

Wednesday April 6

PREMIER LECTURE SERIES



09.00 - 09.40
Patrick Watt Memorial Lecture

Professor David Rimm
Yale University, USA

QUANTITATIVE *IN SITU* ANALYSIS OF PROTEIN EXPRESSION

Dr David Rimm
Yale University, USA

Tissue microarray technology has emerged as a powerful tool for measuring tumor-specific protein expression and linking it to clinical outcome. Automated quantitative analysis of the tissue spots take the technology one step further, removing observer bias, providing standards for quality control and providing for high throughput analysis. We have developed a platform (PM1000) and software (AQUA) that allow rigorous *in situ* assessment of protein expression with the accuracy of an ELISA assay. We have used this platform on a series of large cohort tissue microarrays to discover a range of relationships between protein expression and outcome that are not discernable using conventional pathologist based analysis. We believe this sort of quantitative, objective validation will facilitate the translation of tissue biomarkers from the research lab to broad clinical usage.

Biography

Dr. David Rimm, is an Associate Professor in the Department of Pathology at the Yale University School of Medicine. Dr. Rimm is Director of the Yale Cancer Center Tissue Microarray Facility and Program Director of the Yale Cytopathology Fellowship Program. His time is divided 25% to clinical service work in cytopathology, 75% time to research efforts. His lab group (12 researchers) focuses on tissue biomarkers, including predicting outcomes and response to therapies

in breast and colon cancer and melanoma. A subset of his group studies the cell and molecular biology of cell adhesion and growth factor receptors. He is currently supported by 8 grants from both public and private sources. He is an author of over 90 peer-reviewed papers and 3 patents and is the scientific founder of Histometrix, a digital pathology company. He has served as a consultant for a number of companies including Genentech, Thermo-Electron, Becton Dickinson, Cytoc, and Tripath Oncology.



09.40 - 10.20
Applied Biosystems Lecture

Professor Burkhard Brandt
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ASSEMBLING WHOLE GENOME EXPRESSION ANALYSIS DATA FROM SNAPSHOTS OF CANCER CELL CONDITIONS TO DECIPHER STAGES OF DIFFERENTIATION

Burkhard Brandt, Dirk Kemming, Nicola Tidow,
Horst Bürger, Reinhard Voss, Eberhard Korsching

DNA microarray technology and complete sequencing of the whole human genome enables the study thousands of genes, and the analysis of the whole genome, in parallel. This approach has been applied to immortal and fast growing cancer cells which represent model systems to study covariates such as cellular morphology, proliferation activity, regulation of apoptosis, growth factor self-sufficiency, insensitivity to anti-growth factor signals, cell motility, tissue invasion and distant metastasis. The goal is to identify genes which are the key players at switchboards of the intracellular signalling pathways. To perform this task economical in time and costs the false negative and false positive results have to be minimized in this extreme multiple testing problem. As a technical basis, improvements have been realized in the novel Applied Biosystems Human Genome Survey

Microarray (HGSM). The system uses 60mer oligonucleotides and chemiluminescence for detection increasing the sensitivity and specificity of expression. This was experimentally confirmed by a comparison of HGSM data and Real-Time RT-PCR. Pearson-correlation of 0.92 to 0.63 was found for main cancer genes, e.g. EGFR, Her2/neu, estrogen receptor α , progesterone receptor, urokinase-type plasminogen activator, and plasminogen activator inhibitor-1. In order to study pathways by microarray analysis which represent distinct stages of differentiation specific signalling, esp. those regulating cancer cell invasiveness and motility, we established cellular models expressing EGFR, Her2/neu and genetic modifications by vectors expressing an truncated and an perturbed Her2/neu defect receptor as well as intracellular Her2/neu signalling inhibitors. All cell lines have been evaluated for phosphatidylinositol- and inositolphosphate signaling, PLC- γ 1 activation, Ca²⁺-oscillation, actin reorganization and 3-dimensional migration. Applying a new biomathematical approach, we identified stable subsets of genes involved in the phosphatidylinositolphosphate and inositolphosphate metabolism, that explain the switch in cellular differentiation, consequently to a motogenic phenotype. For control the microarray data of the invasive breast cancer cellular models and genetically modified counterparts were re-analysed for the expression level and the significance of the difference in expression for the key molecule Her-2/neu simultaneously with all other genes. Two way hierarchical clustering of the expression data from these genes in breast cancer tissues from patients with complete clinical records clearly demonstrated distinct groups of cellular differentiation and clinical outcome. Therefore, our approach might be used as a classification tool to sort cancer into various clinically relevant subgroups that is not currently possible with other methods. The analysis of gene expression patterns may also be used to screen for individual genes that are differentially expressed between cancer tissues that respond and do not respond to a given therapy regimen. In conclusion, the novel Human Genome Survey Microarray generates highly reliable and accurate data when performing whole genome expression profiling which might become a useful tool for clinical diagnostics.



