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Plenary Sessions

Keynote Lecture

PL1
FROM RNAi SCREENS TO MOLECULAR FUNCTION: INSIGHTS INTO THE CANCER CELL, STEM CELL INTERFACE THROUGH SYSTEMS BIOLOGY

F. Buchholz
Max-Planck-Institute for Molecular Cell Biology and Genetics, Dresden, Germany

Cancer research has, so far, primarily taken the form of intensive investigation of individual genes and a small number of interactions between genes. However, the identification of a large number of oncogenes and tumor suppressor genes in recent years has illustrated that cancer is much more a problem of the cell system than a problem with a single gene. Hence, it is becoming clear now that cancer can only be fully understood, and therefore combated, when the process of cellular transformation is understood at the systems level. To become cancerous, a cell has to change in many ways, overcoming numerous safeguards that normally keep renegade cells in check. For this reason, a comprehensive understanding of cellular transformation is required to find the best ways to treat this disease.
We are using RNAi screens and large-scale protein-tagging (TransgeneOmics) to obtain profiles of stem/cancer relevant processes to obtain a more comprehensive picture of cellular transformation. Examples of our work to investigate similarities and differences of stem cells and cancer cells will be presented.

Plenary session 1: Genetic Evolution of Cancer

PL02
INVASIVE GROWTH: A GENETIC PROGRAM CONTROLLED BY THE MET ONCOGENE

P. M. Comoglio
IRCC - Institute for Cancer Research-Turin, Italy

Metastasis follows the inappropriate activation of a genetic programme termed invasive growth, which is a physiological process that occurs during embryonic development and post-natal organ regeneration. Burgeoning evidence indicates that invasive growth is also executed by stem and progenitor cells, and is usurped by cancer stem cells. The MET proto-oncogene, which is expressed in both stem and cancer cells, is a key regulator of invasive growth. MET encodes the tyrosine-kinase receptor for "Scatter Factor", a sensor of adverse microenvironmental conditions (such as hypoxia) and drives cell invasion and metastasis through the transcriptional activation of a set of genes, including those controlling blood coagulation. In cancer cells the MET tyrosine kinase stimulates cell scattering, invasion, protection from apoptosis and angiogenesis, thereby acting as a powerful expedient for dissemination. In some cancers, MET has been genetically selected for the long-term maintenance oft he primary transformed phenotype, and those cancers appear to be dependent on (or 'addicted' to) sustained MET activity for their growth and survival. Because of its dual role as an adjuvant, pro-metastatic gene for some tumour types and as a necessary oncogene for others, MET is a promising target for therapeutic intervention. Recent progress in the development of molecules that inhibit MET function will be discussed and their application in the subset of human tumours potentially responsive will be considered.

PL3
LINEAGE ANALYSIS OF SINGLE CANCER CELLS

L.I. Shlush
Faculty of Medicine and Research Institute, Technion – Israel Institute of Technology, Haifa, Israel

The depth of prokaryotic cells – the number of cell divisions since the zygote can be assessed using a panel of around 100 short tandem repeats (STRs), which accumulate mutations during replication. Using phylogenetic methods applied to STRs variation our group reconstructed the population genetic structure of single cells in mouse. Lineage analysis of single cancer

cells might shed light on the evolutionary process of carcinogenesis, and uncover tumor heterogeneity and population stratification in cancer cell populations. We have recently reconstructed a lineage tree of single mouse lymphoma cells and adjacent normal cells obtained by laser microdissection. Analysis of the reconstructed lymphoma tree revealed the monoclonality of the tumor and its age which was estimated to be 5 month old. Our current work is focused on cell lineage analysis of human acute myeloid leukemia (AML). We hypothesized that leukemic cells at diagnosis will be deeper than cells at relapse, due to resistance to chemotherapy of quiescent leukemic cells. We analyzed the lineage tree of 3 leukemic patients revealed their monoclonality heterogeneity and depth; we compared the cell depth of the patients at diagnosis to one patient in relapse and found that leukemic cells at diagnosis were deeper. The ability to obtain data regarding the phenotypical appearance, genotypic profile, and lineage position of single cells in a tumor may prove to be a powerful new tool for cancer research. It could be used in both animal models and human cancers to investigate basic aspects of carcinogenesis, such as timing of tumor initiation, progression from premalignant to malignant states, analyzing the relapsing clone, physical growth patterns of tumors, clonal evolution within cancer cell populations, identification of tumor heterogeneity and new mechanisms for tumor progression, timing of metastasis formation, and the mechanisms underlying differential response to therapy.

PL4
EVOLUTIONARY MEASURES OF HETEROGENEITY AS PREDICTORS IN BARRETT`S ESOPHAGUS

L. Merlo[1], N. Shah[2], C. Maley[1]
[1]*The Wistar Institute, Philadelphia, PA, USA*
[2]*University of Pennsylvania, School of Medicine, Genomics and Computational Biology Graduate Group, Philadelphia, PA, USA*

Cancer progression is, at its core, an evolutionary process. Successive beneficial mutations provide a selective advantage, allowing clones carrying these mutations to expand in a neoplasm. The evolutionary dynamics of mutation, selective sweeps and clonal expansion can lead to varying levels of genetic heterogeneity in a cancer. We show that measurements of this genetic heterogeneity are predictive of progression to cancer in Barrett's Esophagus, a premalignant condition that is a precursor to esophageal adenocarcinoma. We have examined loss of heterogyzosity at 19 loci on two chromosomes and mutation and methylation of the TP53 and CDKN2A loci. We find that a variety of heterogeneity measurements are predictive of progression, regardless of the type of loci used (selective, neutral, LOH, or all loci) to calculate diversity. Genetic heterogeneity measurements are robust predictors of progression in this system.
Heterogeneity measurements encompass a variety of evolutionary processes that are not specific to Barrett's Esophagus and thus may be generalizable predictors for other premalignant conditions.

Plenary session 2: Cancer Genomics

PL5
GENOMICS OF ORAL CANCER

D. Albertson
Cancer Research Institute, Comprehensive Cancer Center and Department of Laboratory Medicine, University of California San Francisco, USA

The 5-year survival rate for patients with oral squamous cell carcinoma (SCC), at 40%, is among the worst of all sites in the body and has not improved over the past 40 years. Problems in oral cancer management include identification of tumors with metastatic potential, prediction of recurrence after surgical excision of lesions, second primary tumors and identification of dysplastic lesions at risk for progression to cancer. It is generally accepted that oral SCC develop via accumulation of genetic and epigenetic changes in a multi-step process with aberrations being frequently recognized in pre-malignant lesions. Therefore we have applied array CGH to study the utility of DNA copy number measurements for biomarker discovery to better predict the behaviors of this disease. This presentation will review our work on the copy number landscape of oral cancers and pre-cancers, our approach to studying the functional consequences of overexpression of candidate oncogenes, and how these latter studies have now focused attention on molecular characterization of the oral SCC stroma.

PL6
UNCOVERING MOLECULAR MECHANISMS IN HUMAN BRAIN TUMORS

P. Lichter
Deutsches Krebsforschungszentrum, Heidelberg, Germany

Identification of genomic and transcriptomic alterations have greatly contributed to revisions of tumor classification schemes and the identification of pathogenically relevant molecular pathways. We performed comprehensive molecular profiling on the level of the genome, the transcriptome and the epigenome, employed to the same samples of human brain tumors. Subsequently, these data were integrated and related to clinical parameters. Emerging candidate genes have been subjected to functional tests in dedicated cellular systems by means of ectopic expression as well as gene knock-down strategies and subsequent assays for cell viability, proliferation, apoptosis and cell migration. This approach allowed us to further elucidate pathomechanisms in human astrocytoma and oligodendroglioma and to uncover novel factors relevant to cell cycle control and cell migration. Notably, we identified i) signatures distingiuishing two subgroups of primary glioblastoma, ii) a pathogenic pathway downstream of TP53 regulated by DNA methylation in astrocytoma, iii) selective pathway activations in glioblastoma of long term survivors, and iv) genetic alterations in pediatric low grade astrocytoma that affect targeted molecular therapy procedures. Furthermore, novel algorithms to classify and to stratify pediatric and adult medulloblastoma patients will be presented. Possible consequences of these findings for the management of brain tumor patient will be discussed.

