether the differentiation-suppressive epigenetic factors are up-regulated in the nullipotent stem cells, we compared the protein expression profile of DNMT3B between N2102Ep line, which is the nullipotent stem cell used for the reference of the pluripotent-like state, NT2/D1 and human embryonic stem cells. We showed that the nullipotent N2102Ep cells exhibited the highest expression level of DNMT3B. In addition, the expression level of EZH2, SUZ12 and RBBP4, the subunits of polycomb repressive complex 2 (PRC2), was similar. Surprisingly, EED was not detected in both nullipotent and the malignant pluripotent stem cells by mean of immunoblotting, whereas its transcript existed. The level of tri-methylation of histone H3 lysine 4, 9 and 27 was also comparable between N2102Ep and NT2/D1. This result suggests the involvement of DNMT3B but not PRC2 for the establishment of the nullipotency. Further studies have been focusing a role of DNMT3B in maintaining the nullipotent state.

P58 Epigenetic and genetic programs of osteogenic mesenchymal stem cell differentiation: a genome-wide integrative approach

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During malignant transformation the finely tuned processes of differentiation and proliferation get out of balance. Understanding the normal balance is central in both oncology and normal biology. We here aim to identify genetic and epigenetic programs that control stemness and govern normal bone formation during mesenchymal development. These programs will be compared to those identified in bone cancer, specifically osteosarcomas, in order to reveal cancer specific events.

To study normal bone development, we have generated an immortalized human mesenchymal stem cell line that can be differentiated to the osteogenic lineage, evidenced by upregulation of osteogenic specific genes, induction of alkaline phosphatase activity and calcium deposition. Using high-throughput technology we have identified a subset of mRNAs and miRNAs that are up- or downregulated during osteogenic differentiation. The CpG methylation status of more than 14,000 gene promoters, including more than 900 cancer-related genes and 144 methylation hot-spots in cancer, have been mapped. Another epigenetic layer of information is contained in the covalent modification of histone N-terminal tails. Using chromatin immunoprecipitation combined with next-generation sequencing technology (ChIP-Seq) we are mapping the genome-wide profiles of histone H3 tri-methylation of lysines K4, K9 and K27 and acetylation of K9 at various stages of differentiation. Using bioinformatic approaches the different layers of information will be integrated to reveal regulatory networks governing stemness and osteogenic differentiation. In parallel, a corresponding set of data are being generated from a large panel of osteosarcoma cell lines, xenografts and primary tumors. Ultimately, data from normal and cancer cells will be compared to identify a set of genes specifically changed in osteosarcomas with the epigenetic regulatory mechanisms underlying cancer specific deregulation. Finally, we aim to develop a molecular staging tool for osteosarcomas, based on their differentiation status in the mesenchymal developmental hierarchy.
P59 Integrative analysis of genome-wide genetic and epigenetic changes in human osteosarcomas

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Osteosarcomas are the most common primary malignant tumors of bone. As part of EuroBoNeT (www.eurobonet.eu), an EU Network of Excellence on bone tumors, we have access to a large collection of clinical samples and resources for preclinical studies. One such resource is a collection of 20 osteosarcoma cell lines, which are being thoroughly characterized in the same way as the clinical samples.

Using different microarray technologies, we have analyzed genetic and epigenetic changes genome-wide in the EuroBoNeT panel of 20 human osteosarcoma cell lines. DNA copy number changes have been mapped at high resolution using the Affymetrix Genome-Wide Human SNP Array 6.0, and we have identified a number of highly recurrent regions. In addition, we have analyzed over 27,000 CpG sites for methylation status using Illumina HumanMethylation27 BeadChip. Global miRNA expression data have been obtained using Agilent miRNA Microarray, whereas genome-wide mRNA expression data have been analyzed using Illumina HumanWG-6 Expression BeadChip.

By integrating different levels of genome-wide information, i.e. DNA copy number changes, loss of heterozygosity, mRNA and miRNA expression and DNA promoter methylation, we aim to identify important genes and transcriptional networks for osteosarcoma development. The identified genes and networks will be investigated in a tumor panel at a later stage. The EuroBoNeT osteosarcoma cell line panel will serve as a well-characterized genetic and epigenetic model system for basic and preclinical studies.

P60 In search for stem cells in myxoid/round cell liposarcoma, MLS/RCLS

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Soft tissue tumours constitute a large and heterogeneous group of neoplasm. They are derived from mesenchymal tissues i.e. fibrous tissue, adipose tissue, bone tissue, skeletal muscle, blood vessels, and peripheral nerves. The most common type of soft tissue tumors are liposarcomas. Among these tumors the myxoid liposarcoma/roundcell liposarcoma, MLS/RCLS is one of the most common.

MLS/RLS have unique histological and cytogenetic features. More than 95% of MLS/RLS carry the FUS-DDIT3 and a few per cent carry the EWS-DDIT fusion oncogene.

MLS grows inter-or intramuscularly with a strong predilection for the thigh region. In skeletal muscle there is a distinct population of myogenic progenitors called satellite cells. These cells specifically express neural cell adhesion molecule, NCAM (CD56, Leu-19) on the surface and can be used for separation of a myogenic cell culture into NCAM positive populations consisting of satellite cells and NCAM negative populations containing a heterogeneous group of cells.