10.20 - 11.00

Dr Hilary Russell

Centre for Cancer Research and Cell Biology, Queen's University Belfast, UK

SEPTINS, THE CYTOSKELETON AND CANCER

Septins are an evolutionarily conserved group of GTP-binding and filament forming proteins that belong to the large superclass of P-loop GTPases. While originally identified in yeast as cell division cycle mutants with cytokinesis defects, they are now known to have diverse cellular roles which include polarity determination, cytoskeletal reorganization, membrane dynamics, vesicle trafficking, and exocytosis. Septin proteins form homo- and hetero-oligomeric polymers which can assemble into higher order filaments. They are known to interact with components of the cytoskeleton, i.e. actin and tubulin. There are at least 12 human septin genes, and although information on expression patterns is limited, most undergo complex alternative splicing with some degree of tissue specificity. One model suggests that septins act as regulatable scaffolds where the stoichiometry of septin associations, modifications, GTP status, and the interactions with other proteins allow the regulation of key cellular processes. An increasing body of data implicates the septin family in the pathogenesis of diverse disease states including neoplasia, neurodegenerative conditions, and infections. Our research has focused on the SEPT9 gene which encodes up to 18 transcripts. We have shown that ovarian epithelial neoplasia is associated with increased levels of individual transcripts in particular SEPT9_v1 and_v4*. We are therefore interested in determining the functional consequences of altered SEPT9 expression in epithelial cells and how this might contribute to aspects of the neoplastic phenotype. Evidence will be provided that SEPT9 can modulate remodelling of both the actin and microtubule components of the cytoskeleton. We find that SEPT9 overexpression causes enhanced motility, decreased polarity and resistance to microtubule-interacting drugs – phenotypes associated with neoplasia.

Biography

Hilary Russell studied Genetics and Microbiology at Queen's University Belfast and completed her PhD in Biochemistry in 1984. After a period of postdoctoral research in the Department of Genetics, Trinity College Dublin, she returned to Queen's and established her own

research group in 1988. She is currently a Senior Lecturer in Oncology in the Centre for Cancer Research and Cell Biology. The focus of her research is the molecular aetiology of epithelial ovarian cancer in particular, the role of the SEPT9 gene which the group identified by positional cloning. She also has a strong interest in research ethics and is Vice-Chair of one of the three Research Ethics Committees in Northern Ireland.

References.

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- [3] Ian G. Macara, Richard Baldarelli, Christine M. Field, Michael Glotzer, Yasuhide, Hayashi, Shu-Chan Hsu, Mary B. Kennedy, Makoto Kinoshita, Mark Longtine, Claudia Low, Lois J. Maltais, Louise McKenzie, Timothy J. Mitchison, Toru Nishikawa, Makoto Noda, Elizabeth M. Petty, Mark Peifer, John R. Pringle, Phillip J. Robinson, Dagmar Roth, S.E. Hilary Russell, Heidi Stuhlmann, Manami Tanaka, Tomoo Tanaka, William S. Trimble, Jerry Ware, Nancy J. Zeleznik-Le, Barbara Zieger. 2002. Mammalian septin nomenclature. *Mol Biol Cell*. 2002 Dec;13(12):4111-3.
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FREE PAPER SESSION 1

Location: PFC G07

01 11.30 - 11.45

GENOME WIDE (ARRAY CGH AND SAGE) AND QUANTITATIVE MICROSCOPY FOR THE ANALYSIS OF LUNG CANCER AND ITS PRE-CURSORS

Dr Calum MacAuley, Dr Kim Lonergan, Dr Cathie Garnis, Dr Bradley Coe, Dr Bryan Chi, Dr Martial Guillaud, Dr Alexei Doukine, Dr Jean le Riche, Dr Stephen Lam, Dr Wan Lam
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Genetic alterations and expression changes are known to be associated with the neoplastic process. Recently developed tools enable the genome wide evaluation of genetic copy number and known gene and novel gene expression changes from the minute lung samples available as part of lung bronchoscopic examinations. Additionally advances in automated microscopy have enabled the automatic identification of cancer and pre-cancerous cells in stained sections.

