Poster presentations (Mon, 24 Sep, 14:00–17:00)

Translational research

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Real time RT-PCR 3-gene expression signature predicts survival in early-stage squamous cell lung cancer

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Background: Around 50% of early stage squamous cell lung cancer (SqCLC) patients will have recurrence after surgery, with distant metastasis being the main cause of treatment failure. Adjuvant chemotherapy improves survival, but the absolute gain is modest and largely limited to stage II-IIIA. High-risk patients may be selected by gene expression profiles and considered for adjuvant chemotherapy.
Methods: Of the consecutive series of 174 NSCLC patients who underwent complete surgery between 2000 and 2004 we selected 66 stage I-IIIA SqCLC patients (64 pts were stage I and II and 2 pts stage IIIA): 33 pts who developed distant metastases and 33 who were free of distant relapse after a median follow-up of 37 months (range, 24–64 months). Snap frozen primary tumor specimens were obtained at the time of surgery. Sections were taken from blocks of tumor tissue for RNA extraction, and gene expression of 29 genes was assessed by RT-PCR using low density arrays. Expression values were dichotomized using the median as a cut-off value.
Results: The univariate analysis identified 10 genes with significant prognostic value: CSF1, EGFR, CA IX, PH4, KIAA0974, ANLN, VEGFC, NTRK1, FN1, INR1. In the multivariate Cox model, CSF1 [HR = 3.5, p = 0.005], EGFR [HR = 2.7, p = 0.02], CA IX [HR = 0.2, p < 0.0001] and tumor size >4 cm [HR = 2.7, p = 0.02] emerged as significant predictors of survival. A risk score based on the expression of CSF1, EGFR and CA IX was 70% accurate in predicting death risk. This model also performed well in predicting development of distant metastases, with 64% sensitivity and 73% specificity. Biologically significant correlations were observed between some of these genes. For example, high levels of PH4 were related to low or no expression of CA IX (r = −0.33; p = 0.007).
Conclusions: Overexpression of CSF1 and EGFR, and downregulation of CA IX was strongly associated with poor prognosis in SqCLC. Simultaneous assessment of the expression of these genes defines the group of high-risk SqCLC patients who might derive the highest benefit from adjuvant chemotherapy.

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Expression of microRNA-451 is associated with disease-free survival in gastric cancer patients treated with chemoradiotherapy after gastric resection

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Purpose: This study was conducted to evaluate the potential association of miRNA expression with outcome after chemoradiotherapy in patients with resected gastric cancer.
Material and Methods: Data of expression of 250 mature miRNAs were obtained by real-time PCR from paraffin-embedded tumor tissues from patients with gastric cancer stage III treated with surgery following radiation therapy plus 5-fluoruracil/leucovorin based chemotherapy. After median-global normalization, we identified the miRNAs whose expression were significantly related to disease-free survival of the patients. The expression of these miRNA was validated using U6B as an endogenous control and including other independent set of patients.
Results: Our results shown that miRNA expression analysis can be tested in archival paraffin-embedded tissues. Low expression of miR-451 is associated with lower time disease-free survival by univariate analysis as well as multivariate analysis (p = 0.000; risk ratio = 4.74; 95% CI = 1.3–17.3). The miR-451 expression was confirmed as prognostic factor independently of stage including in the study 17 new patients of stage II and IV (p = 0.010, risk ratio = 3.3; 95% CI = 1.3–8.5). Moreover, quantitative data of miR-451 was statistically lower in patients with recurrence of disease than in patients without recurrence.
Conclusions: Our results suggests that miRNA expression can be considered as prognostic markers in gastric cancer patients.

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SNS-314, a selective Aurora kinase inhibitor with potent, pre-clinical antitumour activity, shows broad therapeutic potential in combination with standard chemotherapeutics and synergy with microtubule targeted agents

