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Plenary Sessions

Plenary session 1: Cancer Genomics

PL1
EXPLOITING THE GENOME AND TRANSCRIPTOME FOR INDIVIDUALIZED CANCER DIAGNOSIS AND TREATMENT STRATIFICATION

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Genetic markers for disease progression of cervical dysplasia:
Invasive cervical carcinomas almost invariably carry extra copies of chromosome arm 3q, resulting in a gain of the human telomerase gene (TERC). We therefore decided to explore whether gain of 3q and genomic amplification of TERC can predict progression from CIN1 and CIN2 to CIN3 and invasive carcinoma. We applied FISH with a triple-color fluorescent probe set to a series of 59 previously stained Pap smears for which repeat Pap smears and clinical follow-up were available. The samples included (1) CIN1 and CIN2 lesions that progressed to CIN3, (2) CIN1 and CIN2 lesions that regressed spontaneously, and (3) normal Pap smears from women who subsequently developed CIN3 or cervical cancer. We now show that CIN1/CIN2 lesions that progress to CIN3 lesions or cancer revealed a gain of 3q. Our data therefore proof that 3q gain is required for the transition from CIN1 and CIN2 to CIN3 and that it predicts progression. None of the spontaneously regressing CIN1/CIN2 lesions showed this genetic aberration. Of note, 3q gain was found in 33% of cytologically normal Pap smears from women who were diagnosed with CIN3 or invasive cervical carcinoma after a short latency.
Response prediction of patients with rectal cancer treated with radiochemotherapy:
There is a wide spectrum of tumor responsiveness of rectal adenocarcinomas to preoperative chemoradiotherapy ranging from complete response to resistance. We therefore investigated whether gene expression profiling can assist in stratifying patients into responders or non-responders. Pretherapeutic biopsies from 30 patients with locally advanced rectal adenocarcinomas were analyzed using microarrays. Class comparison was used to identify genes that were differentially expressed between responders and non-responders. Responders and non-responders showed significantly different expression levels for 54 genes. When we applied LOOCV to predict response to therapy, we were able to correctly predict the tumor behavior in 83% of patients. Our results suggest that pretherapeutical gene expression profiling may assist in response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy and in prediction of disease free survival if validated in larger independent studies.

PL2
FUNCTIONAL GENETIC APPROACHES IDENTIFY CANCEROUS MIRNAS

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MicroRNAs (miRNAs) are potent post-transcriptional regulators of protein coding genes. Patterns of mis-expression of miRNAs in cancer suggest key functions of miRNAs in tumorigenesis. However, current bioinformatics tools do not fully support the identification and characterization of the mode of action of such miRNAs. To perform genetic screens for novel functions of miRNAs we developed a library of vectors expressing the majority of cloned human miRNAs and created corresponding DNA barcode arrays. In a screen for miRNAs that cooperate with oncogenes in cellular transformation we identified miR-372 and miR-373, each permitting proliferation and tumorigenesis of primary human cells that harbor both oncogenic RAS and active wild type p53. We provide evidence that these miRNAs are potential novel oncogenes participating in the development of human testicular germ cell tumors by numbing the p53 pathway, thus

allowing tumorigenic growth in the presence of wild type p53. Recently, we have used a novel functional genetic approach and identified miR-221 and miR-222 (miR-221&222) as potent regulators of p27Kip1, a cell cycle inhibitor and tumor suppressor. Interestingly, high miR-221&222 levels appear in signatures of poor prognosis cancers. Using miRNA-inhibitors we demonstrated that certain cancer cell lines require high activity of miR-221&222 for the maintenance of low p27Kip1 levels and continuous proliferation. Thus, high levels of miR-221&222 promote cancerous growth by inhibiting the expression of p27Kip1. Last, we performed experiments to uncover metastasis promoting miRNAs. The results of this effort will be presented.

PL3
FROM ONCOGENOME MINING TO FUNCTIONAL VALIDATION OF CANCER GENES

Giovanni Tonon
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Cancer is a genetic and epigenetic disease that results from lesions affecting genes, microRNA and ultimately pathways whose dysregulated expression causes the constellation of growth, differentiation and cell death alterations leading to tumor development. Different lesions at the DNA level have been linked to cancer, including somatic point mutations, chromosomal rearrangements and epigenetic changes. The urgency in identifying the genes affected by these changes and causally linked to cancer is underscored by the success in specific tumors of targeted therapies, that is, of compounds directly influencing altered genes and pathways. Sifting through the genes mutated or residing within regions of amplification or deletions and separate the subset causally implicated in oncogenesis from by-stander genes not endowed with carcinogenic potential is a major challenge facing the cancer community. To this end, the application of genomic and computational tools can greatly reduce the number of candidate targets and therefore help the selection of subsets of genes that could enter more in-depth experimental validations. A case in point is represented by the identification of gains and losses emerging from the analysis of tumor DNA with novel technologies, such as array comparative genomic hybridization (aCGH). Indeed, in both hematological and solid tumors, the extent of genomic rearrangements in each sample is often daunting. To reduce the dimensionality of these changes, several approaches have been proposed, including statistical measures of recurrence of specific lesions across samples or filtering the different lesions through different clinical parameters, including survival and drug resistance. Once a filtered list of genetic lesions is available, the next step is to identify the bona-fide cancer genes within these regions. One of the most promising approaches in this regard stems from the merging of aCGH with gene expression profiling (GEP), which has allowed us to reduce the number of potential candidate genes residing within genomic lesions by 75% in a collection of multiple myeloma patients.
While several challenges lie ahead, including more relevant in vitro and in vivo experimental systems, genomic analysis and implementation of different computational tools will hopefully help to design more effective therapies against cancer.

Plenary session 2: Bioinformatics

PL4
ANALYSIS OF CALLED ARRAYCGH DATA: CLUSTERING, TESTING AND INTEGRATION WITH GENE EXPRESSION DATA

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Array CGH log-ratios resulting from feature extraction are often converted back to discrete states representing loss (< 2 copies), normal (2 copies), gain (3-4 copies), and, possibly, amplification (> 4 copies). This process is referred to as `calling'. This discretization poses a problem for typical downstream statistical data analyses, since standard methodology, mainly developed for analysis of mRNA expression arrays, focuses on continuous rather than discrete data. Moreover, the strong correlation between subsequent clones or oligos is often neglected in the analysis.
Recently, we have proposed several easy-to-use algorithms which are tailored to analysis of called aCGH data. A new clustering algorithm, termed 'Weighted clustering of aCGHdata' (WECCA), uses a new distance metric and allows for weighting areas of the genome differently (eg small amplifications obtain more weight). It outperforms existing algorithms when the clusters are associated with survival. Secondly, a statistical testing approach is introduced that allows for detection of DNA

regions (which is a collection of subsequent oligos or clones) that differ between 2 or more clinical groups. By focusing on regions rather than individual array elements statistical power is gained and interpretation of the results is more natural.
Finally, a new approach is presented for associating the called DNA data with mRNA expression data. We developed a procedure that tests for such an association per gene. One point of criticism on 'calling' is that of potential information loss for elements of which the log-ratio lies between that of a normal and an aberrated state. We overcome this criticism by incorporating the confidence of the call into the test procedure.
All procedures are illustrated on several cancer genomics data sets. The software is freely available and has been applied by a fairly large group of cancer biologists.