Methods: 1) A CGH array designed to provide complete overlapping coverage of the human genome was used to examine genetic changes in neoplastic lung tissue. 2) SAGE was used to examine expression changes seen in a large set (40+) of lung tissue libraries. Selection of material for these technologies requires an estimate of the purity of the samples. 3) A lung pathology workstation for the analysis of preinvasive neoplastic bronchial samples has been adapted to estimate the percentage of neoplastic cells in H&E stained sections. 4) Cytology microarrays were used to evaluate the expression of some of the identified gene candidates from 1) and 2).

Results: Arrays of ~30K amplified BAC DNA clones spanning the whole genome has been constructed. The impact of tissue heterogeneity and DNA quality/quantity in the generation of an accurate CGH profiles have been assessed. CGH profiling of a panel a collection of microdissected lung neoplastic specimens identified recurrent alterations. The repeatability and robustness of SAGE on limited lung samples has been demonstrated. More than 500 14mer tags of which 411 mapped to genes were found to be more than 50x over expressed between normal and cancer tissue. A small subset of the

possible screening markers have been evaluated on a set of cytology microarrays constructed from 60 BAL samples from subjects with and without lung cancer. The performance of a few of these markers approach that required for a usable screening test.

O2 11.45 - 12.00

PREMALIGNANT LARYNGEAL TUMORS AND APPARENTLY NORMAL MUCOSA FROM HEAVY SMOKERS SHOW RECURRENT GENE COPY NUMBER LOSSES AS REVEALED BY MLPA

Dr Mario Hermsen, Dr Nuria Rodriguez Prado, Dr José Luis Llorente Pendas, Dr César Alvarez Marcos, Dr Carlos Suárez Nieto

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Introduction. Long-term exhibition of the epithelium to factors such as tobacco and alcohol induces morphologic changes, ranging from pre-neoplastic hyperplasia and dysplasia to carcinoma in situ and invasive squamous cell carcinoma. This process is accompanied by the accumulation of genetic abnormalities. Gene copy number gains and losses were studied in 32 premalignant and 10 'normal' mucosa samples of the larynx using multiplex ligation probe amplification (MLPA). The aim was to find genetic changes involved in early stages of tumor development.

Methods. Thirty-two premalignant tissues of the larynx were obtained from patients that carried no other malignancy and 10 samples of 'normal' laryngeal mucosa from patients that were operated for other surgical reasons. The MLPA probe mixture was set up to detect copy number changes of 42 genes that according to the literature may play a role in the development and progression of head and neck squamous cell carcinoma.

Results. Preliminary data analysis of the premalignant cases revealed a number of recurrent losses. Frequent losses were observed for p53 (17p13), CDKN 2A and 2B (both 9p16), CDKN 2D (19p13) y CASP6 (4q25). Interestingly, we also found some cases with loss of EMS1 (11q13), a gene known to be involved in amplifications. All 'normal' samples analyzed so far (n=7) showed loss of p53 and losses of DCC and BCL2

(both 18q21). In addition, some cases showed losses of CDKN 2D and of CASP6.

Conclusion. Genetic changes are already present in apparently normal mucosa from heavy smokers. Invariantly, these changes concerned losses. Loss of p53 and DCC/BCL2 may represent very early changes in the development of squamous cell carcinoma. In premalignant tumors, there are more genes showing losses, but at lower frequencies. Loss of 3p as reported previously in oral cancer was not found.

