Friday April 8

PREMIER LECTURE SERIES
Location: PFC G07

PL9 09.00-09.40
Professor Kenneth Hillan
Genentech Inc, CA, USA

EGFR MUTATIONS IN NON-SMALL CELL LUNG CANCER: PREDICTIVE AND PROGNOSTIC IMPLICATIONS
Kenneth J. Hillan & David A. Eberhard
Genentech, Inc., South San Francisco, CA, USA

Lung cancer is the most common cause of cancer death worldwide, and the majority of cases are associated with cigarette smoking. Non-small cell lung cancer (NSCLC) arising in smokers has a different spectrum of molecular abnormalities than does NSCLC arising in non-smokers, suggesting differences in molecular etiology, pathogenesis and possibly prognosis. For example, KRAS mutations occur in 20-40% of NSCLC, are strongly associated with smoking, and have been associated with poor prognosis in some studies but not in others. Molecular abnormalities in NSCLC also represent promising therapeutic targets, since present chemotherapies for advanced or metastatic NSCLC have modest efficacy and considerable side effects. The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is expressed in the majority of NSCLC. The efficacy of EGFR inhibitors in preclinical models together with their favorable toxicity profiles, have led to their clinical development in NSCLC and other indications. Erlotinib (Tarceva) and gefitinib (Iressa) are small-molecule inhibitors of the EGFR tyrosine kinase that showed activity in NSCLC as single agents in phase II trials. A US single arm phase II study of erlotinib demonstrated an objective response rate of 12.3%, and gefitinib provided response rates of 10.4% in non-Japanese patients and 27.5% in Japanese patients. Non-smokers have a markedly higher response rate than smokers. However, randomized phase III studies of gefitinib or erlotinib in combination with chemotherapy failed to demonstrate an increase in efficacy for EGFR inhibitors over chemotherapy alone. One suggested explanation for this may be that cytostatic agents could cause a schedule-dependent antagonism of cytotoxic drugs by inhibiting progression through the cell cycle and apoptosis. Alternatively, patients were not selected to enrich for likelihood of response to an EGFR inhibitor in these trials. In contrast to HER2 testing for trastuzumab therapy, EGFR expression does not predict for sensitivity to inhibitors in preclinical models or in tumors from treated patients, and hence there is no molecular method for patient selection.

Recent reports have associated somatic mutations in the tyrosine kinase domain of EGFR in a subset of NSCLC with sensitivity of the tumors to gefitinib (Lynch et al., 2004; Paez et al., 2004). Paez et al. found tyrosine kinase domain mutations were restricted to EGFR (out of the 47 tyrosine kinases tested) in a panel of 119 lung cancers. The frequency of heterozygous mutations (amino acid substitutions and deletions) was found to be 2% in the American population and 26% in Japanese patients. Lynch et al. reported a prevalence of mutations of 8% (2/25) in unselected patients. Overall, somatic mutations in EGFR were reported in 13 of 14 patients who responded to gefitinib and in none of 11 patients who were treated and did not respond. Notably, patients with EGFR mutations had negative or remote smoking histories. In functional studies, the mutations resulted in increased activity of EGFR and increased sensitivity to inhibition by gefitinib. Thus, EGFR mutation may define a subset of tumors that are highly dependent on activated EGFR signaling and particularly responsive to EGFR inhibitor therapy. These initial studies did not present data regarding the prognostic significance of these activating EGFR mutations outside the setting of EGFR inhibitor treatment or whether the objective responses in EGFR mutants translate into increased survival. Notably, one of the published gefitinib-responsive patients lacked any mutations in EGFR exons 18-21, indicating that EGFR mutations are not necessary for patients to derive clinical benefit from EGFR inhibitor therapy.
**Biography**