The aim of the study is to investigate adipogenic differentiation in normal skeletal muscle tissue cells to further understand the transformation to lipoblastic tumor development in this compartment. Micro array analysis of gene expression in NCAM positive and negative muscle cells and MLS/RCLS cells revealed that MLS/RCLS cells express genes specific for both NCAM positive and NCAM negative cells.
P61  Isolation and Characterisation of Mesenchymal and Epithelial CSC

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A tumour consists of a heterogeneous mixture of cells. The “cancer stem cell” hypothesis suggests that a small population of stem-like cells (CSC) continuously produce the differentiated cancer cells that make up the bulk of a tumour. CSCs have stem cell like properties, such as the ability to self-renew to produce more stem cells when the pool is depleting, for instance after damage, or to produce progenitor cells which can differentiate into the required cell types in response to damage or homeostasis. Furthermore, CSCs can function as a “seed” of a new tumour, either causing relapse or metastasis. This is due to enhanced activity of membrane pumps and enzymes that confer resistance to toxic reagents, mediating resistance to chemotherapy. Moreover, CSCs are quiescent or slowly cycling and enter the cell cycle only to produce more stem cells if the pool is depleting, or to produce progenitor cells which can undergo rapid cell divisions and differentiation into required cell types in response to damage or homeostasis.

We take advantage of these characteristics to isolate CSC from osteosarcoma and liposarcoma cell lines, xenografts and patient material. Moreover, the stem cells have also certain surface epitopes that we utilize for enrichment of the CSCs.

CSCs from epithelial tumours harbour mesenchymal traits and the trans-differentiation of epithelial cells into mesenchymal cells (EMT) induce CSC properties. The architectural transcription factor HMGA2 has been shown to play key roles in regulation of stem-ness and differentiation during embryogenesis, and recently also in CSCs. HMGA2 is normally only expressed during embryogenesis, but is aberrantly re-expressed in cancers where the expression correlates with tumour aggressiveness. Interestingly, the HMGA2 protein is necessary and sufficient in transforming growth factor-beta (TGF-β)-induced EMT, and we are investigating the role of HMGA2 during the generation of CSC through EMT.

P62  Epigenetic basis for oncogenic transformation of bone marrow mesenchymal stem cells

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Spontaneous oncogenic transformation of mesenchymal stem cells (MSCs) occasionally takes place in vitro and is potentially a risk factor in the context of expansion of stem cells and their use in therapeutic intervention. Our goal is to elucidate this transformation process from an epigenetic angle. Focus is on the epigenetic modifiers establishing and maintaining histone modifications and CpG methylation patterns and on changes in genomic distribution of these marks. We show that transformed MSCs (TMCs) display upregulation of both mRNA and protein levels of the PcG proteins EZH2 and SUZ12, as well as the histone deacetylases SIRT1, HDAC1 and HDAC2. TMCs also show increased mRNA levels of DNMT1 and DNMT3B. In correlation to this, tumor suppressor genes with promoter CpG islands known to be silenced by DNA hypermethylation or EZH2 (PRC2) in cancer, such as E-cadherin, p16, p14ARF and MGMT, are repressed in TMCs. The tumor suppressor p21 and RIZ1, a histone methyltransferase for H3K9 that is important for gene silencing of oncogenes, are transcriptionally downregulated upon transformation. To account for mechanisms controlling transcriptional deregulation during MSC transformation, we are setting up a gene-focused and genome-scale mapping of DNA methylation and histone modification, before and after transformation.
P63  Spontaneous malignant transformation of mesenchymal stem cells is associated with a partial mesenchymal to epithelial transition

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We have previously reported the spontaneous malignant transformation of mesenchymal stem cells isolated from human bone marrow (1). The transformed mesenchymal stem cells (TMC) have an epithelial phenotype with high cytokeratin expression. They fulfill several characteristics of a mesenchymal to epithelial transition (MET), such as downregulation of mesenchymal markers, upregulation of epithelial markers, decreased mobility/invasiveness and reorganization of the cytoskeleton.

MET is often referred to as the reverse of epithelial to mesenchymal transition (EMT), however, the mechanism of MET is not well known. MET is an essential developmental process and has been extensively studied in kidney organogenesis and somitogenesis. However, it has been suggested that MET also is the mechanism used by metastasizing cancer cells when forming a secondary tumor.

We have characterized the transformed mesenchymal stem cells, and found that they are morphological similar to epithelial cells. Several epithelial markers, such as various cytokeratins and syndecan-1, are upregulated in TMC. These molecules are not expressed by normal human mesenchymal stem cells (hMSC). In comparison to hMSC, TMC show increased cell proliferation and decreased cell mobility.

TMC continue to express some mesenchymal markers, relating them to their cell of origin. We believe that this is partly related to the in vitro situation and lack of stimulation from an in vivo microenvironment. TMC express both vimentin and N-cadherin, however, these are not restricted to mesenchymal cells, as these molecules are also found in some epithelia. TMC do not show the classical N- to E-cadherin switch, however, they do show a weak downregulation of N-cadherin as well as a significant downregulation of cadherin-11. We are currently investigating how this pattern of expression is influenced by the microenvironment in an in vivo model.


P64  Identification and analysis of canine and feline cancer stem cells

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The recently described cancer stem cell theory opens up many new challenges and opportunities to identify targets for therapeutic intervention. However, the majority of cancer related therapeutic studies rely upon rodent models of human cancer that rarely translate into clinical success in human patients. Naturally occurring cancers in dogs and cats have a cancer incidence similar to humans (1 in 3) and share biological features, including molecular targets, telomerase biology and tumour genetics. Our central hypothesis is that canine and feline cancers are stem cell diseases. Here we show that primitive cells can be isolated from canine and feline cancer cell lines by cell culture methods and by magnetic sorting for the putative cancer stem cell markers CD34 and CD133. Concurrently these cells express the stem cell markers (Oct4 and Nanog) and show resistance to cytotoxic effects of common cancer chemotherapy drugs and ionising radiation. This data indicates that failure of clinical therapy to eradicate tumours in dogs and cats is through the survival of cancer stem cells, which we have shown to be resistant to conventional therapies. Similar results have been found in human studies. We propose that combining rodent studies with comparative cancer studies in the cancer bearing pet population, may offer the opportunity to develop a greater understanding of cancer biology in the natural setting and evaluate the development of novel therapies that could translate into human medicine.
P65  Quantitative Measurement of the Activation of Signaling Pathways Using Two-Color Infrared Fluorescent Western Blotting and Cell-Based Assays