O3 12.00 - 12.15

APPLICATION OF AN INTEGRATED BAYESIAN APPROACH TO THE DETECTION OF COPY NUMBER CHANGES IN ARRAY CGH IMAGES

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Bayesian methods have been widely applied to data analysis problems in the life sciences. Their application to copy number change detection has however been limited to the post processing of primary data extracted from microarray images by more traditional block box image processing techniques. This combination of approaches is sensitive to variability; both from experimental and operator sources, in the primary data.

In this presentation we describe a novel approach which combines the three major stages of arrayCGH analysis; extraction of primary data, normalisation and copy number change detection, in a single integrated solution. The Bayesian model based approach is introduced as a means of improving the measurement of the underlying biological quantities while at the same time estimating the likely variability associated with those measurements. The importance of a single statistical framework as a means of carrying forward knowledge of this variability through to the detection of copy number changes is illustrated by examples of how detected copy number changes may be qualified by confidence estimates; meaningful within the clinical context.

This presentation will also summarise the experiment undertaken in the pre-conference workshop organised by Bauke Ylstra. This will illustrate how an integrated Bayesian approach removes operator intervention from the analysis of arrayCGH data thereby making this powerful technology accessible to individuals without a strong background in microarray image analysis and the associated mathematical techniques.

04 12.15 - 12.30**HIGH RESOLUTION ANALYSIS OF DNA COPY NUMBER CHANGES WITHIN THE 20q AMPLICON IN ADENOCARCINOMAS OF DIFFERENT ORIGIN**

Dr B. Carvalho, Mr J. Coffa, Miss T.E. Buffart, Dr M.A.J.A. Hermsen, Dr N. Anders, Miss S. Mongera, Miss C. Postma, Prof J.P. Schouten, Dr B. Ylstra, Prof G.A. Meijer

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Introduction. Chromosomal instability (CIN) is the most prevalent type of genomic instability in solid tumours. The resulting chromosomal aberrations affect genes in molecular pathways that are critical to tumour biology. One of these is gain of 20q, which frequently occurs in adenocarcinomas of different origin. 20q gain is associated with progression from colorectal adenoma to carcinoma, and is related with poor clinical outcome in gastric, breast, and colorectal cancer.

We aimed to identify DNA copy number changes at gene resolution on chromosome 20q in a series adenocarcinomas of different origin in order to elucidate which genes and pathways are affected.

Materials and methods. We analysed a total of 59 adenocarcinomas (35 colorectal, 11 gastric, 7 Barrett's oesophagus and 6 breast carcinomas) that showed 20q gains by CGH. The analysis was done by MLPA using a dedicated 20q probe mix.

Results. All tumours presented 20q gains as expected. Two amplification areas were detected, one at 20q11.2 and the other at 20q13.2. Genes involved in these amplified regions include BCL2L1 and ZNF217, respectively. ZNF217, a transcription factor, was amplified in all tumour types, but BCL2L1, which has role in apoptosis, was exclusively amplified in colorectal tumours.

Conclusion. In conclusion, ZNF217 is amplified in adenocarcinomas from different origins and is likely to be a key player in carcinogenesis. Moreover, BCL2L1 seems to play a specific role in colorectal tumourigenesis.

0.5 12.30 - 12.45**EXPLORATORY QUANTITATIVE HISTOPATHOLOGICAL ANALYSIS OF ORAL PREMALIGNANT LESIONS (OPLS): ASSOCIATION OF MORPHOMETRIC INDEX WITH HIGH-RISK MOLECULAR PROFILES AND CANCER DEVELOPMENT**

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Objective. The aim of this study was to assess the potential of quantitative histopathology to identify high-risk OPLs by studying its correlation with conventional histopathology, the presence of high-risk molecular profiles and cancer development.

Methods. A total of 131 oral mucosa lesions were analyzed. Feulgen stained sections were analyzed with a modified version of the CytoSavant (Cancer Imaging). Samples from normal oral epithelium and samples from SCC were used to generate a Morphometric Index (MI). This MI was then calculated for all remaining specimens. Samples were also assayed for the presence of microsatellite patterns that have been previously associated with progression risk, using markers on 7 arms (3p, 9p, 4q, 8p, 11q, 13q, and 17p). The lowest risk (RR = 1) was associated with retention of both 3p and 9p (Molecular Risk (MR) Pattern 1). LOH at 3p &/or 9p in the absence of LOH at any other arm had an intermediate level of risk (RR = 3.8; MR2). The highest risk was associated with LOH at 3p &/or 9p in the presence of LOH at any other arm (RR = 33.4; n = 34, MR3). The time course data for these patients has been stratified into those which have developed cancer and those which have not.

