POSTER SESSION

Translational research 1

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Influence of TP53 gene status on treatment response in breast cancer cells

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Breast cancer is a heterogeneous disease for which reliable diagnostic and prognostic markers are still lacking. Currently, estrogen receptor (ER), progesterone receptor (PR) and HER2 statuses are used in the clinic to select patients for anti-hormone and anti-HER2 therapies respectively. However, a significant proportion of patients (up to 30% of patients treated with anti-hormone therapy) will not respond to treatment. It is thus essential to find markers that would predict treatment failures to improve patient management and survival. TP53 gene mutation status has been repeatedly found to be a factor of poor prognosis in breast cancer. It has also been related to poor response to chemotherapy. TP53 is a tumour suppressor gene that is inactivated by mutation in a large proportion of cancers. It encodes a nuclear protein involved in many cellular pathways controlling cell proliferation, cell survival and genomic integrity in response to various types of stress, including DNA-damage caused by most chemotherapeutic agents. While the role of p53 in the cytotoxic effects of chemotherapeutic agents is well characterized, its possible role in the anti-proliferative and apoptotic effects of anti-hormone treatments has not been specifically investigated. In a large series of breast cancers, we have previously shown that TP53 gene mutation status was a factor of poor prognosis independently of the classical clinical markers. In addition, we found an interaction between TP53 mutation status and progesterone receptor expression that suggested a possible influence of p53 status on the response to anti-hormone therapy (Olivier et al., Clin. Cancer Res., 2006). To investigate this hypothesis in a cellular model, we have selected two isogenic breast cancer cell-lines that only differ in their p53 status and we have analyzed possible cross-talks between p53 and ER pathways. We have observed that cells with inactivated p53 are more resistant to the cytotoxic effects of tamoxifen and resume proliferation under estrogen-deprived conditions. Moreover, estrogen-deprivation or ER silencing reduces basal and doxorubicin-induced p53 levels. These results show that cross-talks between p53 and ER pathways do exist and may play an important role in the responses to both anti-hormone and chemotherapy treatments.

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Impact of TP53 and KRAS mutations on cisplatin-based adjuvant chemotherapy in non-small-cell lung cancer

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Background: Cisplatin-based adjuvant chemotherapy improves survival among patients with completely resected non-small-cell lung cancer (NSCLC) (Ronald H. Blum, NEJM 2004), however there are few prognostic and predictive markers to select patients for adjuvant chemotherapy. Mutations of TP53 and KRAS are most frequent in NSCLC. Aim: The purpose of this study is to analysis the impact of TP53 and KRAS mutations in NSCLC patients treated with cisplatin-based adjuvant chemotherapy enrolled in the International Adjuvant Lung Cancer Trial (IALT). Methods: Genomic DNA was extracted from 783 paraffin-embedded sections as described previously. Mutations in exon5 to 8 of TP53 were determined by sequencing and confirmed by another separate sequencing. KRAS mutation was detected by sequencing and confirmed by restriction fragment length polymorphisms analysis. The prognostic and predictive values of TP53 and KRAS status on survival were studied using a Cox model. Results: TP53 Mutation was found in 46% (240/524) of patients. TP53 status had no prognostic or predictive significance in all cancer grouped together or in adenocarcinoma patients. But in non-adenocarcinoma patients, there was a borderline significant interaction between TP53 status and treatment effect on disease-free survival (p=0.05). The effect of chemotherapy was different in TP53 mutated and wild type patients, with a trend for benefit in TP53 wild type patients and a trend for harm in TP53 mutated patients. The prevalence of KRAS mutation was 14% (98/716). The prognostic effect of KRAS on disease free survival was different among 3 histology groups (p=0.03), adenocarcinoma, squamous-cell carcinoma and non- adenocarcinoma/ non-squamous-cell carcinoma, with worst prognostic effect of KRAS mutation vs. wild type among non-adenocarcinoma/ non-squamous-cell carcinoma. Mutation of KRAS was not predictive of the effect of chemotherapy. Conclusion: Patients with non-adenocarcinoma and TP53 wild type appear to benefit from cisplatin-based adjuvant chemotherapy, whereas chemotherapy seems to be harmful in patients with mutated TP53. KRAS mutation is a bad-prognosis factor in non-adenocarcinoma/non-squamous-cell carcinoma patients. Thus mutation detection may help in assigning patients to different treatment protocol. Key words: TP53; KRAS; Cisplatin; Adjuvant Chemotherapy; Non-small-cell Lung Cancer (NSCLC).