PL5
BIOINFORMATIC CHALLENGES IN MICRORNA DISCOVERY

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MicroRNAs are 20- to 23-nucleotide RNA molecules that can regulate gene expression. Currently over 500 microRNAs have been experimentally identified in mammalian genomes, whereas estimates go up to 1000 and beyond. There are two ways to identify microRNAs encoded by a genome. First, computational algorithms that take into account known characteristics can be used to scan the complete genome. Experimental validation will be required to confirm the existence of the candidate microRNA in vivo. Secondly, small RNA from a sample can be cloned and sequenced, for example using massively parallel sequencing technologies. Also, in this latter case, bioinformatic analysis is required to annotated retrieved sequences and classify them as (candidate) microRNAs or as another kind of small RNA.
We have developed a modular data analysis pipeline for the comprehensive computational analysis of small RNA sequencing data. Experimental data generated on Roche/454 (GS-20 and GS-FLX), Illumina (Genome Analyzer) and Applied Biosystems (SOLiD) platforms can be used as input. Output formats are flexible, with up-to-date integration with public resources and ready-to-interpret, allowing the researcher to focus on biological challenges instead of computational problems.

PL6
MINING DATA FOR CANDIDATE METHYLATED GENES

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(No abstract available)

Plenary session 3: Tumor microenvironment

PL7
CHEMOKINE NETWORK OF MONONUCLEAR PHAGOCYTES IN THE HYPOXIC MICROENVIRONMENT

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Hypoxia is a condition of low oxygen tension occurring in inflammatory tissues that creates a special microenvironment conditioning cell physiology. Peripheral blood monocytes (Mn) represent the early leukocyte infiltrate in hypoxic tissues. We investigated the gene expression pattern of human monocytes (Mn) following exposure to hypoxia (1% O2). We identified a group of novel hypoxia-responsive genes related to the inflammatory responses. Among them, we demonstrated up-regulation by hypoxia of the macrophage inflammatory protein-3 (MIP-3a), a CC-chemokine chemotactic for immature dendritic cells, activated/memory T lymphocytes, and naive B cells. MIP-3a mRNA induction was paralleled by increased protein expression and secretion, was associated with gene transcription triggered by the combined effects of HIF1a and NFkB. In vivo data on the expression of MIP-3a in hypoxic human exudates will be presented. MIP-3a induction by hypoxia may represent an important regulatory mechanism recruit dendritic cells (DC) and other leukocytes to the hypoxic site. Maturation, differentiation and mobility of DC in an hypoxic environment is largely unknown. We studied the transcriptome of immature DCs, differentiated from human monocytes under hypoxic conditions (Hi-DCs) or normoxic condition (iDCs). Hypoxia up-regulated genes fell into several pathways associated with cell movement/migration suggesting an improved motility of Hi-DCs. The cytokine-cytokine receptor interaction pathway showed a characteristic and unexpected partition between down-regulated chemokines (CCL24,

CCL23, CCL26, CCL18, CCL14, CCL13) and up-regulated chemokine receptors (CXCR4, CX3CR1, CCR2, CCR3). With the exception of CXCR4, this is the first evidence that the above genes are modulated by hypoxia. Up-regulation of CCR2 and CXCR4 was associated with a strong chemotactic of Hi-DCs but not of iDCs. Shutting down the chemokines production in Hi-DCs may be important to quench the inflammation and the consequent tissue damage and to free the chemokine receptors for optimal performance in driving the cells to a normoxic environment. Hi-DCs represent a class of mobile DCs with a distinct and characteristic phenotype intended for migratory rather than inflammatory activity.

PL8
THE HIF HYDROXYLASE PATHWAY AND THE TUMOUR MICROENVIRONMENT

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Recent studies have defined hypoxia signalling pathways that regulate an extensive range of changes in gene expression via the post-translational hydroxylation of a transcription factor termed hypoxia inducible factor (HIF). HIF is an alfa/beta heterodimeric complex that bind hypoxia response elements at target genes involved in angiogenesis, energy metabolism, matrix metabolism, pH regulation, cell survival and proliferation decisions.
Regulation of HIF by oxygen is mediated by post-translational hydroxylation at specific residues within the alfa-sub-units. HIF prolyl hydroxylation governs proteolytic regulation of HIF whereas HIF asparaginyl hydroxylation modulates interaction with transcriptional co-activators. These hydroxylations are catalysed by a set of non-haem Fe(II) 2-oxoglutarate (2OG) dependent dioxygenases. During catalysis, the splitting of dioxygen is coupled to the hydroxylation of HIF and the oxidative decarboxylation of 2-oxoglutarate. Hydroxylation at two prolyl residues within the central 'degradation domain' of HIF-alfa determines binding to the von Hippel-Lindau (pVHL) E3 ligase complex by a hydrogen bonding mechanism, thus directing HIF-alfa polypeptides for proteolytic destruction by the ubiquitin/proteasome pathway. Since the HIF hydroxylases have an absolute requirement for dioxygen this process is suppressed in hypoxia allowing the HIF-alfa to escape destruction and activate transcription.
Suppression of HIF hydroxylation by genetics events, microenvironmental hypoxia, redox mechanisms, altered cellular metabolism or altered iron status activates HIF in tumour tissues. These mechanisms are amplified by other processes that activate HIF expression at transcriptional and translational levels. The consequences of HIF activation for tumour growth will be discussed together with possible therapeutic strategies.

PL9
HYPOXIA, HIF AND BREAST CANCER

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One of the crucial rate-limiting events during human tumor growth is encountered when tumors outgrow the pre-existing vasculature leading to hypoxia. Intratumoral hypoxia is a common feature of human solid tumor development and hypoxic tumors tend to be more aggressive and are clinically characterized by therapy resistance and a poor prognosis. The cellular response to hypoxia is mediated by the transcription factor Hypoxia-inducible Factor (HIF). I will discuss novel cellular pathways activated in response to hypoxia and HIF signaling and the clinical meaning of HIF activity in breast carcinogenesis.