Results. Using the MI in this pilot data we can correctly identify 94% of the high-grade OPLs while maintaining a specificity of 74%. The MI of lesions with High MR was significantly higher than the MI of lesions with Low- and Intermediate- MR. The MI identified patients which progressed to cancer 77% of the time while correctly identifying the patient which did not progress 78% of the time.

Conclusion. These data support the potential utility of quantitative histology as a phenotypic marker for the presence of high-risk molecular clones and for the progressive potential of oral premalignant lesions. (Supported by grant R01DE13124, NIDCR).

06 12.45-13.00**SPECIFIC KRAS 2 AND APC MUTATIONS IN HUMAN SPORADIC COLORECTAL ADENOMAS ARE ASSOCIATED WITH CHROMOSOMAL INSTABILITY**

Dr Walter Giaretti, Dr Tiziana Venesio, Dr Andrea Sciutto, Dr Angelo Scaramuccia, Dr Davide Malacarne, Dr Mauro Risio

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Introduction. Uncoupling of cell cycle and mitotic checkpoints and inhibition of apoptosis may lead to chromosomal instability (CIN) and aneuploidy. Along with these hypotheses, we investigated the role of KRAS2 and APC mutations in CIN using human sporadic colorectal adenomas (hscAD).

Methods. Multiple samples were obtained from 185 hscAD to provide nuclei suspensions for flow-cytometric analysis of the DNA Index (DI) and isolated DNA for DNA sequencing of KRAS2. In a subgroup of 85 hscAD, morphologic analysis was done to count atypical mitoses, cells with apoptotic bodies, and to separate architectural from cytokeratologic dysplasia. APC mutation spectrum was additionally evaluated by DNA sequencing in 65 hscAD.

Results. KRAS2 and APC mutations and DNA aneuploidy (DI \neq 1) were detected respectively in 42/185 (23%), 20/65 (31%) and 59/185 (32%) cases. Aneuploidy was characterised by near-diploidy (DI $<$ 1.4) in the majority of cases. Incidence of aneuploidy among KRAS2 wild-type, G \rightarrow A transitions, and G \rightarrow C/T transversions were respectively 29%, 22% and 63%. KRAS2 G \rightarrow C/T transversion mutations were strongly associated with aneuploidy as proven by contingency table-analysis (pFisher $<$ 0.005) and by multivariate logistic regression to account for the effects of size, dysplasia, site, type, age and sex (Odd-Ratio=6). G \rightarrow C/T transversions were also associated with a lower level of apoptosis (pChi-Square=0.01), while KRAS2 mutational status was associated with cytokeratologic dysplasia (pFisher=0.02). We have additionally observed association of the APC specific mutations within and downstream the mutation cluster region (MCR) with aneuploidy (pChi-Square=0.006). The corresponding truncated proteins with respect to the proteins for

mutation upstream MCR (approximately at codons lower than 1200) are characterised by the partial conservation of Beta-catenin binding and degradation sites. Multivariate analysis for APC to account for covariates awaits additional cases.

Conclusions. These data suggest that specific KRAS2 and APC mutations in subgroups of hscAD play a role in chromosomal instability and aneuploidy.

13.00-14.00 LUNCH AND NETWORKING

14.00-15.30 POSTER VIEWING AND TRADE SHOW

15.30-16.0 COFFEE AND NETWORKING

FREE PAPER SESSION 2

Location: PFC G07

07 16.00-16.15

ANALYSIS OF BRCA2 POINT MUTATIONS AND EXON DELETIONS/AMPLIFICATIONS IN BREAST AND BREAST/OVARIAN CANCER FAMILIES

Dr Adriana Falchi, Ms Natalie Scott, Ms Velma Hayes, Prof Yvonne Barnett, Mr Ian Garstin, Mr Peter Logan, Prof Patrick Morrison, Dr Antony Bjourson

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Introduction. The Scottish/Northern Irish BRCA1/2 consortium has recently indicated the presence of founder mutations in Northern Ireland including the 6503delTT mutation site in BRCA2 and the 2800delAA in BRCA1. A study in Nottingham has shown that 10% of the 20 Northern Irish families investigated exhibited large deletions and duplications in BRCA1 gene. These findings have implications for the design of a molecular

screening program for BRCA1/2 gene in high-risk breast/ovarian cancer families in Northern Ireland. We describe the analysis of BRCA2 in 60 individuals from Northern Irish cases of familial breast /ovarian cancers.