PL7
GENOMIC APPROACHES TO DISSECTING GASTRIC CANCER HETEROGENEITY

P. Tan
Duke-NUS Graduate Medical School, SG, Singapore

Gastric cancer (GC) is the second highest cause of global cancer mortality. A significant body of epidemiological, clinical, and experimental evidence has revealed that GC is likely a heterogeneous disease comprising multiple different subtypes. In this talk, I will describe how genome-wide approaches can be used to split GC patient populations into biologically and clinically meaningful subcategories, for the purposes of understanding basic mechanisms of GC carcinogenesis and guidance of treatment.

PL8
VIRTUAL MICROSCOPY: A TIPPING POINT IN TISSUE BIOMARKER RESEARCH AND DIAGNOSTIC PATHOLOGY

P.W. Hamilton
Bioimaging and Informatics, Centre for Cancer Research and Cell Biology, Queen's University of Belfast, Lisburn Road, Belfast BT7 1NN, UK

Morphometry, image cytometry and tissue image analysis experienced a surge of enthusiasm in the 1980's and 1990's which subsequently declined as pathologists realised the technical difficulties of introducing quantitative techniques into routine practice. Being considered a "has been" technology, it was quickly superseded by the next wave of enthusiasm surrounding molecular technologies which promised to remove the need for conventional histopathology altogether. Now in the 21st century, image analysis in tissue pathology, or "digital pathology" as it now known, once again finds itself in the spotlight as a technique that could help underpin tissue biomarker discovery and as a practical tool to enhance diagnostic practice in histopathology. What has brought about this resurrection has been the development of Virtual Microscopy which uses specifically designed high resolution scanners to digitally record an image of the whole slide, at diagnostic resolution.

Virtual microscopy is finding a range of applications in domains that use glass slides. Of particular importance is the role of virtual microscopy in tissue-based research and biomarker discovery. We have been developing a range of new tools for the quantitative evaluation of whole slide scans in biomarker discovery. In non-small cell lung cancer (NSCLC) we have developed a range of algorithms which can facilitate the analysis of biomarkers, BAX, BAK, HSSB1, and NOX in lung cancer tissue microarrays (TMAs). These include new methods to de-array TMA samples, to extract regions showing biomarker expression and to quantify immunohistochemical expression. As a means of speeding up the quantitative analysis of TMAs, we have constructed a high performance computing (HPC) platform for the high throughput analysis of tissue biomarkers. This parallelisation of tissue core analysis can significantly speed up TMA analysis, making it a truly high throughput platform for biomarker discovery. Interestingly, this can be applied using the principles of

cloud computing where digital slide hosting and analysis can be carried out remotely on a centralised HPC platform. In addition, we have developed a new imaging tool for distinguishing squamous and non-squamous NSCLC as a means to support the selection of therapy with pemetrexed and cisplatin.
Finally, the revolution in virtual microscopy allows us to again consider the application of digital pathology in routine diagnostic practice, since it overcomes some of the hurdles which prevented its adoption previously. Having a high resolution digital slide allows pathologists to not only make a conventional diagnosis on-screen but to access a range of digital tools to facilitate decision making, including annotation, practical remote consultation, pre-processing, feature extraction and image search. We call this "augmented visualisation" and this will again see the benefits of quantitation being applied more readily in routine histopathology and cytopathology.
The next few years will see some interesting developments in the role of digital pathology, with a critical tipping point where tissue and cell imaging will become an essential tool in tissue-based research and in diagnostic practice.

Plenary session 3: Identification of Molecular Predicitive and Prognostic Factors

PL9
EXPLOITING THE GENOME AND TRANSCRIPTOME FOR INDIVIDUALIZED CANCER DIAGNOSIS AND TREATMENT STRATIFICATION

T. Ried, J. Camps, G. Emons, A. Hummon, B. M. Ghadimi, J. Habermann, K. Heselmeyer-Haddad, G. Auer, M. Grade
National Cancer Institute, NCI/NIH, Bethesda, MD, USA

Despite major improvements in elucidating the genetic changes underlying the initiation and progression of colorectal cancer (CRC), there remains a clinical need to implement novel targeted therapeutic strategies. Proteins that are highly overexpressed in tumor cells have the potential to be selective therapeutic targets. We focused in our analyses on genes on chromosome 13 that were consistently overexpressed. Using cell based model systems that recapitulate the genomic and gene expression changes we have previously observed in primary CRC we applied a functional genomics strategy to identify such anti-CRC targets. We identified 69 genes within the amplified regions that were over-expressed in the tumors compared to matching normal mucosa samples. Next, we validated the expression levels of these 69 genes in 25 colorectal cancer cell lines using real-time PCR, and confirmed over-expression of 44 genes out of these 69 genes. Subsequently, we conducted an RNAi screen in the colorectal cancer cell lines SW480 and HT29. For 15 out of these 44 genes, we observed a decreased cellular viability as a consequence of mRNA silencing. Our experimental strategy led to the identification of genes that were amplified and/or over-expressed in primary colorectal cancers. We surmise that some of these genes represent potential oncogenes residing on chromosome 13q. In order to identify the underlying signaling pathways involved in reduction of viability, we subsequently analyzed the global transcriptomic changes following RNAi using whole-genome microarrays, and could identify the disruption of a variety of pathways.
Recently, expression profiling of breast carcinomas has revealed gene signatures that predict clinical outcome, and discerned prognostically relevant breast cancer subtypes. Measurement of the degree of genomic instability provides a very similar stratification of prognostic groups. We therefore hypothesized that these features are linked. We used gene expression profiling of 48 breast cancer specimens that profoundly differed in their degree of genomic instability and identified a set of 12 genes that defines the two groups. The biological and prognostic significance of this gene set was established through survival prediction in published datasets from patients with breast cancer. Of note, the gene expression signatures that define specific prognostic subtypes in other breast cancer datasets predicted genomic instability in our samples. This remarkable congruence suggests a biological dependency of poor-prognosis gene signatures, breast cancer subtypes, genomic instability, and clinical outcome.

PL10
PREDICTIVE MOLECULAR PATHOLOGY

G. Baretton
University Hospital Carl Gustav Carus, Department of Pathology, Dresden, Germany

New insights into the molecular carcinogenesis of solid tumors have led to the development of targeted therapies. In principle, two approaches are available:
1. antibodies against growth factors (e.g. VEGF) or growth factor receptors (e.g. Her2/new and EGFR)
2. small molecules blocking intracellular signal transduction pathways (e.g. thyrosine kinase inhibitors (TKIs)).
High cost and potential adverse effects of these therapies have prompted drug registration authorities to require predictive, tissue based tests for patient selection.
The introduction of Herceptin into breast cancer therapy can be seen as a prototype of a predictive molecular test, using either immunohistochemistry (IHC) to detect protein over-expression or fluorescence in situ hybridisation (FISH) to detect gene amplification. Nowadays, Her2/new-testing is an integral part of the pathological work-up of breast cancer specimens. Very recently, Herceptin was also approved for the treatment of gastric or GE-junction cancer patients with either IHC 3+ or IHC 2+ and FISH-positivity.
However, the tempting approach of detecting protein over-expression via IHC is not generally transferrable to other pathways, e.g. EGFR expression failed to predict response to anti-EGFR treatment in colorectal (CRC) and non-small cell lung cancer (NSCLC). In CRC, activating KRAS mutations downstream of EGFR turned out to be significant predictors of non-response to anti-EGFR-treatment. Thus, KRAS wildtype status of the tumor became a prerequisite for the eligibility for this therapy. The predictive value of BRAF and other gene mutations (e.g. PI3K, PTEN) downstream of EGFR is still under debate. In NSCLC , mutations of the EGFR gene itself have been shown to predict response to anti-EGFR-treatment with TKIs.
Selection of adequate tumor material and tumor cell enrichment (microdissection) by an experienced pathologist is essential for meaningful results of all further molecular analyses. Since the clinical impact of these test results is paramount, quality control and assurance are crucial. In Germany, this challenge was tackled by the introduction of interlaboratory tests for all molecular analyses by QuIP® (Quality Initiative in Pathology). Our department was involved early on in the establishment of molecular diagnostics and its quality control.