PL10
(TITLE TO BE ANNOUNCED)

Peter Carmeliet
Catholic University Leuven, Leuven, Belgium

(No abstract available)

Plenary session 4: Translating basic cancer knowledge into clinical applications (1)

PL11
TRASTUZUMAB TRIGGERS ERBB2 SIGNALING AND INTERNALIZATION EVENTS

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The ligand-less tyrosine kinase receptor ErbB2 is overexpressed in approximately 30% of invasive breast cancers. Patients with ErbB2 over-expressing breast cancer have substantially lower survival rates and shorter time to relapse then patients without the overexpression. Clinical trials have shown the efficacy of Trastuzumab (HerceptinTM), a humanized monoclonal antibody, directed against the extracellular domain of ErbB2, in the treatment of ErbB2 overexpressing metastatic breast cancer. Nevertheless, nearly all become unresponsive during treatment. We had previously shown that TGFalfa expression by the cancer cells correlated with the acquisition of resistance to Trastuzumab. Further insights into this observation were impaired by the lack sufficient knowledge on the Trastuzumab mechanism of action. Here we report on the effects of Trastuzumab on ErbB2 signaling and internalization in the S-KBR-3 breast cancer cell line. Long term (72 to 120 hours) Trastuzumab treatment of SK-BR-3 leads to a slow degradation of ErbB2, and the induction of a G1 growth arrest after 24 hours of treatment. We than sought for the direct effect of Trastuzumab on ErbB2, by performing shorter kinetic experiments and by analyzing the ErbB2 directly bound to Trastuzumab. Our results show that Trastuzumab induces ErbB2/ErbB1 heterodimerization, phosphorylation of ErbB2-Y1248 and ErbB1-Y1173 residues, and recruitment in lipid Raft domains, within few minutes of treatment. ErbB2 and Trastuzumab undergo cycles of internalization and recycling, stepping through EEA1-endosomes. However, no ErbB2/ErbB1 degradation and ubiquitination is observed. Accordingly, the ubiquitin ligase Cbl binding site ErbB1-Y1045 is not phosphorylated. The activation of the ErbB2/ErbB1 heterodimer is concomitant with increased levels of phospho-Erk1/2, and the phospho-Erk1/2-dependent de-phosphorylation of Akt, linking Trastuzumab activity to the growth arrest.
Our data suggest that Trastuzumab is directly responsible of ErbB2/ErbB1 dependent signaling events eventually leading to growth arrest and receptor degradation. We are currently investigating the role of the receptor internalization recycling events in this framework.

PL12
SYSTEMS BIOLOGY APPROACHES TO UNDERSTANDING GASTRIC CANCER

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Gastric cancer (GC) is the second highest cause of worldwide cancer mortality, yet little is comparatively known about its underlying genetics and key oncogenic pathways. In this talk, I will describe our attempts to characterize GC from a genomics-oriented perspective, in order to better identify cellular interactions in this disease, and potential nodes for pharmacologic intervention. Specifically, I will present our results in establishing the "gastrome"- a consensus gene co-expression metanetwork of GC derived from hundreds of gastric tissues, and how a systematic analysis of this metanetwork can provide insights into various topological and systems-properties of GC. I will then describe how we were able to utilize this metanetwork to elucidate the function of the PLA2G2A gene as a key regulator of GC progression.

PL13
MECHANISMS OF RESISTANCE TO TARGETED AGENTS AND NOVEL THERAPEUTIC STRATEGIES

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In spite of recent advances in cancer cell biology, leading to the introduction of clinically active new drugs, expecially targeting the HER/ErbB family receptors, unfortunately disease control remains unsuccessful due to the presence of constitutive resistance in some patients and the development of acquired resistance also in the responders. Cells rely for growth and survival on a large degree of signalling redundancy and cross-talk among different pathways.

Therefore, the pharmacologic interference with only a single step of these pathways may often results therapeutically insufficient. Consequently, the concept of multitargeting has emerged as a therapeutic strategy in order to avoid the activation of "escape"pathways for tumor cells. The major escape pathways are: 1) the overactivity of tyrosine kinase receptors or signalling proteins alternative to those targeted, including c-MET and IGF-1R; 2) the constitutive activation of downstream mediators, particularly Akt; 3) the increased VEGF expression and angiogenesis. This review focuses on the role of complementary signalling pathways in the development of resistance to EGFR targeting agents and the rationale to combine novel inhibitors as anticancer therapy. Recent studies suggest that the host immune cells and tumor microenvironment not only play a critical role in tumor growth and progression but also may greatly influence the results of targeted agents.
In this regard, in the lecture will be discussed the major implications af all these signalling effectors and tumor compartments in conditioning drug efficacy and how they may help to design more effective therapeutic strategies reducing the chance for resistance.

Plenary session 5: Cancer stem cells and the tumor progression puzzle

PL14
IDENTIFICATION OF STEM CELLS IN SMALL INTESTINE AND COLON BY A SINGLE MARKER GENE LGR5

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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 60-day 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.

PL15
SPECIFIC LEVELS OF BETA-CATENIN SIGNALLING UNDERLIE ORGAN-SPECIFIC CANCER STEMNESS AND MALIGNANT BEHAVIOUR

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The canonical Wnt/beta-catenin signalling pathway plays a widespread regulatory role in embryonic and adult stem cell renewal and maturation. Accordingly, its constitutive activation is among the most common signalling defect in human cancers. Here, we report the generation of a novel mouse model, Apc1572T, carrying a truncating mutation in the Apc tumour suppressor gene. The hypomorphic Apc1572T allele results in intermediate levels of Wnt/beta-catenin signalling activation and in a moderate differentiation defect when compared with other Apc mutations known to predispose to intestinal cancer. Notably, Apc+/1572T mice have no predisposition to intestinal cancer but develop multifocal mammary adenocarcinomas and subsequent pulmonary metastases in both genders. The histology of the Apc1572T primary mammary tumours is highly heterogeneous with luminal, myoepithelial and squamous lineages, and is entirely recapitulated in the lung metastases. Sorting of Apc1572T tumours for the Lin-CD29hiCD24+ combination of cell surface antigens results in the isolation of a relatively small subpopulation of cancer cells earmarked by intracellular beta-catenin accumulation. The Lin-CD29hiCD24+ cells are able to recapitulate tumorigenesis when transplanted in NOD-SCID recipient animals at low multiplicities. Expression profiling of the normal and cancer-associated Lin-CD29hiCD24+ stem cells revealed a striking similarity between the two populations. Our results underline the importance of specific dosages of beta-catenin signalling activation in conferring predisposition to organ-specific tumorigenesis. Moreover, we show that intracellular beta-catenin accumulation, previously shown to earmark a small and non-randomly distributed subpopulation of tumour cells in colorectal cancer and to predict poor prognosis in breast cancer, underlies cancer stemness and metastatic behaviour in the mammary gland.