Methods. The subject of this study were families with history of breast and ovarian cancer, investigated at the Genetic Clinic of Belfast City Hospital as part of BRCA1/2 mutation screening program. All subjects included in the study were negative for point mutations in BRCA1 exon 11 after analysis by protein truncation test (PTT). Sixty DNA samples were analysed for BRCA2 exon 11 mutations by use of direct sequencing. In addition, 27 families negative for BRCA1 and 2 point mutations were analysed for large genomic rearrangements by Multiplex Ligation-Dependent Probe Amplification (MLPA).

Results. A novel putative pathogenic mutation in BRCA2 exon 11 was identified in one patient with breast cancer family history. The single base C->T transition results in substitution of a Serine by a Phenylalanine at codon 2098. In addition, a silent polymorphism designated 6328C>T was detected in another family. This polymorphism is seen in 4% of the general population. The 6503delTT mutation was not identified in the 60 samples analysed. MLPA reliably detected control deletions in BRCA1 exon11. Twenty-seven breast/ovarian cancer families are currently being tested for large deletions and amplifications of BRCA2 exons.

Conclusions. The present study provided initial data suggesting that BRCA2 6503delTT may not represent a prevalent founder mutation in Northern Ireland.

08 16.15-16.30

MOLECULAR DETECTION OF HER2 MRNA-POSITIVE CELLS IN THE PERIPHERAL BLOOD OF PATIENTS WITH OPERABLE BREAST CANCER

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Dr A. Pallis, Dr P. Kanellou, Dr L. Kalmanti,
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Purpose. To evaluate whether the detection of peripheral blood (PB) HER2 mRNA+ positive cells in patients with breast cancer could be another marker of the micrometastatic disease. Patients and Methods: The sensitivity

and specificity of HER2 mRNA detection by nested RT-PCR were investigated using MCF-7 and ARH-77 cells as well as blood from healthy women and patients with colorectal, early and metastatic breast cancer. PB from 216 patients with operable breast cancer, obtained before initiation of any adjuvant therapy, was tested for the presence of HER2 mRNA+ positive cells. *Results.* In dilution experiments the nested RT-PCR assay for HER2 mRNA was capable of detecting up to 105 MCF-7 tumor cells in 105 normal peripheral blood mononuclear cells; no signal was detected with the HER2- ARH-77 cells. No HER2 mRNA+ cells could be detected in the peripheral blood of 31 healthy women, as well as the peripheral blood and the bone marrow of 20 patients with colorectal cancer. Detection rates for HER2 mRNA+ cells in the bone marrow/blood of patients with early or metastatic breast cancer were 36.4%/23.8% and 35.5%/18%, respectively. Double immunostaining with a pan-keratin and a HER2 monoclonal antibody and confocal microscopy analysis revealed co-expression on PB cells in 5 (38.5%) of 13 patients. In addition, FISH analysis of PB tumor cells from 5 patients demonstrated HER2 gene amplification in all patients. Finally, aneusomy cells could be detected by LSI 1, CEP 8, CEP 11 and CEP 11 with chromosomal gain or loss in 2 of the patients. *Conclusion:* The results demonstrate that occult tumor cells expressing HER2 mRNA could be detected in the peripheral blood of patients with operable breast cancer; these cells are not always CK+ indicating that HER2 mRNA detection may represent another marker of the micrometastatic disease.