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There are more than 500 protein kinases and 100 protein phosphatases encoded in the human genome. Protein phosphorylation/dephosphorylation by these kinases and phosphatases is a critical process regulating almost every signal transduction event. The study of protein phosphorylation has largely relied on conventional Western blot and enzymatic kinase assays. Since both assays can assess only one target at a time, an additional step is usually needed for data normalization. We have developed methods employing two-color infrared fluorescent technology for the analysis of signal transduction events. With three different systems (INF-γ, ERK1/2, and Stat3), we have demonstrated the capability of two-color Western blots to simultaneously detect both the phosphorylated protein and the total protein regardless of its phosphorylation status. Furthermore, we have extended the assessment of the phosphorylation state of the EGF receptor using simultaneous direct two-color immunostaining of stimulated and unstimulated cells. Dose-response curves demonstrated that the quantitative cell-based assay is sensitive, highly reproducible and linear over a wide dynamic range. The ability to monitor the activation of signaling pathways by in vitro and in situ assays should greatly facilitate the characterization of pharmacological inhibitors and target validation of specific molecules within the cell.

LICOR Biosciences GmbH will be attending the venue with a booth. You are welcome for a discussion.

P66  Slow Cycling Cancer Stem-like Cells in Pancreas Adenocarcinoma

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Evidence suggests that multiple tumors, including pancreatic adenocarcinoma, display heterogeneity in parameters that are critical for tumor formation, progression and metastasis. Understanding heterogeneity in solid tumors is increasingly providing a plethora of new diagnostic and therapeutic approaches. In this study, focus was put on identifying a subpopulation of stem cell-like, slow cycling tumor cells in pancreas adenocarcinoma cell lines. Using a label retention technique a subpopulation of slow cycling cells (DiI+/SCC) was identified and further evaluated in the BxPC-3 and Panc03.27 cell lines. These slowly cycling cells managed to retain the lipophilic labeling dye DiI, while the bulk of the cells (>94%) did not. The DiI+/SCC population, showed only a partial overlap with the CSC markers CD24+/CD44+, CD133+ and ALDH but they survived chemotherapeutic treatment, and were able to recreate the initial heterogeneous tumor cell population. DiI+/SCCs exhibited an increased invasive potential as compared with their non-label retaining, faster cycling cells (DiI-/FCC). They also had an increased tumorigenicity and displayed morphological changes suggesting that they had undergone an epithelial to mesenchymal transition (EMT). Analysis of DiI+/SCC cells by RT-PCR revealed a selective up-regulation of tell tale components of the Hedgehog/TGFβ pathways, as well as a down-regulation of EGFR, combined with a shift in crucial components implied in EMT. Furthermore, in vivo studies, designed to examine DiI+/SCC and their niche within the confines of the tumor microenvironment, yielded similar results. DiI+/SCC colonies from tumors harvested after 44-54 days were Ki67 negative, expressed the mesenchymal markers N-cadherin and vimentin, and displayed an increased expression of Shh as well as a decrease in EGFR expression after immunofluorescent analysis. Further work to define the DiI+/SCC niche is currently in progress. The presented findings offer an expanded mechanistic understanding that associates tumor initiating potential with cycling speed and EMT in pancreatic cancer cell lines.
P67 Ion homeostasis and cancer
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Specific ion channels are induced in tumors and cancer stem cells and our lab has previously shown that some of these ion channels control self-renewal in stem cells via the DDR pathway (Andäng et al., Nature, 2008). Proliferation was shown to be controlled via a negative feedback loop, by GABA acting on the Cl- ion permeable GABA-A receptor (GABR) ion channel, and may be self-regulatory. Our new data show that the sodium pump, Na,K-ATPase, as well as the K+ ion channel ERG, regulate the cell cycle via overlapping mechanisms.

The cardiac glycoside ouabain is present naturally in the human body and inhibits the Na,K-ATPase pump. We found that low levels of ouabain (<10% pump inhibition) control Na,K-ATPase activity in cancer cells mediating a fully reversible cell cycle block via the DDR pathway without causing DNA damage or apoptosis. Oubain inhibition of pump activity may thus be considered a novel cell cell signaling mediated tumor supressor mechanism.

The ERG gene family of K+ ion channels, has been reported to be upregulated in glioma and implicated in cell proliferation. ERG1 is abundantly expressed in several tumors (such as neuroblastoma) and in GFAP+ glial cells - the putative origin of glioma. Our recent data show that ERG activity is critical for cell survival in stem cells and cancer stem cells. Erg inhibition activated the DDR pathway, blocked cells in S-G2/M -phase and caused induction of apoptosis in a sequential manner.

P68 Effects of Bone Marrow derived Mesenchymal stromal cells on proliferation and expression of stemness related genes in Melanoma cell lines
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Background: Mesenchymal stromal cells (MSCs) play an important role in the development or inhibition of tumor growth. It has been observed that MSCs could specifically migrate into the stroma of metastatic melanomas, but their effects on tumor cells are unclear. In this study we investigated the impact of MSCs on proliferation, phenotype and gene expression profile of melanoma cells in vitro.

Methods: Effects of co-culture with MSC or their conditioned media were tested on HBL and D10 melanoma cell lines. Cells were co-cultured for 6 days and cell proliferation was evaluated by differential cell counting, cell cycle was analysed by PI staining of DNA, immunophenotyping of E Cad, CD133, CD44 and CD24 cell surface markers was performed by flow cytometry and stemness related gene expression was measured by quantitative real time PCR.