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Role of PI3K/Akt /m-TOR pathway in cell response to Cisplatin treatment in Non-Small-Cell-Lung-Cancer - correlation to p53 status

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Combination chemotherapy regimens in NSCLC are usually based on cisplatin, although clinical outcome is poor mostly due to resistance to treatment. Functions that protect against genomic instability (p53 or DNA repair mechanisms) may be implicated in modulating the risk of acquiring resistance to cisplatin. Biochemical alterations such as activation of signal transduction pathways that promote cellular survival may also confer resistance. Clinical studies have shown that activation of PI3K / Akt / mTOR pathway occurs in the majority of pre-cancerous lesions in smokers, and may have predictive significance for non-small cell lung cancer patients. The aim of this study was to elucidate importance of PKB (protein kinase B)/ Akt signaling in NSCLC cells (A549, H1299 and H1975) in mediating resistance to cisplatin treatment, by blockade of signaling cascade either using kinase inhibitors or by down-regulating Akt by siRNA. Data obtained by Western blot using antibodies against Akt and phospho Ser-473 Akt (Cell Signaling Tech.) revealed that wortmannin (PI3K- inhibitor) alone (2 M) and in combination with various doses of cisplatin, inhibits Akt phosphorylation, as observed after 4 h of action. Phosphorylation status of Akt was slightly decreased after 16 h treatment with cisplatin and kinase inhibitors wortmannin, SH-5 (Akt-inhibitor) (10 M). Also, protein level of p53 as well as phosphorylation of p53-Ser-15 were significantly reduced. Dual staining by PI / BrdU (bromodeoxyuridine) and FACS analysis showed that both SH-5 and wortmannin affected cell cycle progression but did not significantly increase the proportion of cell death in comparison to cisplatin alone. Cell survival studies using SRB assay indicated that wortmannin and SH-5 did not sensitize cell to cisplatin treatment. On the contrary rapamycin (inhibitor of m-TOR) induced synergistic effect in combination with cisplatin on p53 wild-type / p53 null / p53 mutant - cell models, when used at sub-toxic concentration (10 nM). Imunocytochemical staining of p53 and confocal microscopy revealed that rapamycin when combined with cisplatin increases nuclear localization of p53, while Akt inhibitor SH-5 antagonized cisplatin action. In conclusion, m-TOR may be a promising target for sensitization of cells to cisplatin treatment independently of functional p53 status in NSCLC cells. Key words NSCLC, cisplatin, p53, PI3K-Akt-m-TOR pathway, rapamycin.

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High resolution melting allows sensitive high-throughput assessment of methylation in tumour samples

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Despite the overwhelming interest in methylation analysis, readily performed methodologies that are both sensitive and specific are not generally available. Methylated DNA and unmethylated DNA acquire different sequences after bisulphite treatment resulting in PCR products with markedly different melting temperatures. We thus aimed to develop the emergent methodology of high resolution melting analysis (HRM) as a sensitive and specific method for the detection of methylation. We used our new strategy of primer design, which corrects for PCR bias and allows us to use annealing temperature to ensure methylation independent amplification [1]. We then developed a panel of locus specific MS-HRM (Methylation Sensitive – High Resolution Melting) assays. Amplification and melting analysis were done in the same tube using a real time PCR

machine with high resolution melting capacity[2]. Reconstruction experiments showed that by manipulating the annealing temperature, methylation could be detected at levels as low as 0.1%. Moreover MS-HRM allowed estimation of the methylation level by using standards with a known unmethylated to methylated template ratio. We used MS-HRM for the determination of the methylation status of the promoter region of a panel of DNA stability and tumour suppressor genes such as BRCA1 and MGMT in cell lines of known methylation status and in panels of cancer specimens. Furthermore we have developed a MS-HRM assay for diagnostic testing of the H19/IGF2 imprinting centre. The changes in methylation status of H19/IGF2 imprinting centre are implicated in etiology of the Beckwith Wiedemann and the Russel Silver syndromes, which clinically demonstrate growth abnormalities and high cancer incidence. The utility of new assay was tested in a blinded study and 100% concordance of MS-HRM assay was obtained with Southern blot analyses (the current diagnostic procedure) of the same locus [3].MS-HRM proved to be highly sensitive, specific and robust for methylation detection. The simplicity and high reproducibility of the MS-HRM protocol has made MS-HRM the method of choice for methylation assessment in our laboratory. It is suitable for both research and diagnostic settings and will be of special utility in multi-centre trials where a reproducible method for methylation analysis is required.References:1. Wojdacz TK, Hansen LL: Reversal of PCR bias for improved sensitivity of the DNA methylation melting curve assay. Biotechniques 2006, 41(3):274, 276, 278.2. Wojdacz TK, Dobrovic A: Methylation-sensitive high resolution melting (MS-HRM): a new approach for sensitive and high-throughput assessment of methylation. Nucleic acids research 2007, 35(6):e41.3. Wojdacz TK, Dobrovic A, Algar E: Rapid detection of methylation change at H19 in human imprinting disorders using methylation sensitive high resolution melting. submitted.