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Background: SNS-314, a selective small-molecule inhibitor of Aurora kinase A, B, and C, has entered a phase 1 clinical trial for the treatment of patients with advanced solid cancers. Aurora kinases play critical roles during mitosis and cytokinesis. SNS-314 demonstrates significant in vivo activity against a wide range of tumor xenograft models. Of importance, SNS-314 shows remarkable tumor growth inhibition using an intermittent dose-schedule which provides potential for combining SNS-314 with other targeted and conventional anti-cancer therapeutics.
Materials and Methods: A colorectal carcinoma cell line, HCT116 with either intact p53 (p53+/+) or suppressed p53 (p53−/−) protein levels, was treated in vitro with SNS-314 in combination with a panel of chemotherapeutic agents using either co-dosing or sequential dosing schedules. High content cell imaging was used to measure the anti-proliferative effects of the compounds.
Results: The most profound anti-proliferative effects were observed with SNS-314 and agents that disrupt microtubule polymerization such as vincristine and nocodazole. Statistically significant synergy was observed in p53 (−/−) HCT116 cells when SNS-314 was co-dosed with high doses of vincristine. Sequential dosing of SNS-314 followed by each chemotherapeutic compound showed significant synergy with vincristine and nocodazole, a trend toward synergy with docetaxel, and additive anti-proliferative effects with carboplatin, gemcitabine, 5-fluorouracil, daunomycin, and the active metabolite of irinotecan, SN38. The synergy observed between SNS-314 and vincristine and the potentiation seen with docetaxel are consistent with the mechanism of action of an Aurora kinase inhibitor that bypasses an activated mitotic spindle checkpoint resulting in mitotic catastrophe and cell death. These results are currently being explored in xenograft models.
Conclusions: SNS-314, a selective Aurora kinase inhibitor, demonstrates significant synergy in colorectal carcinoma cells with vincristine, and additive activity with docetaxel and all other compounds tested. SNS-314, a novel targeted Aurora kinase inhibitor, shows promise for rationally informed chemotherapeutic combinations for the treatment of human malignancies.

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Comparative analysis of microarray testing and immunohistochemistry in patients with carcinoma of unknown primary – CUP syndrome

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Background: Standard pathological examination including immunohistochemistry (IHC) is considered to be gold standard in the evaluation of tissue specimen in cancer of unknown primary. However, the panel of IHC tests is not standardized between different pathologists. Recently a diagnostic microarray gene expression test has been reported to identify the underlying primary malignancy with an accuracy of more than 80%. In this study we compared the microarray test with IHC performed in a central laboratory.
Methods: 92 pts with histologically/cytologically proven adeno- or undifferentiated CUP were randomized in a prospective phase II trial using two different chemotherapy regimen (results have been reported previously, Proc. ASCO 2005, a4089). IHC and microarray testing (CupPrintTM, Agendia, Amsterdam, NL) using paraffin-embedded tissue was performed retrospectively. So far, 27 cases were available for IHC, 15 of these for CupPrint. In 3 of the 15 cases, more than 1 sample was available,

so altogether 18 CupPrint analyses had been carried out. The dropout of 12 cases is explained by paucity of tumor tissue and by insufficient RNA quality, possibly due to long storage time of tissue samples. Results were evaluated and discussed case-by-case on a consensus conference including two independent referees (Folprecht, Buettner). The information yielded by each method was regarded separately and reviewed by clinical experts based on the individual characteristics of the baseline data and clinical course of each patient.
Results. In 11 cases the results of IHC and CupPrint were concordant and matched also the clinical findings. In 1 of these cases, the results would have been beneficial to the patient, as he could have received a more specific chemotherapy. From the remaining 4 samples 1 can not be regarded as CUP anymore, as both IHC and CupPrint strongly favour the diagnosis of serous ovarian cancer, which would also be consistent with the clinical findings. In 2 cases the CupPrint was more concordant with the clinical findings than IHC. In 2 cases IHC was more concordant with the clinical findings than the CupPrint results.
Conclusions. IHC performed centrally led to more informative results than multicenter IHC. IHC and CupPrint microarray testing showed a high grade of concordance. Combination of the results of both methods led to a better definition of the possibly primary tumor, allowing a more specific therapy in some cases.

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Microarray gene expression analysis of human adrenocortical tumours

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Background: Adrenal tumours are common, occurring in 7% of patients over the age of 50 years. Adrenocortical carcinomas (ACCs), however, are rare, with an incidence of two per million population per year. The management of adrenocortical tumours (ACTs) is complex, compounded by the difficulty in discriminating benign from malignant tumours using conventional histology. The identification of a molecular marker which could reliably distinguish between the two groups would be valuable and would lead to improved clinical management of these patients. The aim of this study was to use microarray gene expression analysis to identify molecular markers which would discriminate between ACCs and adrenocortical adenomas (ACAs).
Materials and Methods: RNA was prepared from 6 normal adrenal cortices, 16 ACAs and 12 ACCs. Only samples with an RNA integrity number of 7.5 or greater were used. The samples were hybridised to Affymetrix HGU133plus2.0 genechips. Data analysis was performed with Partek and Affylmgui softwares. Seven genes were selected for validation studies with real time reverse transcription polymerase chain reaction (qPCR). Of these, three genes were also validated by immunohistochemistry (IHC).
Results: Using a cutoff of B > 2 and M > 2 or <−2, 217 genes were found to be significantly differentially expressed between ACCs and ACAs. Of these genes, 120 were unpregulated while 97 were downregulated. On qPCR, all seven candidate genes selected were significantly differentially expressed in ACCs compared to ACAs. All three candidate genes selected for IHC also differed significantly in their protein expression in ACCs when compared to ACAs and normal adrenal cortex.
Conclusion: We identified seven genes which were significantly differentially expressed between ACCs and ACAs using microarray gene expression profiling and confirmed the expression of these genes with qPCR and IHC. With further studies, these genes will provide greater insight into the pathogenesis of ACTs as well as having the potential to be reliable discriminators between ACCs and ACAs.