Results: Co-culture of HBL and D10 melanoma cells with MSCs resulted in a significant dose dependent inhibition of their proliferation. This inhibition was characterized by G0/G1 cell cycle accumulation and was also mediated by MSC conditioned media. The expression of E Cad, CD133 and CD44 surface markers significantly decreased in both cell lines during co-culture with MSC. Expression of OCT4, KLF4, ABCG2, ABCB5 and Nanog in melanoma cells was not modified upon co-culture with MSC, whereas ABCB5 and CD133 gene expression was down regulated. These effects also appeared to be independent from cell-to-cell contact, since they also occurred when co-cultures were performed in trans well plates.

Conclusions: Our results provide the first evidence that MSCs may affect steps of melanoma development including proliferation, expression of cell surface markers as well as stemness related genes. Future studies are warranted to clarify the potential biological role of MSC in melanoma and to characterize soluble factors mediating their effects.
P69 Malignant melanoma cells with tumourigenic properties are common and can not be distinguished by the stem cell marker ALDH

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Tumour initiation and therapeutic resistance in various cancers has lately been linked to the presence of rare cells, cancer stem cells (CSC), which can be distinguished by specific markers and can give rise to non-tumourigenic cells, reflecting hierarchical cell organisation. In malignant melanoma, however, conflicting results have been reported regarding the presence of such cells raising a doubt whether melanoma follows a CSC model. Using Melmet in vitro cultures and in vivo xenografts as models, we have shown that a large fraction, 20-60% of random single melanoma cells display high clonogenicity, self-renewal and abilities to form non-adherent spheroids, i.e properties associated with tumourigenic potential. Besides, unsorted melanoma cells, particularly those derived from spheroid cultures, demonstrated rather efficient tumour initiation in vivo. The proposed “CSC markers” like Aldehyde Dehydrogenase (ALDH), which identifies tumourigenic CSC-like subpopulations in several other cancers, in melanoma could not distinguish clonogenic/tumourigenic cells from cells lacking these abilities. Although, melanoma models and clinical material often harboured a large distinct ALDH+ subpopulation, both ALDH+ and ALDH- cells could generate clones and initiate tumours efficiently. All together indicates that random melanoma cells from different subpopulations possess tumourigenic properties, which opposes the notion about hierarchical cell organisation in this cancer. In conclusion, melanoma cells with characteristics linked to tumourigenicity are common rather than rare and are not restricted to subpopulations expressing proposed “CSC markers”. These observations seem to contradict the traditional view of rare CSCs, but are in line with the biological and clinical features of malignant melanoma – exceptional aggressiveness and notorious resistance to therapy.

P70 Human skin carcinoma cells develop a stem cell hierarchy

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Human epidermis is a permanently renewing tissue and its architecture is maintained by the ability of the interfollicular epidermal stem cells to self-renew and to generate a hierarchy of proliferative and differentiated cells. This is a tightly regulated process and it is presently unclear whether in tumours this process is abrogated or maintained. Having established a long-term organotypic culture model (OTC), we recently developed a modification to allow detection of human epidermal stem cells by their ability to proliferate rarely and thus to maintain label for extended periods – the so-called label retaining cells (LRCs). Taking this approach further, we asked whether human skin carcinoma cells, the immortal but non-tumorigenic HaCaT cells and their Ha-ras-dependent tumorigenic variants (HaCaT-ras II-4 and HaCaT-ras RT3) would establish a similar stem cell hierarchy as normal keratinocytes. These studies showed that non-tumorigenic HaCaT cells cultivated in OTCs develop an epidermis-like epithelium very similar to that of normal keratinocytes. Interestingly, also the HaCaT-ras II-4 cells, which form locally invasive squamous cell carcinomas when injected s.c. into nude mice, develop a highly organized and well differentiated epidermis-like epithelium with LRCs in the basal layer of the epithelium. On the other hand, the HaCaT-ras RT3 cells which are able to metastasize in vivo, form an epithelium where tissue organization is largely disturbed. Nevertheless, label experiments identify LRCs randomly localized within the epithelium. These studies unequivocally demonstrate that also tumour cells of different stages of malignancy establish a stem cell hierarchy with only few undifferentiated slowly cycling LRCs, and a majority of proliferating and differentiated cells. Suggesting that the LRCs might be the cells resistant to chemotherapeutic treatment, this human model gives us a unique opportunity to study tumour recurrence and tumour stem cells.
**P71 Stromal fibroblasts enhance the self renewal properties of transformed oral keratinocytes**

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**Objective:** The aim of this study was to investigate the role that stromal fibroblasts, one of the major components of tumour stroma, have on self renewal properties of transformed epithelial cells derived from normal mucosa, oral dysplasia and oral squamous cell carcinoma (OSCC).

**Materials and Methods:** Single cells from oral cancer and dysplasia-derived cell lines and primary OSCCs were investigated for their clonogenic potential on various substrates. Student's t test was used to analyze the data using the SPSS15 software package.

**Expression of various stem cell-related markers was assessed by qPCR and immunohistochemistry.**

**Results:** The number of colonies formed in the clonogenic assay at 1 cell per well in 48 well plates, that estimates the self-renewing properties of epithelial cells grown in vitro in culture, was not significantly different between the various conditions used to grow carcinoma-derived cells from established cell lines. The percentage of clonogenic cells increased significantly when dysplasia-derived cells, and primary normal and carcinoma oral epithelial cells were grown on primary fibroblasts. Similarly, when cells were grown on extracellular matrix derived from fibroblasts. CD44 and other stem cell-related markers (Oct4 and Bmi-1) were found by qPCR up to 5 times fold up-regulated in colonies of transformed keratinocytes isolated by laser microdissection from co-cultures with stromal fibroblasts than cells from colonies captured from monocultures grown alone.