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Robust and absolute quantitation of PSA in clinical human sera using Protein Reaction Monitoring (PRM)

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The field of proteomics has led to the discovery of numerous protein biomarkers that subsequently need to be verified and validated to evaluate their clinical use with a statistically significant number of patients. At this stage, ELISA test development is a bottleneck as antibody design and generation is time-consuming. To overcome this barrier, we propose to use an alternative assay, called Protein Reaction Monitoring (PRM). PRM associates a robust and automated sample preparation and a mass spectrometry-based detection. Briefly, crude human sera are reproducibly depleted, fragmented and fractionated using a robot. Peptides resulting from specific protein fragmentation are subsequently separated using a robust micro LC column and quantitated using a triple quadrupole mass spectrometer in selected reaction monitoring (SRM) mode. As a proof of concept, we demonstrated the absolute quantitation of a biomarker model, the Prostate Specific Antigen (PSA). In patient sera, PRM doses were compared to automated ELISA quantitation (Vidas TPSA). Between 4 to 30 ng/ml, PRM and ELISA presented an excellent correlation (r2 = 0.94(with similar accuracies and precisions. As a consequence, PRM-based assays can now be considered as valuable alternative assays for proteomic biomarker validation.

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An inflammatory breast carcinoma signature is associated with reduced relapse free survival in patients with non-inflammatory breast cancer

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Background. We hypothesize that a gene expression profile characteristic for Inflammatory Breast Cancer (IBC), an agressive form of breast cancer associated with poor patient survival, might be related to tumour aggressiveness in non-IBC (nIBC).

Materials and methods. RNA from 19 IBC samples and 40 nIBC samples was hybridized onto Affymetrix chips. A gene signature predictive of IBC was identified and applied onto 7 publicly available gene expression data sets (1157 nIBC samples) with survival data of 881 nIBC samples (4 data sets). Samples were classified as "IBC-like" or "nIBC-like". Relapse Free Survival (RFS) was compared between these groups by the Kaplan-Meyer method. We classified the 1157 nIBC breast cancer samples according to other prognostically relevant gene signatures and compared these classifications with the IBC signature classification. Cox regression analysis was performed to identify the most predictive signature with respect to RFS.

Results. Patients with an "IBC-like" phenotype demonstrate a shorter RFS interval in all 4 data sets (p=0.049, p=0.032, p<0.0001, p=0.0005). Classification according to the IBC signature is significantly (p<0.0001) associated with the cell-of-origin subtypes-, the Wound Healing Response (WHR)-, the Invasive Gene Signature (IGS)-, the Genomic Grade Index (GGI)- and the Fibroblastic Neoplasm Signatures (DTF/SFT). Breast tumours having an "IBC-like" phenotype generally belong to the Basal-like (32.8%), ErbB2-Overexpressing (22.6%) or Luminal B (29.6%) subtypes, have an activated WHR (71.6%), express the IGS (75.7%), are less frequently of the DTF phenotype (44.7%) and have a GGI of 3 (71.1%). Significant associations (p<0.0001) were found between the IBC signature and tumour grade, ER status, ErbB2 status and patient age at diagnosis. Cox regression analysis on the entire data set of 881 nIBC samples identified the IBC signature as an independent predictor of RFS (RR=1.532, C.I.=1.100–2.133, p=0.012), together with the WHR and GGI.

Conclusions. We demonstrate that nIBC breast tumours having an "IBC-like" phenotype have a reduced RFS interval. This suggests that IBC and nIBC tumours demonstrate the same phenotypic traits with respect to aggressive tumour cell behaviour. Gene signatures related to tumour stroma and tumour grade add information regarding patient survival. Hence the IBC signature represents a different aspect of aggressive tumour behaviour.

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Poor survival outcomes in HER2 positive breast cancer patients with low grade, node negative tumours

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Background: HER2 status has long been established as a poor prognostic marker for survival in breast cancer and more recently has been validated in numerous adjuvant trials as a predictive marker for response to trastuzumab. However, there remains a small subset of low grade, node negative HER2 positive patients who are currently ineligible for trastuzumab treatment as have been deemed to have no requirement for standard adjuvant chemotherapy.

Methods: We used a cohort of 367, grade 1/2, node negative patients diagnosed between 1980-2002 with full follow-up (median 6.2yrs) and clinicopathological details to assess the impact of HER2 positivity (IHC

Table 1 (Poster 407)

		Events				95.0% CI	
	number in group	HER2 pos	HER2neg	Sig.	Hazard Ratio	Lower	Upper
whole cohort	367	7/19	27/348	<0.001	6.78	2.93	15.69
ER positive	286	3/11	18/275	0.004	6.05	1.76	20.77
ER negative	34	3/5	6/29	0.012	7.97	1.58	40.22
Age<50	66	2/7	3/59	0.030	8.82	1.24	62.69
Age 50-65	170	3/7	11/163	0.001	8.79	2.44	31.70
Age>56	131	2/5	13/126	0.032	5.12	1.15	22.78
Size<20mm	233	5/13	9/220	<0.001	11.75	3.92	35.27
size>20mm	94	2/5	13/89	0.015	7.02	1.45	33.90