PL11
PREDICTIVE CELLULAR PATHOLOGY OF LUNG CANCER

I. Petersen
Institute of Pathology, Jena University Hospital, Jena, Germany

Lung cancer is the leading cause of cancer death worldwide with an overall survival rate of only 15%. Although smoking has the biggest impact for aetiology, there are other risk factors like viral infections which may play a prominent role in certain geographical regions and patient subgroups. The histopathological tumor classification is diverse and has been reduced for a long time by clinicians into the distinction of small cell lung carcinoma (SCLC) and non-small cell cancer (NSCLC). Both cellular types show profound differences in their sensitivity for chemotherapeutic drugs and clinical behaviour. Histological subtyping has meanwhile received renewed attention because of its relevance for individualized tumor therapy. In addition, detection of genetic alterations like EGFR and KRAS mutation being implicated in specific signalling pathways are becoming routine diagnostic test because of their predictive value in targeted tumor therapy. The presentation will highlight the basics of the phenotype – genotype correlations, recent developments in the classification and their influence on prognosis and the prediction of lung cancer pathology.

Honorary Lecture

PL12
CAUSES AND CONSEQUENCES OF microRNA DYSREGULATION IN CANCER

C. M. Croce
Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University Medical Center, Columbus, OH, USA

During the past several years it has become clear that alterations in the expression of microRNA genes contribute to the pathogenesis of most, perhaps all, human malignancies. These alterations can be caused by a variety of mechanisms, including deletions, amplifications or mutations involving microRNA loci, by epigenetic silencing or by dysregulation of transcription factors targeting specific microRNAs. Since malignant cells show dependence on the dysregulated expression of microRNA genes, which in

turn control or are controlled by dysregulation of multiple protein coding oncogenes or tumor suppressor genes, these small RNAs provide important opportunities for development of future microRNA based therapies.

Plenary session 4: Genomics and Proteomics of Breast Cancer

PL13
GENOMICS OF BRCA1/2-MUTATED BREAST CANCERS

J. Jonkers
Division of Molecular Biology, Amsterdam, The Netherlands Cancer Institute, The Netherlands

Breast cancer is by far the most frequent female cancer, affecting one in nine women in Western countries. Familial breast cancers, including those associated with heterozygous germline mutations in the major susceptibility genes *BRCA1* and *BRCA2*, account for 5–10% of breast cancer cases in the western world.
BRCA1-associated breast tumors show a strong overlap with basal-like breast cancers and triple negative breast cancers which, due to the lack of estrogen-, progesterone- or HER2 receptor expression, do not respond to endocrine agents or HER2-targeting therapeutics. This overlap suggests that an important part of the triple-negative tumors may respond to therapeutics that target BRCA1/2-deficient tumors, such as platinum drugs or poly(ADP-ribose) polymerase (PARP) inhibitors. It will therefore be important to develop biomarkers for "BRCAness" in order to identify sporadic breast cancers with BRCA-like features, which are sensitive to PARPi treatment.
To study the role of BRCA1/2 loss-of-function in breast oncogenesis, we have generated GEM models for *BRCA1*- and *BRCA2*-associated hereditary breast cancer based on combined deletion of *Brca1/2* and *p53* in epithelial tissues [1, 2]. The mammary tumors that arise in our BRCA1 mouse model show strong similarity to *BRCA1*-associated breast cancer with respect to high tumor grade, expression of basal cell markers and high degree of genomic instability due to loss of homology-directed double-strand break (DSB) repair [2]. Indeed, treatment of BRCA1-deficient mouse mammary tumors with conventional and targeted therapeutics revealed a selective sensitivity towards agents that directly or indirectly cause DSBs, such as platinum drugs [3] or PARP inhibitors [4].
We have used comparative oncogenomics to identify genomic tumor characteristics associated with BRCA1/2 deficiency in mouse and human breast cancers. We found that both mouse and human *BRCA1/2*-mutated breast tumors show increased numbers of DNA copy number aberrations. Moreover, we observed a strong increase in *TP53* mutations in human *BRCA1*-mutated breast cancers compared to sporadic control tumors due to a selective increase in protein-truncating *TP53* mutations [5]. Interestingly, we find a similar increase in protein-truncating *TP53* mutations in non-hereditary basal-like breast cancers, suggesting that this trait might be a hallmark of BRCAness and a potential biomarker for sensitivity to PARP inhibition.

References:
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PL14
GLOBAL SCREENING TECHNIQUES IN THE CLASSIFICATION OF INVASIVE BREAST CANCER

H. Bürger
Institute of Pathology, Paderborn, Cooperative Breast Cancer Center, Paderborn/Höxter, Germany, Institute of Pathology, University of Utrecht, The Netherlands

Within the last decade the concepts of breast cancer dedifferentiation and progression underwent a significant and substantial change. In the past it was widely believed that the very detailed associations between genetic and morphological changes defined in the Vogelstein model of colorectal cancer pathogenesis can be transferred onto breast carcinogenesis. A multitude of studies seemed to verify this hypothesis. However, with the introduction of global screening techniques, predominantly on the DNA level, it became obvious that this linear model might be oversimplified for breast cancer.
It is now widely accepted that losses of chromosomal 16q-losses characterize in in-situ and invasive breast

cancer tumours with a low tumour grade, irrespectively of a ductal or lobular differentiation and their postulated precursor lesions. Conversely, high grade breast cancer showed this distinct genetic alteration with a significant lower frequency. With the detection of a very distinct distribution of 16q-losses in different grades of invasive breast cancer a concept of multiple, parallel pathways in the pathogenesis of breast cancer emerged, lately also verified on the RNA level by means of global expression profiling and protein expression patterns.
In consequence, it became obvious that the hunt for oncogenes/tumour suppressor genes in invasive breast cancer is pathway specific.

PL15
MOLECULAR ANALYSIS OF MINIMAL RESIDUAL CANCER AND SYSTEMIC CANCER PROGRESSION

C. Klein
University of Regensburg, Division of Oncogenomics, Regensburg, Germany

Metastasis has only recently gained specific scientific attention, while the traditional focus on the primary tumor, regarding its role as surrogate marker for the biology of metastasis and for therapeutic decisions, is still predominant. This can be easily observed in several experimental models as well as in current attempts to select therapy targets for systemic cancer after mutational or molecular profiling of primary tumors. The shared characteristic of such primary tumor-centric concepts is the exclusion of selective adaptation outside the primary tumor. They generally hold that most metastasis-associated molecular traits and therapy targets are acquired or mutated within the primary tumor and then transmitted to disseminating tumor cells. By direct ex-vivo analysis of disseminated cancer cells we found little support for these hypotheses. In general, we document frequently early dissemination and observe that disseminated tumor cells (DTCs), which we isolated from bone marrow or lymph nodes, and primary tumor cells display disparate changes on all levels of genomic resolutions, including point mutations, allelic losses, and genome wide chromosomal rearrangements, providing evidence for ectopic selective adaptation. As DTCs were isolated at the time of surgery or months to years after, they are representative (i) for cells that have disseminated until resection of their source or (ii) for cells that have ectopically survived at least for the period between resection of the primary tumor and bone marrow sampling. DTCs, detected by epithelial markers such as cytokeratin or EpCAM, in bone marrow and lymph nodes are extremely rare (frequency 10^{-5} - 10^{-6}) but associated with metastatic relapse and poor outcome and therefore apparently comprise the metastatic precursor cells. While the genomic characterization of DTCs has generated important insights into the time point of dissemination and provided evidence of selection and mutation outside the primary tumor, it will be important to describe their phenotype and determine the molecular mechanisms of ectopic survival and outgrowth. To address these issues we have developed single cell gene expression profiling and started to isolate and phenotype DTCs.