**Conclusion:** This study brings functional and molecular evidence for enhanced self-renewal properties of transformed oral epithelial cells after interaction with stromal fibroblasts.

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**P72 Transforming growth factor beta (TGF\(\beta\)) increases expression of both epithelial-mesenchymal transition (EMT) and stem cells markers in oral carcinomas**

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**Background:** TGF\(\beta\) is known to induce EMT in epithelial cells. More recently it has been shown that it also has a potential for enhancing self-renewal properties of cancer cells.

**Aim:** To test the ability of TGF\(\beta\) to simultaneously enhance the population of self-renewing cells and the EMT, in both normal and carcinoma-derived oral epithelial cells.

**Materials and methods:** Primary cells isolated from normal oral mucosa and oral squamous cell carcinomas (n=4) were collected from patients after informed consent. Cells were grown in standard culture conditions, but without calcium or epidermal growth factor, and treated with TGF\(\beta\) (1ng/ml). After 72h, cells were lysed, RNA was extracted and qPCR performed for EMT and stem cell related markers. For immunohistochemistry, cells were grown on cover slips, fixed after 5 days in 4% buffered formalin, and double immunostained for CD44 and vimentin.

**Results:** Expression of both EMT and stem cell-related markers showed, as detected by qPCR, considerable variation between cells from different patients both in controls and in their reaction to TGF\(\beta\) treatment, ranging from no difference between treated and non treated samples to 8 folds difference for some of the EMT markers. Of note is that similar differences were detected in samples from normal mucosa. Immunostaining showed a significant increase in the number of cells positive for vimentin (p= 0.020) and CD44 (p= 0.008), used as EMT and stem cell markers, respectively. Double immunostaining revealed that TGF\(\beta\)-treated carcinoma cells expressed significantly (p= 0.001) higher proportion of double CD44 and vimentin positive cells as compared with non-treated controls.

**Conclusions:** Although there were considerable differences between the responses of each cell strain to TGF\(\beta\) treatment, oral carcinoma cells showed increase in the concomitant expression of EMT and stem cell-related markers after TGF\(\beta\) treatment.
P73  Cancer-associated fibroblasts promote epithelial mesenchymal transition associated with stemness in prostate carcinoma cells

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Carcinoma are the most frequent human malignant tumors and several lines of evidence support the notion that the growth and the invasive potential of carcinoma cells are influenced by host stromal cells, collectively called “reactive stroma”. The aim of this study is to explore the role of cancer-associated fibroblasts (CAFs) in epithelial mesenchymal transition (EMT) of prostate carcinoma cells. EMT is a key developmental program, leading to achievement of a motile/invasive phenotype of tumor cells, often activated during cancer invasion and metastasis. We used CAFs from human patients with prostate benign adenomas or aggressive carcinomas. Analysis of the reciprocal interplay between prostate carcinoma cells and CAFs revealed a key role of tumor cell-derived interleukin-6 (IL-6) in fibroblasts activation. In turn, prostate carcinoma activated fibroblasts, through secretion of metallopeptase (MMP)-2 and -9, elicit a clear EMT in cancer cells, correlated with increased invasion through proteolytic degradation of ECM. The reciprocal interplay between CAFs and tumor cells was also demonstrated in vivo: we found that only tumor cells stimulated by CAFs gave tumors and allow lung micrometastases. In agreement, CAF-induced EMT leads prostate carcinoma cells to enhance expression of stem-cell markers, as well as their ability to form prostaspheres and to self-renew. In general terms, our findings suggest that the paracrine interplay between CAFs and cancer cells leads to an EMT-driven gain of stem cell properties, thus enhancing their aggressive characters.

P74  Irradiation response in prostate cancer stem cells

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A paramount issue with prostate cancer is secondary tumours (often metastases) that are resistant to current chemotherapies and radiotherapies, an explanation for which may be the existence of cancer stem cells (CSCs). Prostate CSCs are a rare subset of cells that are defined by a combination of cell surface markers (CD133+/α2β1 integrinhi/CD44+). Prostate CSCs are capable of self-renewal, differentiation and invasion in vitro (Collins et al., 2005) and tumour initiation in vivo (unpublished data).

We determined the response of primary prostate CSCs to ionizing radiation (IR) ex vivo. Primary prostate (benign and cancer) epithelial stem (SC, CD133+/α2β1 integrinhi/CD44+) and committed basal (CB, CD133+/α2β1 integrinhi/CD44+) cells were exposed to 2Gy of IR to induce a DNA damage response in each cell population. Immunofluorescence microscopy was used to quantitate DNA damage response proteins (γ-H2AX, 53BP1, phosphorylated ATM/ATR substrates, phospho-Chk2Thr68) as intranuclear foci at initial and residual sites of DNA breaks. In both BPH and cancer cells, we found that the SCs consistently had a lower percentage of cells containing initial foci (30min post-IR), compared to the TA and CB cells. At 24h post-irradiation there was a reduced percentage of cells positive for foci and reduced numbers of foci in the TA and CB cells in almost all samples suggesting signs of repair. Whilst there were also these signs of repair in BPH SCs, in the prostate cancer SCs there was an increase in the percentage of cells positive for foci at 24h, indicative of a delayed or defective DNA damage response in prostate CSCs.

Further studies, including comet assays to measure DNA damage directly and apoptosis and clonogenic recovery assays to measure survival of PCSCs compared to the other cell types, are now in progress.

P75 Characterization of Cancer-Initiating Cells derived from prostate malignancies

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Objective: Cancer-Initiating Cells (CIC), also called Cancer Stem Cells, might represent novel targets of therapeutic relevance. However their relative rarity and the frequently small size of prostate cancer (PCA) clinical specimens prevent their use in studies addressing functional features and sensitivity to drugs. In this context, the use of established cancer cell lines could represent a convenient alternative. Here, we investigated the presence and the characterization of CIC in PCA cell lines and clinical specimens.