Dr Kenneth Hillan came to Genentech in 1994 to study the role of hepatocyte growth factor in liver regeneration. He joined the Pathology department as a scientist in 1996, was promoted to director of Pathology in 1998, and was named senior director of Research Operations in 2001. In 2002, Hillan was promoted to vice president, Research Operations and Pathology. In July 2003, Dr Hillan was named vice president, Development Sciences. In this position, he is responsible for managing the collaboration between Research and Development as the company moves promising molecules from research into development. In addition, Dr Hillan manages the Research Pathology department and its ongoing work in oncology, immunology, vascular biology and other therapeutic areas. While at Genentech, Hillan’s key research contributions have included the study of VEGF and EG-VEGF in experimental models and human disease, and the application of quantitative analysis of molecular markers in-situ in human tissue microarrays. These quantitative technologies have been used extensively as part of the selection of candidate antigens in the company’s Tumor Antigen Project and are being applied in the study of molecular markers that might predict response to a number of Genentech’s pipeline therapeutics. Dr Hillan is on the editorial advisory board of the Journal of Pathology and is an ad hoc reviewer for the Journal of Clinical Pathology and the American Journal of Pathology and Liver. He has published more than 40 articles in peer-reviewed journals. He also is an Honorary Professor of Molecular and Therapeutic Pathology at the University of Leeds.

**Biography**

Dr Paul Harkin is a Senior Lecturer in Molecular Oncology and a Co-Director of the Genomics Core Facility within the Cancer Research Centre at Queen’s University Belfast. Prior to this appointment he had been a Research Fellow in Medicine at Harvard Medical School and the Massachusetts General Hospital where he developed his initial interest in the emerging field of Functional Genomics. Since returning to N. Ireland Dr Harkin has built a substantial lab team focused on understanding the role of the BRCA1 gene in the development of breast cancer. The BRCA1 research group is specifically interested in the clinical application of basic research in this area and the use of functional genomic-based approaches to achieve these aims. He has published his work in some of the worlds leading research journals and has been invited to speak at over 40 National and International conferences and Research Institutions. Dr Harkin sits on several National committees and funding boards and acts as a reviewer for a number of major research journals including Nature Genetics and The Lancet.

**PL10  09.40-10.20**

Dr Paul Harkin  
Centre for Cancer Research and Cell Biology, Queen’s University Belfast

**UNCOVERING BRCA1 REGULATED SIGNALLING PATHWAYS BY MICROARRAY BASED EXPRESSION PROFILING**

We have evaluated the role played by BRCA1 in mediating the phenotypic response to a range of chemotherapeutic agents commonly used in cancer treatment. We provide evidence that BRCA1 functions as a differential mediator of chemotherapy induced apoptosis. Specifically, we demonstrate that BRCA1 mediates sensitivity to apoptosis induced by antimitotube agents but conversely induces resistance to DNA damaging agents. These data are supported by a variety of experimental models including cells with inducible expression of BRCA1, siRNA mediated inactivation of endogenous BRCA1 and reconstitution of BRCA1 deficient cells with wild type BRCA1. Most notably we demonstrate that BRCA1 induces a 10-1000-fold increase in resistance to a range of DNA damaging agents in particular those that give rise to double strand breaks such as etoposide or bleomycin. In contrast BRCA1 induces a greater than 1000-fold increase in sensitivity to the spinelle poisons, paclitaxel and vinorelbine. FACS analysis demonstrated that BRCA1 mediates G2/M arrest in response to both antimitotube and DNA damaging agents. However, PARP and caspase-3 cleavage assays indicate that the differential effect mediated by BRCA1 in response to these agents occurs through the inhibition or induction of apoptosis. Microarray based expression profiling has identified a panel of BRCA1 regulated genes that are now being evaluated as potential effectors of BRCA1 induced resistance to DNA damage and sensitivity to antimitotube agents.
Array-CGH is nowadays the method of choice for studying DNA copy number changes. However, the lack of commercial availability of the arrays is a disadvantage. Until now, BAC and cDNA clones can be obtained from, for example, the Sanger Center, BACPAC Resources, InVitrogen and Research Genetics. However, array preparation still needs to be performed by the investigators themselves. Commercial providers like Affymetrix, Agilent and NimbleGen are developing oligo array CGH platforms, of which we will show examples. In addition, we have introduced spotted oligonucleotides, recently used in expression profiling, as probes for array-CGH. Meanwhile we have optimized these protocols for human and mouse 20-30K oligo array CGH. We show that oligo-CGH can detect chromosomal aberrations with high accuracy and greater spatial resolution, compared to other current array-CGH platforms. The use of an oligo array CGH platform is particularly interesting for implementation into the clinic. Furthermore, we have developed smoothing algorithms. Improved algorithms enable us to distinguish amplifications from gains. Using these algorithms enables the development of cross-platform/meta-analysis of array-CGH studies, which will be implemented in our Oracle based array-CGH database and be made publicly available.