09 16.30-16.45

LABEL-RETAINING EPITHELIAL CELLS IN MOUSE MAMMARY GLAND DIVIDE ASYMMETRICALLY AND RETAIN THEIR TEMPLATE DNA STRANDS

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It has been postulated that the stem cells of somatic tissues protect themselves from mutation and cancer risk by selective segregation of their template DNA strands. Self-renewing mammary epithelial stem cells that were originated during allometric growth of the mammary ducts in pubertal females were labeled using [3H]-thymidine (3HTdR). After a prolonged chase during which much of the branching duct morphogenesis was completed, 3HTdR-label retaining epithelial cells

(LREC) were detected among the epithelium of the maturing glands. Labeling newly synthesized DNA in these glands with a different marker, 5-bromodeoxyuridine (5BrdU), resulted in the appearance of doubly labeled nuclei in a large percentage of the LREC. In contrast, label-retaining cells within the stroma did not incorporate 5BrdU during the pulse indicating that they were not traversing the cell cycle. Upon chase, the second label (5BrdU) was distributed from the double-labeled LREC to unlabeled mammary cells while 3HTdR was retained. These results demonstrate that mammary LREC selectively retain their 3HTdR-labeled template DNA strands and pass newly synthesized 5BrdU-labeled DNA to their progeny during asymmetric divisions. Similar results were obtained in mammary transplants containing self-renewing, LacZ-positive epithelial cells suggesting that cells capable of expansive self-renewal may repopulate new mammary stem cell niches during the allometric growth of new mammary ducts.

010 16.45-17.00

FUNCTIONAL RELEVANCE OF THE COLLAGEN-RECEPTOR DDR1 IN TUMOR METASTASIS

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Introduction. The interaction between malignant cells and the extracellular matrix plays an important role in directing tumor growth, invasion and metastasis. Our previous work showed that discoidin domain receptors (DDR) are a family of tyrosine kinase receptors that are activated by native, triple-helical collagen. Of importance, DDR1 is expressed in epithelial cells including the mammary gland epithelium and is overexpressed in ductal breast carcinomas. From the 5 isoforms currently known of DDR1, isoforms a, b and c are active enzymes while DDR1d and DDR1e are kinase-dead isoforms.

Methods and results. To investigate the role of DDR1 in human breast cancer, we stably expressed the a-, b- and d-isoform in the metastatic breast cancer cell line MDA-

MB-231, which lacks endogenous DDR1 expression. Cell clones overexpressing full-length DDR1 responded with sustained receptor activation upon collagen stimulation. We found that the expression of all three different isoforms increased the adhesion of MDA-MB-231 cells to collagen approximately 1.4 fold compared to mock-transfected cells, while cell proliferation remained unchanged. To examine its *in vivo* function in immunocompromised mice, DDR1-overexpressing cells were co-transfected with EGFP and either intracardially injected or orthotopically implanted into the mammary fat pad. For a period of one month, tumor cell growth and dissemination as well as the formation of bone metastases was monitored twice per week in anaesthetized animals using the Kodak Image2000 station. We observed a higher incident of bone tumors in mice injected with cells overexpressing DDR1b than control-transfected cells. Volumetric computed tomography (VCT) as well as Faxitron analysis were used as alternative methods to quantify the extent of tumor load and metastasis.

Conclusion. Our data provide compelling new evidence that DDR1 is involved in basement membrane attachment and invasion of human breast tumor cells.

PREMIER LECTURE SERIES

Location: PFC G07



PL4 17.00-18.00

Professor Peter Krammer
*Tumorimmunology Program,
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DEATH AND ANTI-DEATH

CD95, a member of the tumor necrosis factor (TNF) receptor superfamily induces apoptosis upon receptor oligomerization. The receptor and its ligand are important for apoptosis of peripheral T cells, for downregulation of an immune response and most likely, at least in part, also for peripheral T cell tolerance. In AIDS, apoptosis mediated by this system might contribute to the depletion of T helper lymphocytes. Likewise, in diseases in which liver cells

are destroyed the CD95 system might play a major role.

In a search to identify the intracellular signalling pathway of CD95 several molecules coupling to oligomerized CD95 were immunoprecipitated from apoptosis-sensitive human leukemic T cell and lymphoblastoid B cell lines. The following binding molecules were only associated with aggregated and not with monomeric CD95: phosphorylated FADD (MORT1) and caspase 8. Thus, caspase 8 was identified as the most CD95 receptor proximal protease which starts the cascade of protease reactions important for CD95-mediated apoptosis. Association of FADD and caspase 8 with CD95 was not observed with C-terminally truncated non-signalling CD95. FADD and FLICE did also not associate with a CD95 cytoplasmic tail carrying the lprcg amino acid replacement. FADD and caspase 8 form a death-inducing signalling complex (DISC) with the CD95 receptor and are, thus, the first CD95 associating proteins of a signalling cascade mediating apoptosis. The function of the DISC is discussed in detail, particularly with respect to its role in sensitivity and resistance to apoptosis.