Materials and methods: In vitro studies were performed on PC3, Du145 and LNCaP PCA cell lines. Spheroid formation was explored by using polyHema coated plates, or culture in serum-free media. Surface marker expression was assessed by flow cytometry, Aldehyde dehydrogenase (ALDH) activity with Aldefluor® technology, and clonogenic capacity by limiting dilution analysis. Expression of “stemness” genes was evaluated by quantitative rtPCR. In vivo experiments were performed by subcutaneous injection of tumor cells in NOD/SCID mice.

Results: Expression of CSC markers was found to be heterogeneous among the different cell lines and the clinical specimens investigated. CD133+ cells were virtually undetectable in the 3 cell lines. CD44+/CD24- subpopulation of Du145 cell line displayed a higher expression of Oct4A, ABCG2 or Nanog genes as compared to its double positive counterpart. This subset did not show improved clonogenic or spheroid formation capacities. Both subsets equally induced tumor growth in all injected mice. In contrast, ALDH1 bright DU145 cells expressed higher levels of stem-associated genes and displayed an increased tumorigenic capacity in vivo, as compared to the ALDH1 low subpopulation. Interestingly, a sizeable ALDH1 bright population could also be detected in PCA clinical specimens.

Conclusions: These results indicate the possible presence of CIC cells in an established PCA cell line. Expression of CIC markers appears to be associated with a higher expression of stemness genes of potential clinical significance.

P76 Expression of stem cell marker CD133 during neuroendocrine transdifferentiation of prostate cancer cells

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Patients with prostate cancer usually benefit from the conventional anti-androgen therapy at the beginning of the treatment. But the disease recurs in more severe and aggressive manner and cancer cells become androgen-independent. The role of neuroendocrine cells and process of neuroendocrine transdifferentiation (NED) in acquisition of androgen-independence is still under investigation.

Several studies show that there is a subpopulation of stem-like cells within the prostate cancer cells. This subpopulation can be usually characterized by expression of CD133 or CD44 antigen. In the present study we characterized expression of CD133 in LNCaP cells cultivated in the absence of androgens and undergoing NED. Interestingly, NED leads to expression of vimentin, potential marker of senescent cells. Unlike expression of NED marker neuron-specific enolase, which increases in whole population, the expression of vimentin increases only in subpopulation of transdifferentiated cells. Using detection of marker of senescence - senescence associated β-galactosidase we confirmed that induction of NED is associated with senescence.

Using flow cytometry, we detected expression of CD133 in subpopulation of LNCaP cells. Size of this subpopulation is not affected a lot by androgen ablation. Using cell sorter, we sorted out this subpopulation and examined other characteristics of these cells.

Out results show that NED leads to expression of vimentin in subpopulation of transdifferentiated LNCaP cells and to induction of senescence. We observed a subpopulation of CD133 positive cells in LNCaP cell line and we examined potential changes in this subpopulation in response to induction of NED.

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P77 Normal and malignant epithelial cells with stem-like properties have an extended G2 cell cycle phase that is associated with apoptotic resistance

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Background
Cells with stem-like properties have been isolated from epithelial cancers and their resistance to apoptosis-inducing stimuli has been related to carcinoma recurrence. The aim of this study was to investigate the mechanisms of resistance to apoptosis-inducing agents of cells with stem-like properties in both normal and malignant human epithelia.

Methods
Cells isolated from fresh human head and neck carcinomas (n=11), cell lines derived from head and neck, prostate and breast human carcinomas (n=7), and from normal human oral mucosa (n=5), were exposed to various apoptosis-inducing stimuli (UV, Tumour Necrosis Factor, Cisplatin, Etoposide, and Neocarzinostatin). Flow cytometry for CD44 and epithelial-specific antigen (ESA) expression, colony morphology, tumour sphere formation and rapid adherence assays were used to identify the subset of cells with stem-like properties. Apoptosis, cell cycle and expression of various cell cycle checkpoint proteins were assessed (Western Blot, qPCR). The role of G2-checkpoint regulators Chk1 and Chk2 was investigated by use of debromohymenialdisine (DBH) and siRNA.

Results
In both cancer biopsies and carcinoma cell lines the subset of cells with stem-like properties showed a significantly lower rate of apoptosis and a significantly higher proportion of cells in G2-phase of the cell cycle. Pulse-chase with iododeoxyuridine (IdU) demonstrated that CD44high carcinoma cells spent longer time in G2, even in un-treated controls. These cells expressed higher levels of G2 checkpoint proteins, and their release from G2 with DBH or Chk1siRNA increased their rate of apoptosis. Low passage cultures of normal keratinocytes were also found to contain a subset of CD44high cells showing increased clonogenicity, and a similar pattern of G2-block associated with apoptotic resistance.

Conclusions
These data indicate that both normal and malignant epithelial cells with stem-like properties show greater resistance to apoptosis associated with extended G2 cell cycle phase, and that this property is not a consequence of neoplastic transformation.

P78 Effect of MAPKs and PI3K on function of MDR-associated ABC transporters

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There are three main types of ABC transporters in mammals that were found to cause multidrug resistance (MDR) of cancer cells when over-expressed – MDR1, MRP1, and BCRP (reviewed in Li et al. 2007). Recently it has been published that expression of those ABC transporters could be regulated by mitogen activated protein kinases (MAPKs), namely the p38 and ERK subtype (Katayama et al. 2007), and also PI3K/Akt pathway (Liang et al. 2009). Those kinases were found to be able to modulate the activity of some transcription factors that bind into ABC transporters genes promoters, i.e. NFkappaB, AP1, or p53 (Scotto et al. 2003). We aim to connect effects of selected chemotherapeutics on function of ABC transporters with activity of particular kinase and its targets.