Biography
Dr. Ylstra has graduated in 1995 with work he performed at the Free University in Amsterdam (NL) and the University of Vienna (A). His post-doc at UC-Berkeley (CA, USA) focused on the identification of stable mRNA molecules on a genome-wide scale. In 1998 he accepted a position at UC-San Francisco (CA, USA), where he developed and implemented micro-array analysis for Cancer Research. Currently, he is the head of the Micro Array Facility at the VU University Medical Center Amsterdam (NL). In Amsterdam he works on the technological and practical implications of genomic RNA and DNA techniques into the clinic.

References.
[1] Oostlander AE, Meijer GA, Ylstra B (2004) Microarray-based comparative genomic hybridization and its applications in human genetics. J Clin. Genetics. (In Press).
[2] Carvalho B, Ouwerkerk E, Meijer GA, Ylstra B. (2004) High resolution microarray comparative genomic hybridization analysis using spotted oligonucleotides. J Clin Pathol. 57: 644-646.
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FREE PAPER SESSION 6  
(PARALLEL 1)  
Location:  PFC G07

027  11.30-11.45

ERYTHROPOIETIN RECEPTOR ON NON-SMALL CELL LUNG CARCINOMA; A DRUG TARGET

Dr Anita Yakkundi, Dr Christine Le Sann, Miss Elaine Dunlop, Dr Zhanzhong Shi, Dr Nicole Kaftzik, Prof Sabine Flistch, Prof John Mann, Prof Terence Lappin
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Introduction. Erythropoietin (Epo) is the primary inducer of red blood cell formation. The Epo receptor (EpoR) is mainly expressed on the erythroid progenitor cells. EpoR is also expressed in a wide range of tumour types and may play a role in tumorigenesis (1). We report here characterisation of EpoR on non-small cell lung carcinoma (NSCLC) cell lines and attempt to target the neoplastic cells with erythropoietin-drug conjugates. We have used transferrin as a model system for screening of drug conjugates.

Methods. EpoR expression on NSCLC cell lines; H-23, H-157 and H-838 was characterized using RT-PCR and western blotting. Drugs DN1, DN2, DN3 were synthesized bearing the maleimidobenzoyl (aminocaproyl), maleimidobenzoyl and maleimido-caproyl spacers and conjugated to thiolated transferrin and Epo. The cytotoxic effects were measured using the MTT assay. Live cell imaging for drug uptake was performed using the Zeiss Axiovert 300m system.

Results. Expression of EpoR message as well as protein was detected in all three NSCLC cell lines; H-23, H-157 and H-838 was characterized using RT-PCR and western blotting. Drugs DN1, DN2, DN3 were synthesized bearing the maleimidobenzoyl (aminocaproyl), maleimidobenzoyl and maleimido-caproyl spacers and conjugated to thiolated transferrin and Epo. The cytotoxic effects were measured using the MTT assay. Live cell imaging for drug uptake was performed using the Zeiss Axiovert 300m system.

The Epo muteins N24C and N38C were expressed in E.coli, purified, folded in vitro and conjugated to DN2. The resulting Epo-DN2 conjugate was cytotoxic to all 3 NSCLC cell lines tested at a concentration lower than the free drug. Live cell imaging of the Epo-drug conjugates is currently under investigation

Conclusion. We have synthesized two drugs bearing different spacer groups, which are effectively cytotoxic as protein conjugates in the NSCLC tumour model.

References.
[1] Farrell and Lee (2004), Oncologist 9 (suppl. 5): 18-30.

028  11.45-12.00

EFFECT OF ADJUVANT TAMOXIFEN AND LETROZOL ON THE FATE OF PERIPHERAL BLOOD CK-19 mRNA+ CELLS IN PATIENTS WITH EARLY BREAST CANCER

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Purpose. To evaluate the effect of adjuvant tamoxifen (TAM) and letrozol (LETR) on the occult peripheral blood (PB) CK-19 mRNA+ cells in correlation with the clinical outcome.