Pathological Society Lecture

PL16
METHYLATION ANALYSIS OF NIPPLE FLUID FOR EARLY DETECTION AND PREVENTION OF BREAST CANCER

P. van Diest
University Medical Center Utrecht, Dept. of Pathology, Utrecht, The Netherlands

Breast cancer is the leading cause of cancer death in women in the Western world. In the Netherlands, the incidence is about 12,000 per year, which means that eventually every ninth woman will get breast cancer. The most well-established risk factor is the presence of a germline mutation in the BRCA1 or BRCA2 genes, which indicates a life time risk of 45-80% to get breast cancer. Regular screening by clinical breast examination, mammography and/or Magnetic Resonance Imaging (MRI) is offered to these high-risk women, but one out of four breast tumors are missed by these screening modalities. The most effective form of primary prevention for high-risk women is bilateral mastectomy, which gives a considerable breast cancer risk reduction and is highly mutilating, so many women opt out and those who decide to undergo prophylactic surgery prefer to postpone it as much as possible. This bears a significant risk of developing invasive breast cancer in the mean time. In contrast, the procedure has to be seen as over-treatment in the 15%-55% of BRCA carriers that would never have developed breast cancer. Up to now no procedures are available that accurately predict who of these high-risk women will and who will not develop breast cancer nor, in the first group, at what age the cancer will occur.

A new primary prevention modality for these high-risk women could very well be found in the analysis of nipple fluid. Nipple fluid, that contains breast epithelial cells, free DNA and proteins secreted by them, is produced in small amounts in the breast ducts of non-lactating women and can be collected in a non-invasive way by vacuum-aspiration. Genetic analysis of nipple fluid is performed in various cancer centers throughout the world. However, difficulties in obtaining sufficient amounts of cells in nipple aspirates for genetic analysis has hampered this type of research. We were the first to prove that intranasal administration of a small dose of oxytocin enables harvesting nipple fluid in almost all women. At present, we run a prospective clinical trial where we monitor epigenetic changes in nipple aspirates, to optimally time preventive breast surgery in high-risk women. At present, we have over 150 patients enrolled. In parallel, we develop new methylation markers and validate nipple aspirate methylation analysis by comparing methylation in aspirates with that in the primary tumor of invasive breast cancer patients.

Plenary session 5: Experimental Therapy and Clinical Applications

PL17
ATTACKING TUMOR RADIORESISTANCE IN 3D

N. Cordes
OncoRay - Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Dresden, Germany

Cell adhesion-mediated radio- (CAM-RR) and chemoresistance (CAM-DR) of tumor cells essentially contributes to treatment failure and tumor recurrence. To unravel the molecular mechanisms and to identify novel approaches to overcome this obstacle, three-dimensional (3D) cell culture models provide an ideal basis for the ex vivo analysis of human tumor cell lines growing in a more physiological extracellular matrix (ECM) microenvironment. A thorough examination of integrin subunits identified beta1 integrins as central determinant of the cellular radiation survival response and as potential anticancer targets. Combining 3D clonogenic survival assays and in vivo tumor growth experiments with multiproteinkinase assays, whole genome gene expression analysis using DNA microarray, genetic modulation of beta1 integrin and its downstream targets, and histological beta1 integrin expression analysis in tumor biopsies enabled a detailed examination how this integrin subunit functions in cellular radioresistance of head and neck squamous cell carcinoma cells.

PL18
A COMBINED FUNCTIONAL AND SYSTEMS BIOLOGY APPROACH IDENTIFIES COLORECTAL CANCER GENES AS NOVEL POTENTIAL THERAPEUTIC TARGETS

M. Grade[1,2], A.B. Hummon[2], J. Camps[2], G. Emons[1], M. Spitzner[1], J. Gaedcke[1], P. Hoermann[2], R. Ebner[2], H. Becker[1], M. J. Difilippantonio[2], B. M. Ghadimi[1], T. Beissbarth[1], N. J. Caplen[3], T. Ried[2]
[1]*Dep. of General and Visceral Surgery, University Medicine, Georg-August-University, Göttingen, Germany*
[2]*Section of Cancer Genomics and*
[3]*Gene Silencing Section, Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*

Background: Despite the implementation of sophisticated therapeutic strategies into clinical practice, colorectal cancer is still a major cause of cancer death in the Western world. Thus, establishing novel therapeutic options remains of considerable clinical interest.
Materials and Methods: Since amplified and/or over-expressed genes represent promising targets for therapeutic intervention, we previously profiled a series of 90 primary colorectal cancers and 50 matched normal mucosa biopsies using gene expression microarrays. Towards functional validation, mRNA expression levels of 25 genes, identified as showing significant levels of over-expression in the primary tumors, were established in 25 colorectal cancers cell lines by real-time PCR. We then systematically silenced a subset of these genes using RNAi analysis, and screened for siRNA duplexes that reduced cellular viability.
Results: We could first confirm that the majority of genes were similarly deregulated comparing primary colorectal cancers and cell lines. Using RNAi analysis, we could subsequently show that silencing seven out of 11 highly up-regulated genes reduced the viability of SW480 cells. Among these hits we identified prominent oncogenes such as *MYC* and *HMGA1*. This effect was independently confirmed for up to four

different siRNA duplexes, and in HT-29 and DLD-1 cells. In order to identify the signaling pathways involved in reduction of viability, we subsequently analyzed the global transcriptomic changes following RNAi against five candidate genes using whole-genome expression microarrays, and identified the disrupted pathways. By comparing the expression levels of the RNAi signature genes with their respective expression levels in a set of primary rectal carcinomas, we could independently recapitulate these defined RNAi signatures, therefore establishing the biological relevance of our observations.
Discussion: This combined functional genomics and systems biology approach led to the independent identification of *RRM2* and *TACSTD2*, two genes that were very recently discovered as therapeutic targets, and *HMGA1*, and we subsequently unveiled the deregulation of the underlying signaling pathways. In addition, we identified *RPS2* and *NOL5A* as novel promising molecular targets.