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Serum adipokine levels in colorectal cancer patients

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Purpose: Leptin, a product of the ob gene involved in the control of food intake and energy expenditure, may act as a potent mitogen and anti-apoptotic cytokine in colon cancer cell lines and promotes the invasiveness of familial adenomatous colonic cells. Adiponectin, in turn, may exert protective actions through its anti-proliferative and anti-angiogenetic effects. Despite a significant amount of in vitro data, direct and convincing evidence about their role in the development of colorectal cancer (CRC) is not available. Thus, the aim of this study was to evaluate the possible associations between leptin, adiponectin and clinicopathological variables of CRC.
Methods: Baseline serum leptin (DBC Inc.), adiponectin (BioVendor Inc.), TNF-alpha (R&D Systems) and carcinoembryonic antigen (CEA, Abbott Labs.) levels were analyzed in 90 patients with histologically diagnosed primary (Stages A: 7, B: 34, C: 19 and D: 13, with a single resectable liver metastasis) or metastatic (liver: 8, peritoneum: 5, lung: 1 and multiple: 3) CRC treated at “Tor Vergata” Clinical Center and followed for a median period of 3 years. The study was performed under the appropriate ethics approvals, and informed consent was obtained from each patient.
Results: Serum leptin and adiponectin levels in patients with CRC were 8.8 ng/ml [median, interquartile range (IQR): 3.7−17.6) and 8.06 μg/ml (IQR: 5.66−9.34). Of interest, median leptin (10.9 ng/ml), but not adiponectin levels of metastatic CRC were higher than those observed in primary CRC patients (7.7 ng/ml, $p = 0.034$). Leptin inversely correlated with adiponectin ($p = 0.002$) and directly correlated with TNF levels ($p < 0.05$) in all patients. In metastatic CRC only the correlation with TNF was retained. Of interest, 47% of non metastatic CRC had leptin levels above the median compared with 71% of metastatic patients ($p = 0.07$). Median follow-up of metastatic CRC patients was shorter (12.6 months) in patients with high leptin levels compared to those with normal levels (21.7 months, $p = 0.07$). Cox proportional hazard regression model including age, sex, leptin, adiponectin, TNF and CEA levels showed that leptin was an independent predictor for overall survival in metastatic CRC (Cox-Mantel test 2.03, $p = 0.042$).
Conclusions: These results suggest that serum leptin levels might have a role in the biology of CRC and may be regarded as a useful prognostic indicator in metastatic disease.

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Cetuximab-induced thymidylate synthase inhibition is associated with EGFR expression

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The monoclonal antibody cetuximab directed against the epidermal growth factor receptor (EGFR) is an attractive agent for targeted therapy in advanced colorectal cancer (CRC), especially when combined with 5-fluorouracil (5-FU)-based chemotherapy. However, the mechanisms of cetuximab activity as chemosensitizer remain poorly understood. Using proteome-fluorescence-based technology we found that cetuximab is able to suppress the expression of thymidylate synthase (TS) which is involved in the mechanism of 5-FU action. Caco-2, HRT-18, HT-29, WiDr and SW-480 CRC cells were found to express different levels of EGFR. SW-620 was used as EGFR-negative cell line. Only in EGFR-expressing cells cetuximab is able to inhibit TS expression. Combination treatment with cetuximab and 5-FU revealed an antitumor response that is closely correlated with the level of EGFR expression. Moreover, no correlation was seen between constitutive TS expression, cetuximab-induced TS downregulation and response either to 5-FU alone or in combination with cetuximab. We demonstrated that only high level of EGFR expression is important for the synergistic effects between cetuximab and 5-FU in the investigated cell lines and may represent a potential marker of response to cetuximab/5-FU-based chemotherapy in patients with advanced colorectal cancer.
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Prognostic significance of Ki-67 expression in sporadic desmoid tumor

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Background: This study is conducted to evaluate the treatment outcome of sporadic desmoid tumor patients, and its association with Ki-67 expression.
Materials and Methods: From April 1999 to July 2005, 44 patients were pathologically diagnosed with primary sporadic desmoid tumor at Seoul National University Hospital. Among these, we analyzed the medical records of 38 patients and performed immunohistochemical staining for Ki-67 expression. Tumors were located in extra-abdominal areas (23 cases), abdominal walls (11 cases), and intra-abdominal areas (4 cases). Clinical or pathologic tumor sizes ranged from 1 to 13.5 cm in largest linear dimension