Patients and Methods. Patients were treated with TAM (group TAM: n= 117 pts), LETR (LETR: n= 34pts) or sequential TAM (2 or 3 years) and LETR (3 or 2 years) (TAM/LETR: n= 46pts) after completion of adjuvant chemotherapy. CK-19 mRNA+ cells were detected by real-time RT-PCR. Results: Post-chemotherapy, CK-19 mRNA+ cells could be detected in 39 (33.3%), 13 (38.2%) and 14 (30.4%) patients treated with TAM, LETR, and TAM/LETR, respectively. Hormone treatment resulted in undetectable levels of PB CK-19 mRNA+ cells in 33 (84.5%) TAM and 11 (85%) LETR patients. In the TAM/LETR group, LETR resulted in undetectable levels of CK-19 mRNA+ cells in 12 (80.3%) patients treated with TAM, LETR, and TAM/LETR, respectively. Hormone treatment resulted in undetectable levels of PB CK-19 mRNA+ cells in 33 (84.5%) TAM and 11 (85%) LETR patients. In the TAM/LETR group, LETR resulted in undetectable levels of CK-19 mRNA+ cells in 12 (80%) of 15 patients with CK-19 mRNA+ cells after adjuvant TAM. The median duration of CK-19 mRNA-negative peripheral blood was higher in patients receiving LETR (LETR and TAM/LETR groups) than in those receiving TAM (TAM group) (p=0.0075). The incidence of relapse
was 11.7% (TAM), 14.3% (LETR) and 8.5% (TAM/LETR). The probability of relapse was significantly higher (p = 0.0025) for patients with detectable CK-19 mRNA+ cells than for patients without CK-19 mRNA+ cells before or during adjuvant hormone treatment. CK-19 mRNA+ cells during hormone treatment was associated with a lower overall survival (p=0.0394).

Conclusions. We conclude that: 1) PB CK-19 mRNA+ cells are sensitive to TAM and LETR; 2) TAM-resistant CK-19 mRNA+ cells are sensitive to LETR; 3) the elimination of PB CK-19 mRNA+ cells with hormone treatment seems to have a positive effect on both disease-free interval and overall survival.

029 12.00-12.15

EGFR ACTIVATION DETERMINES RESPONSE TO COMBINED ZD-1839 (IRESSA) AND CYTOTOXIC DRUG TREATMENT IN COLORECTAL CANCER AND NON SMALL CELL LUNG CANCER CELL LINES

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Introduction. ZD-1839 (Iressa), an EGFR tyrosine kinase specific inhibitor, is active in 12-19 % of NSCLC patients, previously treated with chemotherapy. Despite single agent activity, addition of ZD-1839 to chemotherapy (INTACT1-2), did not improve overall survival or response rate. Recently, EGFR activating mutations have been linked with sensitivity to ZD-1839 in monotherapy. However, up to now, there are no established predictive markers for response to combination of ZD-1839 with chemotherapy. In order to determine the predictive value of phospho-EGFR (a measure of EGFR activation), we examined the cytotoxic effects of ZD-1839 in a panel of human NSCLC cells (H23-H1157-H322-H460-H727-H838-A549-Calu 6) and CRC cells (HCT 116-p53 wt and null-H630-RKO-LoVo) alone and in combination with 5-fluorouracil and the platinum compounds oxaliplatin and cisplatin.

Methods. Cell viability was assessed using MTT. Apoptosis was measured by flow cytometry, PARP- and caspase 8 cleavage. Phospho-EGFR was detected by Western Blotting.

Results. In our panel of CRC and NSCLC cell lines, we were able to distinguish two different phenotypes: 1. In cell lines where we found an increased EGFR activation post-drug exposure, the interaction between ZD-1839 and this agent was supra-additive. 2. In cells where we found no change or decreased EGFR activation following the cytotoxic drug, an antagonistic or additive interaction between the two compounds was observed.

Conclusions. These novel findings indicate that post-treatment EGFR activation levels may predict response to ZD-1839 in combination with 5FU and platinum compounds in CRC and NSCLC cell lines. We are currently investigating the mechanism of upregulation of phospho-EGFR in response to chemotherapy.