First, we selected chemotherapeutic drugs for their specificity using WST-1 proliferation assay and cell lines over-expressing particular transporter. Roscovitin is a substrate of MDR1 protein, Camptothecin and Actinomycin D are substrates of MRP1 protein, Valinomycin of BCRP protein, and Doxorubicin and Geldanamycin are general substrates of all the studied ABC transporters. We evaluated their effect on function and expression of ABC transporters in A549 cells using “Dye Exclusion Assays” by means of flow cytometry (JC1 was used as a fluorescent substrate of MDR1, Calcein AM for MRP1 and Bodipy-prazosin for BCRP). Selected cytotoxic drugs were shown to activate studied kinases to a different extent. In the next step we used pharmacologic inhibitors of studied kinases to assess how they affect the up-regulated function of ABC transporters – p38, JNK, and Akt inhibitors generally block the effect of cytotoxic drugs. In the next step we aim to asses involved transcription factors.
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Venue
The symposium takes place at Holmenkollen Park Hotel Rica, Oslo. The lectures are held in Saga Hall.

Holmenkollen Rica Hotel was built in the Norwegian "dragon" style over 100 years ago. It is situated 350 meters above the centre of Oslo, affording a unique view of the city and fjord. The hotel is surrounded by fresh air, along with peace and quiet and is situated right next to the Holmenkollen Ski Arena and Nordmarka forest.

Conference hotel
Holmenkollen Park Hotel
Kongeveien 26
0787 Oslo, Norway
Tel + 47 22 92 20 00
www.holmenkollenparkhotel.no

OSLO – The capital of Norway
Between the Oslo Fjord and the green hills of Nordmarka lies the Norwegian capital. The combination of city life and easy access to outdoor activities makes Oslo a unique destination. Even in the city centre, the nearest park is never more than a few blocks away. A ten-minute boat ride from the centre takes you to lovely beaches on the Oslo Fjord islands. In the winter Oslo has hundreds of kilometers of cross-country trails within the city boundaries, in addition to several ski centres. In 2007 Reader's Digest ranked Oslo as number two on a list of the world's greenest, most liveable cities. At the same time Oslo has everything you would expect from a capital. The city offers an abundance of attractions, shopping possibilities and a flourishing cultural life, and a choice of restaurants which is almost unparalleled in Scandinavia.

CONFERENCE
Registration
On the 2nd of December, 10.00 – 13.00 registration will be by the hotel reception at the main entrance of the hotel. 3rd and 4th the registration and information will be outside the lecture area (Saga Hall).

Posters
Posters should be delivered at the poster desk next to the registration. The posters will be mounted by us. We kindly ask the poster owners to take the poster down after the poster-session is over.

Check-in
From 15.00.
Keys for the hotel rooms have to be picked up at the hotel reception.
Mini-bar is not included in the price of the hotel, and has to be paid by the participant upon departure.
Check-out
Before 12.00 (am)

Secretariat /Information desk
There will be an information desk outside the lecture hall, staff will be there during breaks. Secretariat can be reached at +47 91 81 11 39 or + 47 90 08 71 39

Telephone
Switchboard Holmenkollen Park Hotel Rica (+47) 22 92 20 00
This number can be used for important messages for meeting participants, alternatively (+47) 91 81 11 39

Lunch
Lunch is offered all days of the meeting in “Galleriet”, close to the main entrance, at the specific time announced, and dependent on wearing of the meeting badge.

Refreshments
Coffee, tea, water, fruit and other snacks will be offered in the area outside the lecture hall during breaks.

Hotel facilities
Internet
Codes for wireless internet have to be picked up at the hotel reception
Swimming pool and SPA
Holmenkollen Park Hotel SPA has a first class SPA-lounge. Conference participants can get a reduced price by referring to the rebate code ONKFORUM. See info at www.holmenkollenspa.no.

The small swimming pool and sauna are available to everyone staying at the hotel.

PHOTOGRAPHY AND INTERNET
Please note that photography during sessions will not be allowed, and we ask all participants not to be on-line during session.

CURRENCY
In Norway the currency is Norwegian Kroner (Norske kroner), NOK
It is not possible to pay with € (cash).
Many international bank cards allow withdrawal of local currency from all the minibanks (ATMs). You may also exchange your money before you travel, at arrival at the airport or at Oslo S (Oslo Central Station).

FOREX Bank at Oslo Sentral Station, Airport Express Train terminal exchange money.
Telephone: +47 22172265. Opening hours Mon-Fri 07-20, sat 09-17, Sun 10-17.
Exchange rate: 1 NOK = 8.4 € (23.11.09)

CREDIT CARDS
Most places in Norway accept Master Card and Visa Card.
Social Events

EACR Key Note Lecture and Poster-session
2nd of December at Institute for Cancer Research Building, the Norwegian Radium Hospital, Montebello. Street address: Ullernchausseen 70, 0310 Oslo

There will be buses from Holmenkollen Park Hotel for all participants at 18.30
Welcome by Erik Boye at 19.00
EACR Key Note Lecture by Hans Clevers at 19.15

Tapas, posters and mingling 20.00 - 22.00

Bus back to hotel at 22.00

Poster-session
Posters are handed in at registration. The organization committee will mount the posters.
When the poster session is over, we kindly ask each poster owner to take down the poster and take care of it.
About 50 posters will be presented at the poster session.

Poster prize
There will be three poster prizes: one first prize and two second prizes.
Oslo Cancer Cluster is sponsoring the poster prices, and Bjarte Reve (CEO of OCC) will give out the prices.