PL16
MUTANT STEM CELLS AND THE FIXATION AND THE FIXATION AND SPREAD OF MUTATIONS IN THE GASTROINTESTINAL EPITHELIUM

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Our current concepts of the development of cancer begin with the development of multiple cumulative epigenetic and genetic alterations that ultimately transform a cell, or indeed a field of cells in, for example, an epithelial sheet such as the epidermis or tracheobronchial mucosa. These early genetic events lead to clonal expansion of genetically-modified cells in a particular field. Subsequently further mutation and selection in one or more of these cells will drive them towards the fully-developed malignant phenotype. In several instances, a population of cells with such early genetic changes remains in the epithelium, the concept of field cancerization. Usually, but not always, such field changes can diagnosed morphologically: examples would include the pre-malignant actinic keratosis in the epidermis after chronic sunlight exposure and the squamous metaplasia that occurs in the tracheobronchial tree of smokers.

Tumors in the gastrointestinal tract are common: colorectal cancer is amongst the most frequent of human tumors, and carcinoma arising in Barrett's esophagus is increasing rapidly in the Western world. Pre-malignant lesions are well-recognised in the human gut - dysplasia in the context of ulcerative colitis, and of course the colorectal adenoma, so often the precursor of cancer. In the stomach and in Barrett's esophagus, it is intestinal metaplasia which has been incriminated as the main pathway in the development of cancer in these epithelia.

Important questions which then arise include: where do the mutant cells originate in the intestinal crypts, gastric glands or esophageal epithelium? Once established, how do these mutant cells then colonize the crypt or gland? And then once a crypt or gland is filled with mutant cells, how do they then spread within the epithelium to form the aforesaid field lesion? Intrinsic to thee questions are the stem cells in this system: we would proposed that it is a cell or cells the stem cell population which is first mutated and then, with or without selection, fills the stem cell niche with mutant stem cells - the process of niche succession. The next step is where the mutant stem cell colonizes the entire crypt or gland, - clonal conversion, where the entire unit is filled with these cells. In some instances, such as intestinal metaplasia, this lesion is now recognizable. This single crypt or gland then has to spread within the epithelium to form the pre-cancerous field.

We have used mutations in the cytochrome oxidase 1 gene (cox1), which is encoded mainly by the mitochondrial genome, as a clonal marker. We detect cox 1 and a nuclear-encoded oxidative enzyme, succinic dehydrogenase, by double enzyme histochemistry and mutations by sequencing the entire mtDNA genome, and have been able to examine these concepts of filed canzerisation in the human gut experimentally. We have been demonstrated that the progeny of a morphologically normal human colonic crypt or gastric gland stem cell that contains an mtDNA mutation can expand to occupy a whole crypt or gland: that neighboring mutated crypts and gastric glands have the same genotype, which is different from adjacent cytochrome c oxidase-positive crypts and glands; that mutated crypts in the process of fission share the same mutated mitochondrial genotype not present in neighboring cytochrome c oxidase-positive: that mutated crypts/glands are clustered together throughout the colon and stomach, and that patches of cytochrome c oxidase-deficient crypts increase in size with age. We have made similar observations in intestinal metaplasia in the stomach. We thus demonstrate definitively that crypt fission is the mechanism by which mutations spread in the normal human colon and stomach. and that this then further expands by fission to form a patch or field.

We conclude that niche succession, clonal conversion and crypt/gland fission as the mechanisms by which the fixation and spread of mutations occur in the colon and stomach. However, the spread of mutations in Barrett's esophagus is extremely complex, involving the evolution of multiple clones. Reasons for this will be discussed.

Plenary session 6: Chromosome territories – function and aneuploidy

PL17
LINKING THE LINEAR DNA SEQUENCE ORGANIZATION ON METAPHASE CHROMOSOMES WITH 3D CHROMATIN ARCHITECTURE IN INTERPHASE NUCLEI OF NORMAL AND MALIGNANT CELLS

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Metaphase chromosomes show a linear organization with clusters of low and high gene density, of different replication timing, DNA composition and compactness, reflected by a consistent chromosome specific banding pattern. Until recently there was a lack of comprehensive data on how the distinct segments of metaphase chromosomes are folded in the variably shaped chromosome territories (CTs) in interphase nuclei both of normal or malignant cell types.
We performed multicolor 3D-FISH in different cell types using whole chromosome painting probes and BAC-pools comprising genomic regions of different gene density and transcriptional activity from chromosomes 11, 12, 18 and 19. By confocal microscopy and elaborated quantitative image analysis we analyzed the 3D chromatin arrangement in these cell types. Chromatin during interphase is found spatially arranged in a distinct radial pattern with many (though not all) cell types showing a preferential localization of gene-dense and early replicating DNA in the nuclear interior and of gene-poor and later replicating DNA at the nuclear envelope. Such patterns were found evolutionary conserved over several hundred millions of years, illustrating that the radial arrangement of chromatin in the interphase nucleus represents a basic principle of nuclear architecture and pointing to a functional relevance in the context of epigenetic mechanisms of gene regulation. For several human cell types we could show that regional gene density within windows of several Mbs is a decisive parameter for the radial arrangement of chromatin in the nucleus, while the influence of transcriptional activity per se has remained a matter of discussion.
A significant fraction of tumor cells show complex chromosomal re-arrangements and the nuclear morphology and chromatin texture characteristic for a given normal cell type can change drastically during malignant transformation, e.g. by a relocalization of heterochromatin. We have compared the three-dimensional higher order chromatin architecture in normal and tumor cells. Our findings demonstrate the overall maintenance of a radial gene-density correlated chromatin arrangement in tumor cell nuclei irrespective of chromosomal arrangements observed in the different cell types. Preliminary data suggest that tumor related inversions appear to influence the nuclear topology of involved loci to a greater extent compared to chromosomal translocations.