PL19
EFFECT OF COAGULATION AND INFLAMMATORY PROCESSES ON METASTASIS: POSSIBILITIES FOR DETECTION

R. Muschel, A. Gil-Bernebe, S. Ferjancic, J. Im, A. Gal, L. Balathasan, T. Tapmeier, W.S. Carbonell, S. Serres, N. Sibson
University of Oxford, Gray Institute, Oxford, UK

Establishment of metastases depends upon a vascular supply similar to that in formation of primary tumours. Metastasis and primary tumour establishment differ however in that hematogenous metastasis is initiated in the blood stream. The interactions of the tumour cells in metastases with the blood supply thus has features not entirely analogous to that of promary tumours.
In the brain tumour cells extravasate, in groups and then use the pre-existing brain vasculature as a scaffold for growth and invasion. The engagment of the tumour's beta1 integrin with the extracellular matrix provides essential interactions of tumour cell growth, adhesion and invasion in the brain.
The direct engagement of the tumour cells with the vessels led us to speculate that some endothelial activation markers might be induced. This was the case. We will present some evidence suggesting that the induction of such markers can be used as a surrogate marker to detect mciroscoic metastases in whole animals.
The interaction of the tumour cells with vessels also brings the coagulationsystem into consideration. It has long been known that anticoagulation will inhibit metastasis in experimental model systems. We have begun to explore the mechanisms through which clotting facilitates metastasis. Small clots form around tumor cells as they arrest in the pulmonary capillaries. These clots only persist for the initial 24h after arrest yet inhibition of coagulation inhibits metastasis. Expression of tissue factor can enable clot formation around tumor cells and expression of the natural TF inhibitor (TFPI) in B16F10 tumor cells expressing TF inhibits clot formation and subsequent metastasis. This clot formation leads to the recruitment of myeloid cells. These are CD11b+ and CX3CR1+ but mainly cd11c- cells. Inhibition of clot formation prevents the recruitment of these cells and inhibits the survival of the tumor cells. Depletion of these cells or use of CD11b knockout mice led to greatly decreased numbers of myeloid cells surrounding the clots and greatly decreased tumor cell survival. Thus we conclude that cd11b+ cells are essential for the survival of metastatic tumor cells in the lungs and the integrin itself appears to be required for the recruitment of the cells. Our results put forward the concept that the induction of clot formation by metastatic tumor cells serves to recruit cd11b+ myeloid cells that are essential for the survival of the tumor cells. This work has important implications for the concept of the pre-metastatic niche.

PL20
ONCOLYTIC VIRUSES IN TREATMENT OF CANCER PATIENTS

A. Hemminki
CGTG, Biomedicum, Helsinki, Finland

Advanced, recurrent and metastatic cancers remain difficult to treat effectively and it is unlikely that currently available modalities with solve the issue. Novel approaches such as oncolytic viruses might be useful in the context of cases currently incurable. Such viruses are able to infect, replicate in and lyse tumor cells. Recent data from clinical trials has shown that they are safe and can be effective when combined to standard therapy. However, single agent antitumor efficacy would benefit from improvement. This talk will introduce some strategies that are being used to improve tumor cell transduction and antitumor efficacy, including arming of the virus with immunostimulatory transgenes. Furthermore, an overview of the most important clinical approaches

with oncolytic adenoviruses will be given, including our own Advanced Therapy Access Program, where 170 patients have been treated with promising results including evidence of efficacy in more than half of patients. Safety, efficacy, virological and immunological data will be presented.

Plenary session 6: Telomeres and Chromosomal Instability

PL21
MDS AND AML: HOW TO DEFINE COMPLEXITY

B. Schlegelberger[1], G. Göhring[1], C. Niemeyer[2]
[1]*Hannover Medical School, Institute of Cell and Molecular Pathology, Hannover, Germany*
[2]*University Hospital, Department of Adolescent and Pediatric Medicine , Freiburg, Germany*

Cytogenetic characteristics, together with specific molecular lesions, provide important prognostic information in MDS and AML. In particular, a complex karyotype is associated with a very poor prognosis. Usually, a complex karyotype is defined as ≥ 3 or sometimes ≥ 5 chromosome aberrations. Recently, a score for prognostically unfavorable acute myeloid leukemia (AML) with multiple chromosome aberrations was proposed. This monosomal karyotype (MK) index defines a karyotype with at least two autosomal monosomies *or* one single autosomal monosomy in the presence of one or more structural aberrations (1). So far, the different cytogenetic scores to identify patients with a very poor prognosis have not been evaluated prospectively in MDS. We therefore analysed 192 patients with advanced childhood MDS, consecutively registered in the prospective studies EWOG-MDS 98 and EWOG-MDS 2006. We defined a complex karyotype as ≥ 3 chromosome aberrations including at least one structural aberration. In childhood MDS, monosomy 7 is the most frequent chromosome aberration. When these patients were treated with stem cell transplantation, patients with monosomy 7 and patients with a normal karyotype did not differ in their overall survival (OS). Moreover, patients with clonal evolution of monosomy 7, i.e. a clone with monosomy 7 and another clone with monosomy 7 and additional aberrations, had similar OS as patients with other karyotypes. Therefore, in the case of childhood MDS, clonal evolution of monosomy 7 was excluded from the new definition of a structural complex karyotype. A considerably higher number of patients with a very poor prognosis were recognized using our new definition of a complex karyotype than by the traditional definition (≥ 3 or ≥ 5 chromosome aberrations) or by the presence of a monosomal karyotype. This cytogenetic subgroup may benefit from new therapeutic approaches.

PL22
GUARDING GENOME STABILITY: MITOTIC KINASE FUNCTIONS AND LESSONS FROM AN INHERITED ANEUPLOIDY AND CANCER PREDISPOSITION SYNDROME

G. Kops
UMC Utrecht, Physiological Chemistry, Utrecht, The Netherlands

High fidelity chromosome segregation relies on the activity of various mitotic kinases. Most have multiple functions and a subset is particularly important for chromosome biorientation and the mitotic cell-cycle checkpoint. Research in the lab is aimed at understanding the signaling networks that are controlled by this subset of kinases. One of its members is BubR1, a protein kinase encoded by the BUB1B gene. Biallelic mutations in BUB1B cause an inherited aneuploidy and cancer predisposition syndrome. We have studied the impact of the various disease-associated BubR1 mutations on chromosome segregation and BubR1 function, and the results provide a coherent model for how these mutations cause the level of chromosome segregation errors observed in patient cells. These studies have also provided surpising new insights into the molecular workings of human BubR1. Finally, full inhibition of the kinases that control biorientation and the mitotic checkpoint causes massive chromosome missegregations and thereby kills cells in culture and organisms early in development. We have explored the possibility of using inhibition of these kinases to kill tumor cells and present evidence that this strategy may discriminate between cancerous and non-cancerous cells.

PL23
COMMON FRAGILE SITES

A. Rump, V. Langhof, E. Schrock
Technische Universität Dresden, Institute for Clinical Genetics, Dresden, Germany

Fragile sites are chromosomal loci that become visible in the light microscope as gaps or constrictions in chromatids after culturing cells under replicative stress. Depending on frequency of occurrence and mode of induction, fragile sites are divided into two groups: rare and common fragile sites. Rare fragile sites are often associated with disease, occur in less than 5% of the population and are composed of di- or trinucleotide repeats that may cause spontaneous breaks during replication.
Common fragile sites (CFSs), on the other hand, are areas of chromatin that fail to compact even in healthy individuals. Despite immense effort, characteristic sequence patterns that might cause CFSs have not been found so far.
After a short review of the current state of CSF identification and characterization we will present the up to date knowledge concerning the contribution of CFSs to chromosomal instability and cancer. In this context, we will propose and discuss a mechanistic model of CFS induction that has the potential to explain the numerous, sometimes even inconsistent observations that have been made along the way of studying the molecular mechanisms causing common fragile sites.