030 12.15-12.30

COMPLETE REGRESSION OF BRAIN AND SECONDARY LUNG METASTASES OF NON-SMALL-CELL LUNG CANCER (NSCLC) IN RESPONSE TO GEFITINIB IS ASSOCIATED WITH THE COMBINED AMPLIFICATION AND MUTATION OF THE GROWTH FACTOR RECEPTOR (EGFR) IN THE PRIMARY TUMOUR

Dr Richard Schwab, Dr Ferenc Pintér, Dr Karoly Oreskovich, Dr Judit Moldvay, Dr Judit Papay, Dr Janos Strauss, Dr Laszlo Kopper, Dr Gyorgy Keri, Dr Akos Pap, Dr Istvan Petak
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Introduction. Gefitinib is a novel tyrosine kinase inhibitor drug targeting the epidermal growth factor receptor (EGFR). Recently, strong association between mutations of the EGFR tyrosine kinase domain and clinical responsiveness of non-small-cell lung cancer (NSCLC) to gefitinib has been reported. Brain metastasis occurs in 20-40% of patients with NSCLC and is associated with very poor outcome. Genetic testing of brain metastases is usually not possible and the clinical value of testing the archived tumour material of the primer tumour in the management of patients with late metastases is not known.
Materials and Methods. Here, we describe a patient with multiple NSCLC brain and lung metastases. Fluorescent in-situ hybridization (FISH) and DNA sequencing of the paraffin-embedded tissue sample of the three years earlier surgically resected primary tumour was applied to analyze the amplification and sequence of the EGFR gene.

Results. Gefitinib treatment induced dramatic improvement of clinical symptoms and complete regression of brain and lung metastases demonstrated by magnetic resonance imaging after 8 weeks. The patient is still free of tumour over one year since the diagnosis of multiple metastases. FISH analysis detected EGFR gene amplification. Sequencing revealed the presence of L747-S752del, P753S mutation. Random sequencing of PCR product proved the predominance of the mutant allele.

Conclusions. Our results indicate the legitimacy of molecular analysis of the primary tumour in the consideration of EGFR-targeted therapy of brain metastases. These findings also suggest that tumours with amplified mutant EGFR gene represent a sub-type of NSCLC with extreme sensitivity to EGFR-inhibitor therapies.

031  12.30-12.45
DIFFERENTIAL ALTERATION IN THE METHYLATION OF DNA AND GENES IN MOUSE LUNG TUMORS: EFFECT OF BUDESONIDE

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Introduction. DNA of lung tumors is hypomethylated. Modulation of DNA hypomethylation and the methylation of genes by chemopreventive agents might be used as biomarkers in chemoprevention studies. Budesonide, a chemopreventive agent that prevents lung tumors in mice was evaluated for the ability to modulate the hypomethylation of DNA and genes in tumors.

Methods. Lung tumors were induced in strain A mice by vinyl carbamate. Fourteen days prior to sacrifice at 36 weeks, the mice were given 2 mg/kg budesonide in their diet. DNA methylation was determined by dot-blot analysis using an antibody for 5-methylcytosine. Methylation was determined of the differentially methylated region-2 (DMR-2) in the IGF-2 gene and of a CpG island near the start codon of the P21 gene using a bisulfite-modified DNA sequencing procedure. mRNA expression was determined by Real Time RT-PCR.

Results. DNA and the DMR-2 of the IGF-2 gene were hypomethylated in lung tumors relative to normal lung tissue. In contrast, the CpG island of p21 was almost completely methylated in both lung tissue and tumors. Seven days of treatment with budesonide reversed the hypomethylation of DNA and the IGF-2 gene to a level similar to that found in lung tissue, while the methylation of the p21 gene remained mostly methylated. mRNA expression of the IGF-2 gene was increased, while that of p21 was decreased in the tumors. Budesonide decreased the expression of IGF-2 and increased that of p21.

Conclusions. DNA and the IGF-2 gene become hypomethylated, while another gene, p21 remains methylated in tumors. This demonstrates selection of the CpG sites that become un-methylated in tumors. Decreased expression of the p21 gene was not associated with increased methylation of the evaluated CpG island. Budesonide reversed DNA hypomethylation, decreased the expression and methylation of the IGF-2 and increased the expression without altering the methylation of p21.

032  12.45-13.00
MITOMYCIN-C INDUCES UP-REGULATION OF FAS IN VIVO IN TUMOUR CELLS FROM PATIENTS WITH BLADDER CANCER

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Introduction. FAS death receptors on the surface of bladder cancer cells trigger apoptosis upon cytotoxic immune cell activation. Interference with normal FAS signalling through mutation or gene methylation may contribute to tumour immune escape.

Materials And Methods. Prior to surgical resection, bladder washings were retrieved from 6 patients with superficial bladder cancer before treatment and at 2, 4 and 6h post intravesical mitomycin-c instillation.
Expression of FAS protein on tumour cells was quantitatively determined using flow cytometry. Resected tumour tissue from these 6 patients together with tissue from 10 additional patients, including those with high grade and stage disease, was assessed for exon-9 mutations and methylation within the p53 enhancer region of the first intron.