PL18
IDENTIFYING NOVEL CHROMATIN PACKAGING FACTORS

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Human cells package their DNA into chromatin, a nucleoprotein complex. Cancer is a disease of aberrant gene expression. Since gene expression is regulated by chromatin structure it is important to understand how the structure of the chromatin fibre is modulated and how this influences gene expression. The primary structure of chromatin, the nucleosome, is well understood. However the secondary level of chromatin fibre folding, the 30 nm solenoid-like fibre, is much less well characterised, in particular the factors that regulate its conformation. In cancer many genes are mis-expressed and in part this maybe due to an alteration in chromatin fibre structure.
In normal lymphoblastoid cells we have analysed the secondary level of chromatin folding, the 30 nm chromatin fibre, across the human genome. We showed that compact chromatin fibres originate from some sites of heterochromatin (C-bands), and G-bands (euchromatin), whilst open chromatin fibres correlate with regions of highest gene density, R-bands, but not with gene expression. This data shows that the chromatin fibre has the ability to adopt alternate structures in different parts of the genome, but the factors responsible are unknown. Genes most frequently mis-regulated in cancer are found enriched in open chromatin suggesting this environment might be transcriptionally poised and readily influenced by an alteration in chromatin structure.
We have shown that changes in histone modifications and DNA methylation do not greatly influence the secondary-level folding of the chromatin fibre. It has been suggested that RNAs might be a component of the mammalian chromatin fibre and recent studies have

shown that some chromodomain containing-proteins including HP1alpha and CBX7 have the ability to bind to RNA. We have developed a novel approach for identifying chromatin-associated RNAs. Some of the RNAs we have isolated appear to have a nuclear diffuse distribution whilst others are more restricted to nuclear compartments including splicing and para-speckles. We are currently characterising these RNAs and identifying whether they play a role in regulating the 30 nm chromatin fibre or higher-levels of interphase chromatin fibre organisation. The mis-regulation of these RNAs in disease might then be expected to affect the expression of many down-stream genes.

PL19
MITOTIC CHECKPOINT DEFECTS IN CANCER: BOTH CAUSE AND TARGET FOR THERAPY?

Geert Kops
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The genetic trait shared most commonly by solid tumors of all kinds is aneuploidy. Aneuploidy is a manifestation of underlying chromosomal instability, or CIN. CIN can contribute to the transformation process in several ways, including amplification of oncogenes and haploinsufficiency or LOH of tumorsuppressor genes. Faithful chromosome segregation in healthy cells is monitored by the mitotic checkpoint. This checkpoint halts exit from mitosis when chromosome mis-segregations that will lead to aneuploidy are looming. Many cancer cells have a weakened but not completely inactive mitotic checkpoint, and several lines of evidence indicate that this may have contributed to the transformed phenotype of these cells by enhancing CIN. At the same time, complete inhibition of the mitotic checkpoint causes such massive chromosome mis-segregations as to efficiently nduce apoptosis. Thus, checkpoint weakening may contribute to tumor formation, while further inhibition of the checkpoint will kill the cells. I will discuss these various faces of the mitotic checkpoint in relation to cancer and will present our recent advances in using this checkpoint as an anti-cancer therapy.

Plenary session 7: Molecular imaging

PL20
NON-INVASIVE IMAGING OF BIOLOGICAL PROCESSES AND DISEASE STATE

Venu Raman
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Imaging disease progression, from early onset throughout its biogenesis, has become a crucial component to the development and management of highly effective treatment regimens. The term, imaging, refers to any morphological, physical, biochemical, or genetic characteristic that can be visualized and catalogued. For example, the very familiar and well-established field of immunohistochemistry generates very specific images of the molecular environment of live and fixed tissue sections with immunoglobulin based staining protocols. There is an ongoing concerted interdisciplinary effort to extend imaging beyond this “snapshot”approach through the synthesis of novel reagents and the development of new instrumentation along with computer software. The latter provides accurate 3D reconstructions and high resolution images.
Over the past decades, the fields of Magnetic Resonance Imaging, Positron Emission Tomography, Single Photon Emission Tomography, and Optical Imaging are pushing the limits of sensitivity and detection. These technologies facilitate real-time dynamic non-invasive imaging of biological processes in living subjects in both preclinical and clinical settings. This includes non-invasive and longitudinal tracking of variant cells, which can be isolated at precise times of a process such as metastatic progression or developmental differentiation. The subsequent cellular characterizations aid in the understanding of the varied biological changes that occur during the course of these processes. Real-time image-guided pharmaceutical delivery strategies are in use, which allow for optimizing the timings of drug administration. In these protocols, clinicians are able to visualize the accumulation of a component of a treatment regime that mediates the action of a prodrug, for instance, at the site of interest and its clearance from vital organs. Such strategies minimize systemic toxicity of untimely prodrug administration. This type of strategy also presents evidence of treatment efficacy and, thus, an indication as to whether or not a modification or change in treatment is necessary. In this talk, I will bring to the forefront some of the recent advances in several of these areas of molecular imaging

and discuss the general utility that this field is having in both preclinical models and patient application

PL21
2D AND 3D WHOLE BODY OPTICAL IMAGING AND ITS APPLICATIONS, INCLUDING INTRA-OPERATIVE GUIDED SURGERY

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Recent advances for imaging weak visible light sources using CCCD cameras, peltier cooled detectors and micro-plate channel intensifiers allow detection of photon emission from inside the tissues of small animals. Whole body Fluorescent imaging (FLI) and Bioluminescent imaging (BLI) are now applied to study cell- and tissue specific promoters but also to follow trafficking, differentiation and fate of i.e. GFP/RFP and/or luciferase expressing cells, or processes like tumor progression and metastasis, apoptosis, inflammation, protein-protein interaction, hypoxia and angiogenesis, and gene-transfer. Optical imaging and optical reporter systems are also very cost-effective and time-efficient and they are particularly well suited for small animal imaging. Until recently using firefly luciferase as a reporter, BLI was the most commonly used technology for whole body optical imaging. In Cancer Research whole body optical imaging has allowed semi-quantitative measurements of tumor progression and metastasis and treatment response.
Limitations of fluorescence GFP reporter imaging include auto-fluorescence, the requirement of an external source of light and the exponentially decreasing intensity of light with increasing depth of the target. However, a new class of red fluorescent proteins and its more red shifted variants (i.e. mCherry, mPlum) as well as the development of near-infrared dyes and quantum dots that can be coupled to al kinds of ligands, antibodies etc, are providing better deep tissue imaging characteristics (penetration of cm's). These new developments brings optical imaging also into the clinic. Especially intra-operative optical imaging guided surgery using near infrared probes or nano-particles will revolutionize oncological surgery.
Optical imaging has been based on 2D images and, therefore, spatial resolution was poor and quantification difficult and semi-quantitative. 3D optical tomography has now made it possible to better quantify photon emission. In addition, fusing 3D optical images with images obtained from the same animal using MRI or CT allows obtaining structural anatomic information and greatly enhances spatial resolution. Furthermore, structural tissue information obtained by fast CT or MRI also allows generating a tissue atlas that can be used to correct for tissue-dependent photon scattering and absorption. This allows for the first time to obtain real quantitative data.