PL24
ROLE OF TELOMERES IN CANCER

P.M. Lansdorp
BC Cancer Research Centre, University of British Columbia Vancouver, Canada

In order to distinguish a normal telomere from a double strand break, a minimum number of telomere repeats must "cap" each chromosome end. The length of each repeat array will reflect a unique history of addition and losses. Telomere losses are known to occur sporadic as well as with every replication cycle. Losses of telomeric DNA are countered by the telomerase enzyme containing telomerase RNA (encoded by the TERC gene) and a reverse transcriptase protein (encoded by TERT gene) as minimal components. Telomerase levels are high in cells of the germline and immortal cellines and the telomere length is typically maintained in such cells. In contrast, telomerase activity is limiting in most human somatic (stem) cells and as a result the average length of telomere repeats in most somatic cells shows a highly significant decline with age. The hypothesis that loss of telomere repeats acts as a "mitotic clock" and a tumor suppressor mechanism in stem cells is strongly supported by recent studies of patients with mild telomerase deficiency resulting from haplo-insufficiency for either the TERC or TERT gene. Such genetic defects can give rise to various disorders including autosomal dominant Dyskeratosis Congenita (DKC), aplastic anemia, liver fibrosis and pulmonary fibrosis. Other recent studies have revealed that amplification of the hTERT gene is one of the most common genetic abnormalities in various cancers. Paradoxically, it is becoming clear that SNPs within the TERT locus are among the most reproducible risk factors for the development of different types of cancer including lung cancer, acute myeloid leukemia and chronic lymphocytic leukemia. The links between hypo- and hyperproliferative consequences of inborn telomerase deficiencies and SNP's in the TERT gene are poorly understood. It seems plausible that the increased risk of leukemia development in aplastic anemia, myelodysplastic syndrome and Dyskeratosis Congenita, results from stem cell failure. Measurements of the average telomere length as well as the length of telomere repeats at individual chromosome ends in specific cells and tissues are used to study the involvement of telomeres in bone marrow failure, normal aging and tumor biology.

Plenary session 7: Growth and Transcription Factors, Tumor Suppressors and Oncogenes

PL25
***TMPRSS2:ERG* GENE FUSIONS IN PROSTATE CANCER**

S. Tomlins
University of Michigan Medical School, Pathology, Ann Arbor, USA

Recurrent chromosomal rearrangements have been causally implicated in hematologic and mesenchymal malignancies; however, until recently, they have not been well characterized in common epithelial carcinomas. In 2005, we developed a methodology termed COPA to identify candidate cancer targets

with marked over-expression in a subset of tumors (outlier-expression) from gene expression microarray data. This approach led to the identification of fusions of the 5' untranslated regions of the androgen-regulated gene *TMPRSS2* with members of the ETS transcription factor family in prostate cancer. More recently, novel 5' and 3' fusion partners and multiple splice isoforms have been identified. The most common fusion, *TMPRSS2:ERG*, is present in approximately 50% of prostate-specific antigen (PSA)-screened localized prostate cancers and in 15-35% of population-based cohorts. Since the discovery of ETS gene fusions, rapid progress has been made in understanding their genesis, role in prostate cancer, and relevance as biomarkers and potential therapeutic targets. In addition, ETS gene fusions provide a unique tool to understand prostate cancer, and provide insight into prostate cancer precursor lesions, the multifocal nature of prostate cancer, progression to advanced disease and mechanisms of treatment resistance.

PL 26
MYB GENE FUSIONS IN CARCINOMAS OF THE BREAST AND HEAD AND NECK

G. Stenman
Lundberg Laboratory for Cancer Research, Department of Pathology, The Sahlgrenska Academy at University of Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden

Fusion genes are potent oncogenes resulting from chromosome rearrangements, in particular translocations. Most fusion genes have so far been identified in hematological disorders and mesenchymal neoplasms and only a few have been found in carcinomas. We have recently cloned a recurrent t(6;9)(q22-23;p23-24) translocation in adenoid cystic carcinomas (ACC) of the breast and head and neck and shown that this translocation results in fusions involving the transcription factor genes *MYB* and *NFIB*. Detailed analysis of the fusions revealed that they mainly encoded chimeric transcripts consisting of *MYB* exons 1-14 linked to the last coding exon(s) of *NFIB*. The minimal common part of *MYB* lost due to fusion was exon 15 including the 3´-UTR, which contains several highly conserved target sites for microRNAs that negatively regulate *MYB* expression. Deletion of these target sites may thus disrupt the repression of *MYB* leading to overexpression of MYB-NFIB transcripts and protein as well as to activation of critical *MYB* target genes. The fact that all ACCs analyzed expressed the *MYB*-fusion irrespective of whether they were derived from the salivary glands, lacrimal glands, ceruminal glands of the ear, or breast indicate that the fusion is a hallmark of this tumor type and that deregulation of the expression of *MYB* and its target genes are key oncogenic events in the pathogenesis of ACC. Our findings also identify the *MYB-NFIB* fusion as a novel candidate therapeutic target for this often deadly carcinoma.

PL27
ROLE OF CYTOKINES IN LYMPHOID NEOPLASIA CELL SURVIVAL

D. de Totero[1], M. Capaia[1], S. Canevari[2]., A.M. Orengo[1], L.De Cecco[2], M. Fabbi[1], G.Cutrona[1], S.Zupo[1], M. Ferrarini [1], S. Ferrini [1]
[1]*Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy*
[2]*Istituto Nazionale Tumori, Milano, Italy*

B cells of indolent lymphoid neoplasia, such as Chronic Lymphocytic Leukemia (CLL) and Follicular Lymphoma (FL), require interaction with the microenvironment of lymphoid organs and bone marrow for their survival and expansion. In their microenvironment neoplastic B cells establish a cross-talk with supportive cells, including CD40L-expressing T-helper cells, stromal cells and myeloid cells, such as "nurse-like cells". Several cytokines and chemokines as well as cell surface molecules mediating cell-cell interactions are involved in this cross-talk, and their role will be reviewed. Cytokines belonging to the IL-2 family play an essential role in lymphoid cell survival. IL-15 is expressed by bone marrow stromal cells and in lymphoid tissues, and may therefore act as growth factor for CLL B cells. Indeed, IL-15 induces proliferation and rescues CLL B cells from apoptosis. The related cytokine IL-21, instead, does not support CLL B cell proliferation but promotes apoptosis and counteracts the effects of IL-15. In addition, IL-21 also triggers apoptosis of FL cells. The opposite effects of these two cytokines, which share the usage of the common-gamma chain and the kinase JAK3 in their receptor complexes, are related to differential signaling. IL-15 increases tyrosine phosphorylation of STAT5 and of ERK1/2, whereas IL-21 activates STAT3 and JAK1/STAT1 pathways. Pharmacological inhibition of JAK3 or of MEK1/ERK1/2 blocks IL-15-triggered proliferation and survival effects, suggesting that the IL-15 signaling pathways may represent a target in CLL. The two cytokines differentially regulate chemokine gene expression. Indeed, IL-15 up-regulates

the expression of CCL4 and CCL3 by CLL B cells, which attract supportive myeloid cells. IL-21, instead, inhibits the expression of these chemokines and also of CCL17 and CCL22, involved in T cell recruitment. Therefore, differently from IL-15, IL-21 down-regulates chemokines that favour the cross-talk of CLL B cells with supportive cells of the microenvironment. Altogether these data indicate that IL-21 is a negative regulator of CLL and FL lymphoma survival, which may play a role in the "indolent" natural history of CLL and FL, and that rIL-21 may represent a new therapeutic tool in these lymphoid neoplasias.

PL28
A VARIANT EWING SARCOMA: NOVEL TRANSLOCATION INVOLVING THE NFATC2 GENE IN MULTIPLE CASES

K. Szuhai
Department of Molecular Cell Biology and Pathology, Leiden University Medical Center, Leiden, The Netherlands

Ewing sarcoma (ES) is an aggressive sarcoma, and is the second most common bone sarcoma in childhood. Disease specific t(11;22)(~85-90%), t(21;22)(~5-10%), or rarer variant translocations with the involvement of chromosome 22 (~5%) are present. The translocation resulted in chimeric gene formed between the EWSR1 gene and a member of the ETS transcription factor family such as FLI1, ERG. So far, no ES has been identified with a fusion to transcription factors other than ETS. This specificity is important in diagnosing ES.
By using a panel of molecular tools such as multicolour FISH and array-CGH, a ring chromosome containing chromosomes 20 and 22 was identified in two index cases with ES-like histological appearance. Molecular karyotyping showed the translocation and amplification of regions of chromosomes 20q13 and 22q12. Cloning of the breakpoint showed an in-frame fusion between the EWSR1 and NFATc2 genes. The translocation led to the loss of the N-terminal, calcineurin-dependent control region. Consequently, the remaining intact DNA binding domain of NFATc2 is under control of the EWSR1 promoter region permitting oncogenic activation. Intriguingly, in all cases a distinct histological feature was observed. The same phenotype-genotype association was observed in seven ES cases.
In conclusion: a new translocation involving EWS and NFATc2 was cloned that is associated with a histological variant of ES. The NFATc2 transcription factor is not a member of the ETS family of transcription factors. NFTAC2 has well characterized functions in T-cell differentiation and immune response. For the first time a direct involvement of NFATc2 in oncogenesis has been shown.