**Results.** Three of the 6 patients demonstrated significant sustained up-regulation of FAS expression following mitomycin-c treatment as determined by quantitative flow-cytometry and confirmed by immunocytochemistry. Single stranded conformation polymorphism and sequencing revealed no evidence of mutation. Methylation specific polymerase chain reaction detected no methylation in the p53 enhancer region suggesting that other mechanisms must render FAS non functional in bladder cancer.

**Conclusions.** This is the first definitive evidence of FAS up-regulation post MMC therapy in TCCB patients in vivo. This implies a role for FAS in the enhancement of the chemotherapeutic response.

**FREE PAPER SESSION 7**

(PARALLEL II)

**Location:** PFC G06

**033 11.30-11.45**

**MUTATION ANALYSIS OF KRAS2, TP53 AND BRAF GENES IN HUMAN SPORADIC COLORECTAL CANCER PROGRESSION**

Dr Daniele Calistri, Dr Claudia Rengucci, Dr Ian Seymour, Dr Silvia Molinu, Dr Andrea Sciutto, Dr Arturo Lattuneddu, Dr Anna Maria Poliferno, Dr Walter Giaretti

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**Introduction.** Gene mutations in APC, KRAS2 and TP53 are thought to be essential events for colorectal cancer (CRC) development and progression. Recent data, however, indicated that KRAS2 and TP53 mutations in CRC rarely co-existed in the same tumor. Moreover, an inverse relationship between KRAS2 gene activation and BRAF mutations has been demonstrated, suggesting alternative pathways for CRC genesis. Our main goal was to reconstruct the chronological modulation of these gene mutations during CRC progression from Dukes' stages A to D.

**Materials and Methods.** Mutations of TP53, KRAS and BRAF genes were analysed by Single Strand Conformation Polymorphism and/or sequencing analysis in 135 human sporadic CRCs distributed among the different Dukes' stages.

**Results.** Mutations in TP53, KRAS2 and BRAF genes were found respectively at incidences of 39%, 34%, and 4%, with a minimal co-presence of the different mutations. Overall, in the present CRC series, the simultaneous presence of KRAS2 and TP53 mutations was detected in 16 among 135 cases (12%), and only in 1 among 26 cases (4%) with Dukes' A stage. We also observed, in particular, that the frequency of TP53 mutations increased from 12% at stage A to 36% for stage D, suggesting an important role for TP53 in the A-D Dukes' stage progression. Conversely, KRAS2 or BRAF gene mutations were not related to tumour stage or location.

**Conclusions.** The present data indicate that KRAS2 and TP53 gene mutations are rarely simultaneous, suggesting that these mutations may not represent a synergistic evolutionary pathway and that multiple pathways of CRC are possible. Moreover, since mutations in TP53, KRAS2 and BRAF were not present in more than one third of CRCs, it is likely that other mechanisms and pathways, including large scale DNA changes, play a role in CRC progression.

**034 11.45-12.00**

**C-FLIPL REGULATES COLORECTAL CANCER CELL DEATH IN RESPONSE TO CHEMOTHERAPY**

Timothy Richard Wilson, Wendy Louise Allen, Ultan McDermott, Miranda McEwan, Leeona Galligan, Patrick Gerard Johnston, Daniel Broderick Longley

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**Introduction.** Death receptors such as Fas trigger death signals when bound by their natural ligands, mainly through the activation of caspase 8. A key regulator of death receptor signalling is the caspase 8 inhibitor c-FLIP, which is expressed as long (c-FLIPL) and short (c-FLIPS) forms due to differential splicing. We found that activation of Fas-mediated apoptosis in HCT116 colorectal cancer cells following treatment with chemotherapy coincided with complete processing of c-FLIPL to a truncated form (p43-FLIPL) and a ~4-8-fold increased expression of c-FLIPS, suggesting involvement of c-FLIP in regulating drug-induced apoptosis in these cells.

**Methods.** c-FLIPL and c-FLIPS overexpressing cell lines were generated in HCT116 cells and c-FLIP targeted siRNA’s were used to down-regulate c-FLIP expression. The effect of c-FLIP expression on chemosensitivity was assessed by MTT cell viability and flow cytometry assays. Caspase 8 and PARP cleavage were assessed by western blot.