PL22
MRI-BASED MOLECULAR IMAGING USING NANO-PARTICLES

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Magnetic Resonance Imaging (MRI) is a widely used modality for anatomical imaging. The method uses specific properties of the hydrogen nuclei from the water molecules to generate the images. Therefore, MRI can produce images of soft tissues with an excellent signal-to-noise. A large variety of measurement sequences are available that can distinguish between tissues as well as differentiate healthy from diseased tissue. In many cases, the differences between healthy and diseased tissue are not specific. Therefore, techniques to assess diseases at the molecular level are being developed for MRI. However, the detection of molecular or metabolic markers by is hampered by the intrinsic low sensitivity of MR. Therefore, several classes of new specific contrast agents are being developed. One approach uses large spherical particles, referred to as nano-particles that can carry a large amount of 'imagable' MRI agents to a specific molecular site [1]. For example, the surface of such particle can be coated with Gadolinium chelates that will locally change the signal contrast. Local binding is achieved by also incorporating anti-bodies or other ligands on the surface that are specific for biomarkers, e.g. cell surface proteins that are over expressed in diseased tissue. The nano-particles can also be filled with specific atoms that resonate at frequencies different from water. Often, fluorine based compounds are used for this purpose. The MR properties of fluorine required specially optimized sequences. In particular, the different resonance frequencies have to be dealt with properly in order to prevent multiple images from one binding site[2]. Some applications of these specific targeted nano-particles, for example, are the MR imaging of angiogenesis and the early detection of colorectal cancer.

1. A. M. Morawski et al., Magn Reson Med 51, 480-486 (2004). 2. M. Yildirim et al., Proc. Intl. Soc. Mag. Reson. Med. 15 (2007)

Plenary session 8: Translating basic cancer knowledge into clinical applications (2)

PL23
MUTATIONAL AND FUNCTIONAL ANALYSIS OF CANCER ALLELES

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Mutations affecting tumor-associated genes are the hallmark of cancer. We have exploited cancer alleles using two complementary approaches. On one side we assessed cancer mutations as genetic determinants of clinical response and resistance to targeted therapies. On the other we have generated innovative tumour progression models by introducing cancer mutations in the genome of human cells. We will present evidences that these approaches can provide insights into the pathogenesis of cancer and are relevant for therapeutic intervention.

PL24
COMMON FRAGILE SITES CURRENT KNOWLEDGE AND HYPOTHESES

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Common Fragile Sites (CFSs) are chromosomal loci that become visible as chromatid gaps or chromosome breaks after culturing cells under replicative stress. Since they can be induced in a large proportion of healthy individuals, CFSs are considered a normal component of the chromosome structure. Nevertheless, the increased occurrence of CFSs has been linked to cancer and genetic disease mechanisms.
More than 100 CFSs have been identified in the human genome, with the 20 most frequently observed sites accounting for more than 80% of breaks. Although known for more than 20 years now, the molecular cause(s) of fragile site instability has not been resolved so far and are subject of intense research.
We will summarize the current knowledge on CFSs including (i) the mapping of CFSs in different species, (ii) knowledge on DNA sequence and flexibility (iii) the non-random colocalization of active and large genes in regions of CFSs, (iv) the correspondence between CFSs and DNA deletions, duplications and amplifications, (v) the timing of replication in regions containing CFSs, (vi) the integration of foreign DNA in CFSs such as viral DNA or artificial vectors, (vii) the occurrence of micro RNAs in relation to CFSs and (vii) CFSs and genetic disease including cancer.

PL25
ONCOGENE-INDUCED CELLULAIR SENESCENCE: A TWO-EDGED SWORD?

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Oncogene-induced cellular senescence (OIS) represents a growth arrest response to unscheduled oncogenic signaling and is thought to play an important role in the prevention of outgrowth of neoplastic cell clones on the basis of one or a few oncogenic mutations. The phenotype of the OIS cells includes large size, positivity for acid beta galactosidase activity, presence of SAHFs (senescence-associated heterochromatin foci) in combination with complete growth arrest brought about by activation of a tumour suppresor network including INK4A, ARF, p53 and RB. Indirect indications that OIS is effective in the prevention of cancer, derives from tumour-proneness syndromes resulting from germline inactivationg mutations in these genes. Indeed, a senescent phenotype has been identified in several animal models and a couple of growth-arrested neoplastic human lesions, including the melanocytic naevus (mole).

Progression to overt cancer is thought to require evasion or abrogation of the OIS response, and a growing body of evidence supports this notion. However, this does not mean that necessarily every aspect of the OIS response is lost in every cell of growing malignant tumours. Indeed, acid beta galactosidase activity ands p16 immunoreactivity are commonly encountered in some of the cells of clinically overt human cancers. This subpopulation of growth-arrested tumour cells may influence patient outcome, since the OIS phenotype appears to be linked to an anti-apoptotic state, and very recent experimental data suggest that OIS needs to be actively maintained, i.e., does not reflect irreversible transition to a permanent postmitotic state. Transiently senescent cancer cells resisting apoptosis might thus negatively

impact on outcome of systemic therapies that induce apoptotic cell death in tumours.

Plenary session 9: Analysis of tumor cell populations by flow and image cytometry

PL26 (Distinguished Bas Ploem lecture) ROLE OF CYTOMETRY IN CLINICAL AND RESEARCH MEDICINE: NEW TOOLS FOR EVALUATION AND PREDICTIVE MEDICINE

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The field of cytomics broadly defined as the systematic study of biological organization and behavior at the cellular level has begun to mature and establish itself as an integral component in cell biology. The necessary tools for integration of cytomics into the fundamental nature of cell systems analysis are maturing but new tools are demanded to achieve our goals. While there is a long way to go before we have tools that can perform true cytomics analysis, cytometry is a subset of tolls that is tremendously powerful and from which we can extract a significant subset of information about many biological systems.

It is important therefore to realize that new technologies must be developed for cytomics to become a reality and be effective in the clinical setting. For example there will be a need for essential development of new sensor technologies that provide both sensitivity and selection in the visible and near IR spectrum. Secondly, a better integration between different measurement and detection tools will be needed. We simply cannot make independent measurements and hope to integrate these tools easily. Thirdly, in order to analyze the complex data sets resulting from new technology integration a major advance is needed to accommodate analysis of these data sets. Fourthly, chemistries must advance to permit greater selectivity of tracking tools. These will most likely expand beyond fluorescence to accommodate enhanced scatter analysis as well as chemical composition.