Plenary session 8: Molecular, Cellular and 3-D-Imaging

PL29
FLUORESCENCE CORRELATION SPECTROSCOPY: NEW PERSPECTIVES FOR IN SITU BIOCHEMISTRY

P. Schwille
Technische Universität Dresden. Dept. of Biophysics, Dresden, Germany

Optical single molecule analysis has reached a high degree of technical sophistication, with the ability to study single molecular interactions and even conformational changes of freely moving molecules in solution. Fluorescence Correlation Spectroscopy (FCS) is a powerful means for the study of concentrations, translocation processes, molecular association or enzymatic turnovers on time scales from microseconds to seconds. Its variant, dual-color cross-correlation (FCCS), is particularly useful to quantify molecular interactions between distinct species and thus, a very promising tool to quantify molecular complex formation on a single molecule scale. We have in recent years been able to address not only protein-protein interactions in the living cell, to elucidate reaction kinetics and binding constants, but also to reveal complex binding stoichiometry.
During the past years, we applied FCS to a variety of cell-associated phenomena, among them protein-protein binding, enzymatic reactions, endocytosis, and gene delivery. We established one- and two-photon scanning FCS for processes which are too slow for standard FCS observation with a fixed beam. By this, we could bring FCS to the level to investigate fundamental processes in living organisms.
Performing circular scanning FCS on developing embryos of C.elegans, we could show how the motion of labelled proteins is non-uniformly distributed in the cortex during cell polarization. FCS measurements in Zebrafish embryos allowed us to quantitatively elucidate gradient formation of morphogens, as well as their specific interactions with different receptors.

PL30
SELECTIVE EXCITATION LIGHT FLUORESCENCE (SELF)IMAGING IN MICROSCOPY

C. MacAulay
BC Cancer Agency, Integrative Oncology, Vancouver, Canada

Fluorescence imaging is a potential candidate for tissue diagnostics in a wide variety of clinical situations. In order to extract diagnostic information using fluorescence, different approaches may be used. Typically, fluorescence imaging is performed by illuminating the sample at a single excitation wavelength and detecting the emissions at one or more wavelengths. Examples of systems using this technique are: *LIFE Lung Fluorescence Endoscopy System, VELscope or Identafi 3000 for* visualization of mucosal abnormalities that may lead to oral cancer, *conventional fluorescence microscopy systems, and most other fluorescent imaging devices.*
It is important to recognize that there are alternative fluorescence imaging techniques available to extract the relevant diagnostic information, and, in many of the potential applications, it is not yet clear which will be optimal.
In this study, we have built a prototype system for a new fluorescence imaging technique called Selective Excitation Light Fluorescence (SELF) Imaging. In this technique, the sample is illuminated with multiple excitation wavelengths (for example from 400nm to 530nm every 10nm); and one or more emitted wavelength images (for example 550-700nm) are detected. The different emitted images for the different excitation wavelengths are then combined into a single three-color representation using principle component decomposition where the three presented colors are the three first principle components.
Some potential advantages of this imaging technique are: detection of multiple labeled objects in microscopy using only a single filter cube, increasing the number of simultaneous labels which can be used on a single slide as labels are separated by their absorption spectra not just their emission spectra, detection of different components of tissue based on different excitation spectra, etc. This new imaging technique can be used in both microscopy and endoscopy.

PL31
IDENTIFICATION OF POTENTIAL AMPLIFICATION TARGETS IN SQUAMOUS CELL CARCINOMA OF HEAD AND NECK

T. N. Lepikhova, A-K. Anttonen, H. Pehkonen, O. Monni
Institute of Biomedicine, Genome-Scale Biology Research Program, University of Helsinki, Helsinki, Finland

Head and neck squamous cell carcinoma (HNSCC) is known to progress from dysplasia to invasive cancer through well-defined clinical and histological stages. Several genetic lesions are associated with the progression of HNSCC, but the molecular mechanisms contributing to initiation and progression of HNSCC are still poorly known. Our aim is to identify genes which contribute to copy number alterations in HNSCC. We have performed an integrated arrayCGH and gene expression analysis to find genes whose altered expression is associated to gene copy number change. By high-resolution arrayCGH we have characterized nine focal highly amplified regions in which 9-64% of the genes show simultaneous overexpression (Järvinen et al., 2006; Järvinen et al., 2008).
To evaluate the role of these candidates in malignant behavior of HNSCC, we are now carrying out RNAi screens to demonstrate that specific knockdown of a mutant allele triggers cell death or proliferative arrest in oncogene-dependent cell lines. This lowcomplexity approach will allow us to assay for driver genes in each individual amplicon and will potentially also give clues for clinical or therapeutic targets.

Ploem Lecture

PL32
NUCLEAR ARCHITECTURE AND THE TOPOGRAPHY OF NUCLEAR FUNCTIONS

T. Cremer
Chair of Anthropology and Human Genetics, LMU Biozentrum, Martinsried, Germany

The topography of nuclear components, such as chromosome territories, chromatin domains and loops, genes, nuclear bodies, splicing speckles, as well as molecular machineries for transcription, co-transcriptional splicing, DNA-replication and repair is not well understood. The big goal of all genome and

epigenome research is to understand how the presence of one and the same diploid genome is able to regulate the development of an organism and orchestrate specific gene activities in hundreds of cell types. Progress made during the last few years has underlined the importance to understand not only the chromatin language but also the space-time dynamics of higher order chromatin organization in cycling and postmitotic cells. The large gap of knowledge, which must still be bridged from the molecular level to the level of higher order structure, is emphasized by differences of currently discussed models of nuclear architecture. I point out conflicting predictions of these models and review present evidence for the intranuclear location of sites, where major nuclear functions take place, focusing in particular on the topographical implications of gene expression and DNA repair.

Plenary session 9: Individualized Medicine - Colorectal Cancer

PL33
NEW PARADIGMS IN COLORECTAL CANCER PREDISPOSITION

A. Geurts van Kessel, R. Kuiper, R. Venkatachalam, N. Hoogerbrugge, M. Ligtenberg
Radboud University Nijmegen Medical Centre, Human Genetics, Nijmegen, The Netherlands

The human genome is subject to substantial structural variation, including copy number variation (CNV). Constitutional CNVs may either represent benign polymorphic variants or be associated with disease, including cancer predisposition. Rare non-polymorphic CNVs, i.e. DNA lesions that result in gene deletions, inversions and/or fusions, may be responsible for a high cancer risk. Recently, we detected deletions encompassing the last exons of *EPCAM*, a gene located directly upstream of *MSH2*, in several unexplained cases of familial colorectal cancer. Due to these deletions the transcription termination signal of *EPCAM* was lost and, as a result, its transcription was extended into *MSH2*. This so called transcriptional read-through leads to methylation and, thus, silencing of the *MSH2* promoter. This novel mechanism explains the etiology of a recurrent and strongly inherited epimutation. Recently, we obtained additional evidence for such a CNV-associated silencing scenario, suggesting that it may be more prevalent than previously thought. Based on these observations, we anticipate that copy number profiling in unexplained high-risk colorectal cancer families will lead to the discovery of additional cancer-predisposing genes and/or mechanisms.