**Results.** Firstly, overexpression of c-FLIPL, but not c-FLIPS, inhibited apoptosis induced by 5-Fluorouracil (5-FU), oxaliplatin (OXA) and irinotecan (CPT-11) in HCT116 cells. Secondly, siRNA-mediated down-regulation of c-FLIP expression synergistically enhanced apoptosis of HCT116 cells in response to chemotherapy. c-FLIP down-regulation also sensitised p53 wild type RKO, p53 mutant H630 and a p53 null HCT116 daughter cell line to chemotherapy-induced apoptosis. Intriguingly, complete knock out of c-FLIP expression with higher concentrations of siRNA potently induced apoptosis in the absence of chemotherapy in all four colorectal cancer cell lines. Finally, we found that specific down-regulation of c-FLIPL recapitulated the effects of knocking out both splice forms.

**Conclusions.** These results suggest that c-FLIPL expression may determine response to chemotherapies used to treat colorectal cancer. Furthermore, targeting c-FLIPL, either alone, or in combination with existing chemotherapies may have therapeutic potential for the treatment of colorectal cancer.

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**035 12.00-12.15**

**HIGH RESOLUTION ANALYSIS OF THE 8q22-q23, 13q21-q31 AND 20q AMPLICONS ASSOCIATED WITH TUMOUR PROGRESSION IN COLORECTAL CANCER**

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**Introduction.** Colorectal cancer is the second leading cause of cancer death in the western world, with over 4200 deaths per year in The Netherlands. Colorectal cancer arise from adenomas, which in only 5% of cases ever progress to carcinomas. We showed by CGH, that gains at chromosomes 8q22-q23, 13q21-q31 and 20q13 are associated with progression of colorectal adenomas to carcinomas. We aimed to further delimit the boundaries of the gained 8q, 13q and 20q regions, in order to identify putative oncogenes in these regions, involved in tumour progression.

**Materials and Methods.** In total, 32 paired samples from the adenoma and carcinoma compartments of malignant polyps were analysed by array-CGH, using a whole-genome BAC-array with an average resolution of 1Mb and a high resolution at 8q22-q23, 13q21-q31 and 20q13 regions.

**Results.** In addition to gains and losses that had previously been detected by classical CGH, array CGH revealed several other chromosomal alterations. In most cases, the adenoma and carcinoma parts of malignant polyps showed the same pattern of aberrations while in some cases carcinomas presented additional aberrations. Within the 8q, 13q and 20q regions we were able to delimit small regions of amplification. These regions contain several candidate oncogenes, like PTP4A3 (8q) and BCL2L1 (20q), which were also shown to be amplified in colon by multiplex ligation-dependent probe amplification (MLPA).

**Conclusion.** Array-CGH allowed further restriction of the 8q, 13q and 20q gains associated with colorectal adenoma-to-carcinoma progression. Moreover, we identified small regions of amplification not previously detected by classical CGH, which may harbour the genes involved in colon cancer progression.
CONSTITUTIVE SURVIVAL SIGNALS SUPPRESS MUTAGEN-INDUCED APOPTOSIS DURING INTESTINAL EPITHELIAL REGENERATION IN AN IN VIVO MODEL SYSTEM

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Introduction. Failure to execute the apoptosis programme after genotoxic insult may enhance genomic instability and oncogenesis. Regeneration alters the homeostatic growth equilibrium of intestinal mucosa and promotes cancer by unclear mechanisms.

Aims: Using a definitive in vivo model of intestinal epithelial regeneration (IER), we test the hypothesis that constitutively activated cell survival mechanisms associated with tissue restitution during regeneration, may conflict with DNA damage induced pathways of apoptosis.

Methods. Rat small intestinal crypt stem cell aggregates were grafted unto the subcutaneous plane of recipient rats and were retrieved at intervals of 7, 14 and 21 days, that represent early, intermediate or late stages of regeneration, respectively. Animals were treated with N-methyl-N-nitrosourea (MNU) or placebo, 18 hours prior to graft retrieval. Expression of Bcl-2, Bax and p53 by regenerating epithelium was assayed by western blot while apoptosis was assessed by TUNEL assay.