Next generation technologies are constantly being created. The fastest areas are in consumer electronics - technologies that rarely impact clinical medicine. But it has become evident that before we can develop useful relatively low cost predictive tools, we need to integrate the advances in such technologies into the medical diagnostic paradigm.

Together, these advances place the cytomic opportunity into a new dimension for understanding metabolic responses in single cells and ultimately defining new functional populations of cells. The result will be new research tools as well as a toolset for clinical and diagnostic utility focusing on predictive medicine as a fundamental tool in the clinical medicine toolkit.

PL27 NEW PROGNOSTIC PARAMETERS IN COLORECTAL CANCER

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Background: Recent models on metastatic invasion focus on the tumor-"host"interface, in particular the role of the stromal tissue. The biological meaning of the stromal compartments are thought to be part of the process of wound healing in cancer, but there is also strong emphasis that CAF's (cancer-associated fibroblasts) are important promotors for tumor growth and progression. Assuming these models are correct we anticipated that changes in the proportion of stromal compartment in the primary tumor could reflect progression.We therefore investigated if the carcinoma-percentage (CP) of the primary tumor, as a derivative from the carcinoma-stromal ratio, could be applied as a candidate marker to identify patients with poor prognosis for adjuvant therapy.

Methods: In a retrospective study of 63 patients with colon cancer (stage I-III, 1990-2001) the carcinoma-percentage of the primary tumor was estimated on routine H&E stained histological sections. Additionally these findings were validated in a second independent study of 59 patients (stage I-III, 1980-1992).

Neither of the patients had preoperative chemo- or radiation therapy nor adjuvant chemotherapy.

Results: Of 122 analyzed patients 33 (27.0%) had a low CP and 89 (73.0%) a high CP. The analysis of mean survival revealed: overall-survival (OS) 2.13 years, disease-free- survival (DFS) 1.51 years for CP-low and OS 7.36 years, DFS 6.89 years for CP-high. Five-year survival rates for CP-low versus CP-high were respectively for OS: 15.2% and 73.0% and for DFS: 12.1% and 67.4%. High levels of significance were

found (OS p<0.0001, DFS p<0.0001) with hazard ratio's of 3.73 and 4.18.
In a multivariate Cox regression analysis, CP remained an independent variable when adjusted for either stage or for tumor status and lymph-node status (OS: p<0.001, OS: p<0.001).
Conclusions: The carcinoma-percentage in primary colon cancer has shown to be a factor to discriminate between patients with a poor and a better outcome of disease. This parameter is already available upon routine histological investigation and can, in addition to the TNM classification, be a candidate marker to further stratify in more individual risk. Our current research aims to explain the molecular signaling mechanisms underlying this morphological observation, in particular the transforming growth factor-ϐ (TGF-ϐ) pathway, that plays a prominent role in epithelial-to-mesenchymal transition (EMT), used by cancer cells to develop invasive and migratory abilities.

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PL28
MULTIPARAMETER IMMUNOPHENOTYPIC ANALYSIS OF HAEMATOLOGICAL MALIGNANCIES: TECHNICAL ADVANCES

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Immunophenotyping of leukemia and other haematological malignancies has become one of the most relevant clinical applications of flow cytometry. Its utility was initially focused mainly on the characterization of leukemia cells and classification of the disease once diagnosis of leukemia/lymphoma had already been established. Progressively some immunophenotypic markers have been associated with disease prognosis and the panels used were extended. More recently, immunophenotyping of leukemia by multiparameter flow cytometry has proven to be a sensitive and specific method for the identification of leukemia cells even when present at very low frequencies. A new era in which the use of flow cytometry immunophenotyping, extended also for the detection of minimal disease -for staging purposes and to monitor response to treatment- and the diagnostic screening of haematological malignancies, was established. Currently, immunophenotyping of neoplastic hematological cells is more likely based on the use of unique combinations of >3 markers for the detailed comparison between normal and leukemia cell phenotypes rather than the evaluation of single antigen expression. Similarities between leukemia and normal hematopoietic cells allow definition of the lineage and maturation stage of the pathologic cells, and the phenotypic aberrations are frequently associated with underlying genetic abnormalities, this information being of great help in the diagnosis, classification and the prognostic evaluation of haematological malignancies as well as for diagnostic screening, disease staging and monitoring of minimal disease levels. Interestingly, such leukemia-associated phenotypes might also point out potential new drug target molecules for re-inducing cell differentiation/death pathways. Despite these advances and the recognition of new applications of flow cytometry immunophenotyping in the management of patients suffering from different types of haematological malignancies, new applications o flow cytometry are expected to emerge from the development, identification and evaluation of new antigenic markers, in the context of the recent improvements in instrumentation, bead technology, multicolour stainings and multiparameter analyses. In this presentation we will also review those new tools that have recently became available to the study of leukaemia/lymphoma by flow cytometry and their potential applications to the diagnosis and management of patients with haematological malignancies.

Plenary session 10: Translating basic cancer knowledge into clinical applications (3)

PL29 (The Patholigical Society Lecture)
DISCOVERY OF RECURRENT GENE FUSIONS IN PROSTATE CANCER: A NEW CLASS OF BIOMARKERS AND THERAPEUTIC TARGETS

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The characterization of disease-specific, recurrent chromosomal rearrangements in epithelial tumors, such as prostate cancer, is lacking. Using bioinformatics, we examined gene expression data for candidate oncogenic chromosomal aberrations based on outlier gene expression. Gene rearrangements, characteristic of human malignancies, were identified including two

members of the ETS family of transcription factors, ERG and ETV1, as outliers in prostate cancer. Either ERG or ETV1 was over-expressed in prostate cancers (50-70%) with mutual exclusivity across independent gene expression datasets suggesting a functional redundancy in prostate cancer development.
RNA ligase-mediated rapid amplification of cDNA ends (RACE) identified a recurrent gene fusion of the 5' untranslated region of a prostate-specific, androgen-regulated gene TMPRSS2 to ERG or ETV1 in prostate cancers over-expressing the respective ETS family member,, as confirmed by quantitative PCR (QPCR) and sequencing of rtPCR products. Fluorescence in situ hybridization (FISH) demonstrated that 23/29 (79%) prostate cancer samples harbored rearrangements in ERG or ETV1. In vitro studies suggest that androgen-responsive promoter elements of TMPRSS2 mediate aberrant over-expression of ETS family members in prostate cancer. We interrogated the expression of all ETS family members in prostate cancer profiling studies and identified outlier expression of ETV4 in 2/98 cases. Over-expression of ETV4 was confirmed in one case, and fusion of TMPRSS2 and ETV4 loci identified by RACE, QPCR and FISH.
These results suggest an important pathogenetic role for recurrent chromosomal rearrangements in common epithelial tumors with implications for the molecular diagnosis and treatment of prostate cancer. Three subtypes, TMPRSS2:ERG, TMPRSS2:ETV1 and TMPRSS2:ETV4 were identified, suggesting that dysregulation of ETS family member expression via gene fusions with TMPRSS2 may be a generalized mechanism for prostate cancer development.
Novel 5' fusion partners with outlier expression of ETS family members were identified; untranslated regions from a prostate-specific androgen-induced gene and endogenous retroviral element, a prostate-specific androgen-repressed gene, and a strongly expressed housekeeping gene. We recapitluted this shared aberrant over-expression of ETS genes in vitro in benign prostate cells that displayed increased invasion, confirming involvement in prostate cancer development. Identification of distinct classes of ETS rearrangements revealed activation of dormant oncogenes by juxtaposition to tissue-specific or active genomic loci. Subversion of active genomic regulatory elements may permit a generalized mechanism for carcinoma development,. Androgen-repressed and insensitive 5' fusion partners have critical implications for the anti-androgen treatment of advanced prostate cancer.