The CD95 death system plays a role in destruction of liver tissue. In hepatitis cytotoxic T lymphocytes might use the CD95 system to kill infected hepatocytes. In M. Wilson copper overload leads to upregulation of the CD95 ligand that may finally contribute to acute liver failure. In HCC from patients treated with chemotherapeutic drugs the CD95 receptor and ligand are upregulated and may contribute to apoptosis of the tumor or, dependent on the drug sensitivity of the tumor, to the status of the tumor as an immunoprivileged site.

Biography

Prof. Dr.med. Peter H. Krammer was born in Rheydt, Rhineland, Germany. He got his medical training in Freiburg/ Germany, St. Louis/ USA, and Lausanne/ Switzerland. He did his thesis on extracellular streptococcus antigens at the Institute for Microbiology and Hygiene at the University of Freiburg, and investigated the role of small nuclear RNAs at the Institute of Pathology, also in Freiburg. In 1973 at the age of 27 he became a member of the Basel Institute for Immunology and spent almost three fruitful years at the Institute studying T cells and their specificity. From Basel he moved via the Max-Planck-Institute for Immunobiology in Freiburg, where he stayed one year to continue T cells studies, to Heidelberg to the German Cancer Research Center, where in 1976 he started his work in the Division of Immunogenetics. There, again,

his main work was on T cells and T cell clones, their receptor specificities and their activities. Later, in the early 1980s he focussed on T cell-derived cytokines. He investigated the activation of macrophages by macrophage activating factors and in a fruitful, longstanding collaboration with E. Vitetta and her associates from Dallas, discovered IL-4 as a B cell immunoglobulin switch factor. With fondness he remembers his days as a visiting professor in Dallas and the friendliness of the Texans who hosted his stay. In 1984/85, he felt that molecular biology would leave a significant mark on immunology and he spent one and a half year in A. Sippel's laboratory at the Center for Molecular Biology in Heidelberg to learn the thinking and the techniques in this field. In the mid til late 1980s, his interest shifted very much towards negative regulation of tumor cell growth and apoptosis. In this context he and his associates discovered the CD95(APO-1/Fas) system, highlighted by the first publication in Science in 1989. CD95, its signalling machinery and its role in physiology and diseases remained at the center of his interest. Peter Krammer has received numerous prizes for his work, he is reviewer for and in the editorial board of many journals. Presently, he is the Director of the Tumorimmunology Program of the German Cancer Research Center. He runs a large group of scientists and his main interest is sensitivity and resistance in apoptosis and the role of apoptosis in the immune system and in diseases. Peter Krammer has an outstanding list of publications with 261 original papers in international scientific journals and 120 invited reviews and book chapters.

19.00-21.00 VISIT AND RECEPTION AT RANDOX LABORATORIES, CRUMLIN

Radox Laboratories and Biochip array technology

Radox have developed two biochip systems, namely *Evidence* and *Evidence Investigator*. The biochip systems are based on the use of a biochip as a platform for immunoassay measurement. The portfolio of biochip arrays includes thyroid, fertility, tumour monitoring, tumour PSA, cell adhesion molecule, cytokines and growth factors, cardiac, DNA and drugs of abuse. The cytokine and growth factors array allows the simultaneous detection of 13 analytes from a single sample. Two DNA arrays are currently in development, the cardiovascular disease DNA array will detect 27 different polymorphisms and the colorectal cancer DNA array will detect 28 different mutations. Conventional immunoassay techniques are utilised for the

measurement of protein analytes on the surface of a biochip, which results in the specific and simultaneous profiling of protein markers. Rather than having to divide and separately test a patient sample to obtain each test result, biochip array technology offers a means for simultaneous testing of a sample and thus provides a more complete diagnostic profile for each patient.





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