Results. Expression of Bcl-2 and p53 were increased (p<0.01) while % spontaneous apoptosis was reduced in early regenerating epithelium vs late regeneration (3.81 ± 0.79 vs 6.9 ± 1.26 % apoptosis). An apoptosis response to MNU treatment was observed by western blot while apoptosis was assessed by TUNEL assay.

Conclusions. Early IER appears to be associated with suppression of spontaneous and MNU induced apoptosis, in an in vivo model system. These findings could be relevant to the pathobiology of regeneration associated cancer.

MICROARRAY-CGH ANALYSIS OF DIFFUSE AND INTESTINAL COMPONENTS OF MIXED GASTRIC CARCINOMAS

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Introduction. In addition to the main histologic types of gastric cancer, i.e., the intestinal type (glandular) and the diffuse type (isolated cells), a rare third entity exists, the mixed carcinomas, in which both diffuse and an intestinal components can be found. The biology and genetic background of these mixed gastric cancers remains still obscure.

The aim of this study was to analyse, by microarray-CGH, the pattern of chromosomal aberrations in the diffuse and intestinal components from mixed gastric tumours to better understand the genetic origin of the two histological distinct components within these tumours.

Materials and methods. We analysed by microarray-CGH a series of 9 of these rare mixed gastric tumours, from which the two components (diffuse and intestinal) were analysed separately. We used a full-genome array with an average resolution of approximately 1 Mb.

Results. The most common events (frequency ≥33%) observed were gains at 20q, 13q, 7p, 10q, 15q and losses at 3p, 9, 18q, 22q. The two lesions (diffuse and intestinal) from the same tumour do not show exactly the same aberrations, although the majority of aberrations from one tumour are present in both components, suggesting a clonal origin. A high level amplification (more than 4 copies extra) on 8q24.13 was detected in one case, in both components.

Conclusions. Given the low frequency of mixed gastric cancer, and technical issues that make array CGH impossible in a proportion of these lesions, the present series makes an important contribution to our knowledge of these tumours. The pattern of aberrations observed seems to favour a clonal origin, at least in a subset of
cases. In addition, the spectrum of changes observed is not distinct from that in intestinal and diffuse cancers, indicating that the genetic background of the different histology is not related to chromosomal changes.

038 12.45-13.00

EFFECT OF P53 STATUS AND STAT1 ON CHEMOTHERAPY-INDUCED FAS-MEDIATED APOPTOSIS IN COLORECTAL CANCER

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Introduction. The Fas death receptor is a 48 kDa member of the tumour necrosis factor receptor superfamily. Binding of the Fas death receptor to Fas ligand results caspase 8, which in turn activates downstream executioner caspases resulting in cell death. The ability of chemotherapy drugs to induce the Fas receptor has stimulated interest in targeting it with either therapeutic antibodies or peptides to enhance chemotherapy-induced cell death.

Methods. The chemotherapeutic agents 5-fluorouracil (5-FU), TDX, CPT-11 and Oxaliplatin were used. The agonistic anti-Fas antibody CH-11 was used to activate Fas-mediated apoptosis. Apoptosis was measured using cell cycle analysis, MTT and PARP cleavage assay. Cell surface expression of the Fas receptor was measured by flow cytometry. STAT1 targeted siRNA was used to down-regulate STAT1 expression. Real-time RT-PCR was used to quantitate Fas mRNA expression.

Results. 5-FU and Oxaliplatin only sensitised p53 wild-type colorectal cell lines to Fas-mediated apoptosis, whereas CPT-11 and TDX sensitised p53 wild-type, mutant and null cells to Fas-mediated cell death.

Furthermore, CPT-11 and TDX (but not 5-FU or Oxaliplatin) up-regulated cell surface Fas expression in a p53-independent manner. Increased Fas cell surface expression in the p53 mutant and null cell lines by CPT-11 and TDX was not associated with increased total Fas expression, indicating that these agents trigger p53-independent trafficking of Fas to the plasma membrane.

siRNA-mediated down-regulation of STAT1 significantly decreased Fas cell surface expression in response to CPT-11 and TDX, indicating that STAT1 plays a role in drug-induced cell surface trafficking of Fas.

Conclusions. These results suggest that CPT-11 and TDX may be more effective than 5-FU and Oxaliplatin against p53 mutant tumours by sensitising them to Fas-mediated apoptosis. Moreover, our data suggest that CPT-11 and TDX may sensitise colorectal tumours to Fas-targeted therapies irrespective of p53 status.

13.00-14.00 LUNCH