PL30
PANCREATIC CANCER MODELS AND MEDICINE

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Pancreatic ductal adenocarcinoma (PDA) is a common and lethal cancer, responsible for approximately 200,000 cases and deaths annually world-wide. Our inability to intervene meaningfully has been attributed to the poor activity of systemic therapies and to the late stage of disease presentation. We have generated several genetically engineered murine models (GEMM) of PDA to explore the disease etiology and investigate preclinical applications. These GEMMs harbour pancreatic-specific orthologous mutations in oncogenes (KRAS) and tumour suppressor genes (TrP53, p16Ink4a, SMAD4) that are the canonical genetic alterations found in human pancreatic cancer. Importantly, these GEMMs recapitulate the molecular and pathophysiological features of human pancreatic cancer, including the presence of signature biochemical alterations and chromosomal instability, and the development of widespread metastases and cachexia in effected mice. Using such models, we have explored the cellular origins of pancreatic cancer, identified new pathways involved in the genesis of pancreatic cancer, and investigated the therapeutic response of such models to standard treatments. Collectively, our results should illuminate some features of this malignancy and stimulate new approaches to pancreatic cancer patients.

PL31
CONDITIONAL MOUSE MODELS OF BREAST CANCER

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Genetically engineered mouse (GEM) models of human cancer not only permit us to gain a detailed insight into the specific genetic changes that drive tumor initiation and progression [1], but also provide the tools to define the underlying mechanisms of drug response and acquired resistance. Once these processes are understood in sufficient detail it may be possible to design combination therapies that give rise to complete remissions, while at the same time eliminating remnant cells that might elicit recurrent disease.

The focus of our research is on the genetic dissection of human breast cancer through the use of GEM models for BRCA-associated hereditary breast cancer [2,3], and E-cadherin-mutated metastatic breast cancer [4]. These models are used to study (i) investigate genotype-phenotype relations in mammary tumorigenesis; (ii) identify genetic changes underlying breast tumorigenesis using functional genetic screens, array-CGH, transposon tagging [5]; (iii) study the role of innate and adaptive immunity in breast cancer development; (iv) perform tumor prevention and intervention studies with conventional and targeted therapeutics [6]. The utility of GEM models of breast cancer is illustrated by our mouse models for hereditary breast cancer and invasive lobular carcinoma:

Women carrying germline mutations in BRCA1 or BRCA2 are strongly predisposed to developing basal-like breast cancers, which frequently contain TP53 mutations. To study the role of BRCA1/2 loss-of-function in breast oncogenesis, we have generated conditional mouse models for BRCA1- and BRCA2-associated hereditary breast cancer based on combined inactivation of BRCA1/2 and p53 in epithelial tissues [2,3]. The mammary tumors that arise in our BRCA1 mouse model show strong similarity to BRCA1-associated breast cancer in regard to high tumor grade, expression of basal cell markers and high degree of genomic instability [3]. This model may therefore be helpful in predicting chemotherapeutic responses of human BRCA-associated and BRCA-like tumors. Indeed, intervention studies with conventional and targeted chemotherapeutics showed a selective sensitivity of BRCA1-deficient mouse mammary tumors towards agents that directly or indirectly cause double-strand DNA breaks [6].

While metastatic disease is the main cause of death in breast cancer patients, the underlying mechanisms are poorly understood. Loss of E-cadherin is strongly associated with tumor metastasis, as well as with invasive lobular carcinoma (ILC), which accounts for 10-15% of all breast cancers. To study the role of E-cadherin in breast oncogenesis, we have produced E-cadherin conditional mutant mice and crossed these to our conditional p53 mammary tumor model. Combined loss of E-cadherin and p53 resulted in accelerated tumor development and a switch from non-invasive adenocarcinoma with a ductal morphology to highly invasive and metastatic carcinomas, which show strong resemblance to human ILC [4]. Moreover, loss of E-cadherin induced anoikis resistance and facilitated angiogenesis, thus promoting metastatic disease. Our data show that E-cadherin loss may play a dual role in mammary tumor initiation and metastasis formation.

References: 1. Jonkers and Berns. Nat Rev Cancer 2002; 2:251-65. 2. Jonkers et al. Nat Genet 2001; 29:418-25. 3. Liu et al. Proc Natl Acad Sci USA 2007; 104:12111-6. 4. Derksen et al. Cancer Cell 2006; 10:437-49. 5. Theodorou et al. Nat Genet 2007; 39:759-69. 6. Rottenberg et al. Proc Natl Acad Sci USA 2007; 104:12117-22.

PL32
A QUANTITATIVE CHEMICAL PROTEOMICS APPROACH REVEALS NOVEL MODES OF ACTION OF CLINICAL ABL KINASE INHIBITORS

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We describe a novel chemical proteomics approach to profile the interaction of small molecules with hundreds of endogenously expressed protein kinases and purine-binding proteins. This sub-proteome is captured by immobilized non-selective kinase inhibitors (kinobeads) and bound proteins are quantified in parallel by mass spectrometry using isobaric tags for relative and absolute quantification (iTRAQ). By measuring the competition with the affinity matrix, we assess the binding of drugs to their targets in cell lysates and in cells. By mapping drug-induced changes in the phosphorylation state of the captured proteome, we also analyze signaling pathways downstream of target kinases. Quantitative profiling of the drugs imatinib, dasatinib and bosutinib in K562 cells confirms known targets including ABL and SRC family kinases and identifies the receptor tyrosine kinase DDR and the oxidoreductase NQO2 as potent novel targets of imatinib. The data indicate that our approach is a valuable tool for drug discovery.