PL34
BIOLOGY AND CLINIC OF COLORECTAL CANCER

G. Meijer
VU University Medical Center, Department of Pathology, Amsterdam, The Netherlands

Colorectal cancer (CRC) is a common and deadly disease in the Western world. The average lifetime risk to develop CRC is approximately 5%. Each year more than one million people are diagnosed with CRC and about half of them die from this malignancy As such, CRC is one of the leading cancer types in the Western world with respect to cancer incidence rates and cancer mortality rates.
Colorectal cancer is caused by changes in gene function, due to DNA sequence mutations, DNA copy number changes, promoter hypermethylation, altered miRNA expression and other alterations, ultimately disrupting the normal functions of the epithelial cells. Opportunities to interfere with the natural course of the disease and improve clinical outcome exist at the levels of cancer screening, treatment of primary tumor and treatment of metatstatic disease. At all these three levels of clinical needs, a better understanding of the biologic characteristics of the tumors can be translated into new in vitro molecular tests and molecular imaging applications. Examples include stool based DNA testing using methylation markers, cell surface proteomics for identifying molecular imaging targets and prediction of response to systemic therapy in metastatic CRC based on genomic profiles.

PL35
HIGH-THROUGHPUT SCREENS FOR INDIVIDUALIZED TREATMENT OF RECTAL CANCER

B. M. Ghadimi
Departments of General and Visceral Surgery, University Medicine, Georg-August-University, Göttingen, Germany

As result of the CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group that demonstrated a significant reduction of local recurrence, preoperative 5-FU-based chemoradiotherapy is recommended for locally advanced rectal adenocarcinomas. However, the response of individual tumors is not uniform, and ranges from complete response to complete resistance. Integrated into a Clinical Research Unit (KFO 179), we have therefore employed targeted approaches to identify molecular markers for predicting response. We previously analyzed pretherapeutic biopsies from 30 patients with locally advanced rectal adenocarcinomas participating in the CAO/ARO/AIO-94 trial using gene expression microarrays. Responders and non-responders showed significantly different expression levels for 54 genes. When we applied a leave-one-out cross-validation to predict response to therapy, we were able to correctly predict the tumor behavior in 83% of patients. We have now initiated transcriptional profiling of additional patients participating in the ongoing CAO/ARO/AIO-04 trial. Additionally, we expanded our efforts to mutational sequencing of the MAPK-PI3K-signaling pathway, and to systematic analysis of the rectal cancer transcriptome. Furthermore, we have begun to functionally validate our response signature, and preliminary evidence suggests that RNAi-mediated silencing of one candidate gene, which was over-expressed in non-responsive tumors, sensitizes colorectal cancer lines to radiation therapy. In summary, these experiments indicate that pre-therapeutic assessment of the response to preoperative chemoradiotherapy is feasible, and we foresee that the integration of predictive biomarkers into the clinical management of rectal cancer patients will serve as a first step towards a personalized medicine.

PL36
PREDICTION IN COLORECTAL CANCER USING PROTEOMICS

G. Auer[1], U. J Roblick[1,2], T. Ried[3], J.K. Habermann[1,2]
[1]*Karolinska Biomic Center (KBC), Karolinska Institutet, Stockholm, Sweden*
[2]*Laboratory for Surgical Research, Dept. of Surgery, University Clinic Schleswig-Holstein, Campus Lübeck, Germany*
[3]*Genetics Branch, National Cancer Institute, NIH, Bethesda, MD, USA*

Background: Colorectal cancer is the second leading cause of cancer related death. Current clinical practice in colorectal cancer screening (FOBT, Colonoscopy) has contributed to a reduction of mortality. However, despite these screening programs, about 70% of carcinomas are detected at advanced tumor stages (UICC III/IV) presenting poor patient prognosis. Thus, innovative tools and methodologies for early cancer detection can directly result in improving patient survival rates.
Methods: Here we report our results of a comprehensive approach for colorectal cancer biomaker identification in tissue and blood with a main emphasis on two-dimensional gelelectrophoresis (2-DE) and mass-spectrometry analyses.
Results: Proteomics-based technologies enable to identify single proteins and groups of proteins, including isoforms and post-translationally modified variants that are highly correlated to malignant transformation and clinical tumor aggressiveness. In addition, our 2-DE data strongly indicate that macroscopically and microscopically uneffected mucosa of patients later developing familial adenomatous polyposis (FAP) exhibit a specific protein expression pattern, which may allow to diagnose the disease at its earliest stage. This observation indicates for the first time a possibility to discriminate between clinically silent and clinically active hereditary disease. Furthermore, the detection of serum-based protein expression pattern that allow distinction of healthy and diseased patients in the peripheral blood can ameliorate existing screening programs in a minimally-invasive and patient friendly fashion.
Conclusions: Proteomics-based technologies could greatly improve common classification systems, diagnostics and prediction. However, this progress has not yet transferred from bench to bedside but could open the door to a more accurate and target specific personalized medicine with improved patient survival.

Plenary session:

PL37
PRODUCTION OF TORSIONAL STRESS BY RNA POLYMERASE DRIVES LARGE SCALE CHROMATIN DECOMPACTION

N. Gilbert, C. Naughton, N. Avlonitis, L. Mati, C. Hamilton
The University of Edinburgh, Institute for Genetics and Molecular Medicine, Edinburgh, UK

In mammalian cells transcription influences large scale chromatin compaction by an unknown mechanism. To identify the factors responsible we analysed human female active and inactive X chromosomes and showed that transcriptionally active large scale chromatin structures are torsionally constrained and their compaction is topoisomerase dependent. Transcriptional activation is accompanied by a decompaction of large scale chromatin structures correlating to the production of short RNA transcripts upstream of genes by the initiating form of RNA polymerase. Using a novel approach we directly monitored the production of torsional stress in vivo at gene promoters by interchelating biotinylated psoralen into the DNA. Negative supercoils are introduced upstream of genes prior to transcriptional elongation introducing torsional stress and priming the locus for subsequent transcription consistent with the transcriptionally inactive X being in a torsionally relaxed conformation. We propose that torsional stress generated during transcription is transmitted through the chromatin fibre decompacting large scale chromatin structures.

PL38
CHROMOSOMAL ABERRATIONS FOR DIAGNOSIS AND PATIENT TREATMENT

B. Ylstra
VUMC, Dept of Pathology, Amsterdam, The Netherlands

Patient tailored medicine requires matching of the most effective therapy with the molecular characteristics of a cancer. Therefore, we need to recognize the molecular heterogeneity of the individual patient's tumor. Chromosomal copy number aberrations underlie almost all human cancers and offer considerable opportunities to stratify patient samples for therapy. Array Comparative Genomic Hybridization (array CGH) is a high-resolution laboratory technique to detect chromosomal copy number aberrations on a genome-wide scale. The array CGH technique can be applied to archival tissue specimens (formalin fixed paraffin embedded, FFPE). This has allowed us to identify; HSP90 as a therapeutic target for lung cancer, pivotal genes in pathogenesis of colorectal and DLBCL, chromosomal regions responsible for resistance to therapies in colorectal cancers, and different (sub-) types of medduloblastomas, gastric, colorectal, cervical and head&neck tumors. Unsupervised clustering of chromosomal copy number profiles has also allowed us to distinguish subclasses within mammary tumors which are tightly linked to survival in independent datasets. These chromosomal copy numbers can also predict the breast cancer expression subtypes. With these examples I hope to be able to convince you during my seminar that copy number aberrations can serve as a marker for better cancer classification, prognosis, and outcome prediction.