Reception at The City Hall
All participants of the Second Norwegian Cancer Symposium 2009 are invited to a reception by the mayor at the City Hall of Oslo, Wednesday 3rd of December at 19.00. Official invitation card is required; all participants that have signed up for this event have received an invitation card.

There will be bus transport from Holmenkollen Park Hotel at 18.15 to the City Hall.
Please present the invitation card at the entrance
Attire: Formal
Entrance: The City Hall Seaside

The City Hall of Oslo is where the Nobel Piece Prize ceremony is being held.
Transport after the Poster session, Wednesday evening

There will be busses going back to Holmenkollen Hotel at 22.00
One of them (see sign) will stop at Smestad for those who wish to go elsewhere, e.g. with the T-bane (Underground) lines 2 or 6 to the centre. If you wish to leave on your own, you may call a taxi at 02323, or find public transportation as indicated on the map.

Transport after reception in City Hall, Thursday evening

The evening after the reception is free for all participants, there is no organized transport back to Holmenkollen Park Hotel. Take T-bane (underground) from Nationaltheatre Station or take a taxi from the city centre.

Map over area around The City Hall (Rådhuset)
Industry stands

At the Cancer Stem Cell Symposium 2009 you will meet these companies/organizations, they will have stands outside the lecture hall:

- SFI-CAST; Centre for Research-based Innovation (SFI) - Cancer Stem Cell Innovation Centre
- Oslo Cancer Cluster (OCC)
- LI-COR Biosciences UK Ltd.
- QIAGEN
- AME Bioscience
- R&D Systems Europe Ltd
- MILLIPORE AS
- Sanofi-aventis Norge AS
Public transport to/from Oslo City Centre

From the airport
Upon arrival at Oslo Airport, Gardermoen, you can take the Airport Express Train directly to Oslo Central Station (Oslo S) or Nationaltheatret station; trains depart every 10 minutes from the airport. Travel time is approx. 20-25 minutes, and the cost of a single ticket is NOK 170.00

T-bane/underground/metro from the Nationaltheatret Station
Take T-bane/underground/Metro #1 "Frognerseteren" and get off at the Besserud Station. Due to construction work, you have to take a bus one more stop. It is a 1 minute walk from the underground stop to the bus stop. Take bus 1B one more stop to the Holmenkollen stop. The ride will last for approx. 30 minutes. The cost is NOK 36.00 per person. Follow the road uphill towards the Holmenkollen National Ski Stadium. It is a 10 minutes uphill walk.

Cancer Symposium bus transport
We are planning a bus transport from Oslo Central Station to Holmenkollen Park Hotel. This will cost 100 NOK, and have to be paid as cash on this bus. All participants will receive information by e-mail, and will have to register for this bus transport.

Taxi
Airport Taxi
Taxi from the Gardermoen Airport directly to the Holmenkollen Park Hotel Rica. There are several different taxi companies, and the prices could be very different. Please confirm price of taxi before travel. Contact the Airport Taxi stand, next to the arrival gate inside or pre-order at: +47 02323

1 - 4 people: NOK 740.00
5 people: NOK 1060.00
from 6 - 16 people there will be an extra charge of NOK 120,- per person.

Additional charges when travelling the following times:
Weekdays: after 5pm - 6 am
Saturday and Sunday: All day
- additional charge NOK 200.00

Bankholidays
- additional charge NOK 200.00

Taxi from the Central Station (Oslo S)
The taxi journey takes approx 20 minutes, and the cost is between NOK 200.00 - 350.00, depending on what time you will be travelling and number of passengers. The taxi station is at your left hand side when leaving the Airport Express train.
1 - 4 people: about NOK 300,00
5 people: about NOK 550,00
T-bane/Subway/Metro

From Oslo City:
T-bane #1, Frognerseteren, from Oslo to Besserud station (due to works on the Holmenkollen stop). From there a free bus will take you close to Holmenkollen (bus stop on the lower side of the T-bane), or you can walk the 1,3 km (see map). Bus 1B to Holmenkollen stop (one stop)

From Holmenkollen:
Take Bus 1B to Besserud (or walk down to this stop), take T-bane #1 from Besserud T-bane stop to Oslo City Centre.

There are three stops for Oslo City Centre: Nationaltheateret, close to the City Hall) Stortinget and Jernbanetorget (Oslo S).
You will travel with T-bane #1 to/from Holmenkollen Park Hotel and Oslo City, towards Frognerseteran.
Where to buy tickets
You buy tickets/public transport passes from automats at the stations, from kiosks at T-banen or one of the ticket sales stores; Narvesen, MIX, Deli de Luca. *It is NOT possible to buy tickets onboard the T-bane.*

Different tickets

**Single ticket (Enkeltbillett)**
If you are only travelling once.
Costs 36 kroner if you buy it from driver on-board bus, tram, train (you can *not* buy tickets onboard the T-bane). Costs 25 kroner if you buy it before entering the vehicle.

The single ticket has to be stamped at the start of the travel (orange or yellow boxes) to be valid. You can travel for 1 hour with any public transport after stamping.

**Day pass (Dagskort)**
If you are travelling more than once during one day this is cheaper. Costs 65 kroner. The pass is valid for 23 hours after stamping - except for night lines. It is not a personal card, but only one person can use it at the time.

The day pass has to be stamped at the start of the travel (orange or yellow boxes) to be valid. You can travel for 1 hour with any public transport after stamping.

**Flexi Card (Flexikort)**
If you and your companion are travelling more than once, on different days. Costs 180 kroner

You have 8 fields to stamp on, and can travel several people on the same card (one stamp per person) as much as you like on any public transport within 1 hour.

**Taxi**
There are several taxi companies in Oslo, we recommend these companies:
Oslo Taxi: +47 02323
Norges Taxi: +47 08000
Notes