PP70

Cdk4/6 inhibition sensitizes medulloblastoma-derived stem like cells to ionizing radiation

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Background: Treatment and cure of pediatric brain tumors continues to be disappointing and results in long-term damage to the normal brain. Recent evidence suggests that a population of cells with stem-like properties (TSC) have extensive capacity for self-renewal and increased radio- and chemoresistance that may be responsible for treatment failures. Elucidation of molecular mechanisms of TSC resistance to DNA damage will allow for improved therapeutic intervention that may spare normal neuronal cells.
Materials and Methods: TSCs were isolated from medulloblastomas that spontaneously arose in mice haploinsufficient for Patched (Ptc), a component of the Sonic hedgehog receptor, and cultured in serum free media as free-floating spheres. Neural stem cells (NSC) were derived from wild-type and Ptc+/− mice. Cultures were exposed to 2 Gy of ionizing radiation and cell cycle distribution was characterized with flow cytometery. For clonogenic survival assays cells were plated into 96well plate at limiting dilution and treated with PD322991 for 16 hours prior to irradiation.
Results: We found that normal NSC showed sustained accumulation in G1 within two hours of irradiation. In contrast, TSC failed to arrest in G1 and showed a marked but unsustained G2/M arrest eight hours after radiation. By 48 hours after irradiation TSC had re-entered the cell cycle. The Trp53 pathway was intact and functional as indicated by sequencing, appropriate phosphorylation on Serine15, induction of p21 and phosphorylation of Rb, suggesting that the abrogated G1 arrest is not due to mutation within the p53 pathway. Treatment of the cells with a Cdk4/6 inhibitor (PD322991) resulted in an increase in apoptotic TSCs after both 2Gy and 5Gy and decreased clonogenic survival.
Conclusion: We find that murine medulloblastoma-derived TSC escape the G1 checkpoint in a p53 independent manner and continue through the cell cycle without adequate DNA repair. This likely contributes to propagation of therapy-resistant malignant clones. We have found that inhibition of Cdk4/6 sensitizes TSC but not NSC to therapeutic doses of ionizing radiation, suggesting that inhibition of these cyclin dependent kinases during therapy improve outcomes in pediatric brain tumor patients.
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PP107

The molecular basis of the chemosensitivity of cutaneous melanoma to chemotherapy

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Background: Chemotherapy benefits relatively few patients with cutaneous melanoma. The assessment of tumour chemosensitivity by the ATP-based tumour chemosensitivity assay (ATP-TCA) has shown strong correlation with outcome in cutaneous melanoma (Ugurel S et al., Clin Cancer Res 2006; 12: 5454−63), but requires fresh tissue and dedicated laboratory facilities. We have therefore examined whether the results of the ATP-TCA correlate with the expression of genes known to be involved in resistance to chemotherapy, based on the hypothesis that the molecular basis of chemosensitivity lies within known drug resistance mechanisms.
Materials and Methods: The chemosensitivity of a series of 38 cutaneous melanomas was assessed using the ATP-TCA and correlated with quantitative expression of 93 resistance genes measured by relative quantitative RT-PCR in a Taqman Array™ following extraction of total RNA from formalin-fixed paraffin-embedded (FFPE) tissue. The results were standardised against the least variable housekeeping gene of those tested (PBGD), and compared with ATP-TCA results by multiple linear regression using SPSS, using PRESS statistics to avoid overfitting of the results.
Results: There was correlation between ATP-TCA data and gene expression for DTIC (adj $R2 = 0.52$, $p < 0.001$). The genes involved in this model were ABCB4, EGFR, IAP2, CES1. ABCB4 (TAP4/MDR3) is a transporter molecule, while EGFR is epidermal growth factor receptor, IAP2 is an inhibitor of apoptosis, and CES1 is a carboxylesterase.
Conclusion: These data suggest that response to DTIC may be influenced by the ability of melanoma cells to metabolise and transport DTIC metabolites from the cell, as well as their susceptibility to apoptosis, which may be influenced by growth factors in addition to intrinsic anti-apoptotic gene expression. However, the degree of correlation is not as strong as we have observed for more active drugs, which may indicate a lack of key genes involved on the array or difficulty of the ATP-TCA in measuring the efficacy of DTIC.

PP12

Signature of miRNA in TEL/AML1-positive acute lymphoblastic leukemia: potential regulation of CD9 expression

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Background: We have recently shown that the underexpression of CD9 could discriminate TEL/AML1 B-acute lymphoblastic leukemia (B-ALL) from the other B-ALL. After having excluded different underlying mechanisms, we are now investigating the presence of RNA instability and have screened for the presence of miRNAs that would potentially be deregulated in TEL/AML1-positive ALL, focusing on CD9 targeted miRNAs.
Materials and Methods: Bone marrow samples with >80% blast-cells of thirty childhood B-ALL have been studied. CD9 expression and recurrent rearrangements were determined for each sample. Total RNA has been extracted and purified using the mirVana miRNA isolation Kit (Ambion). The cohort was split into a training set A of 20 samples (14 TEL/AML1-positive CD9-negative and 6 CD9 positive ones of whom 2 TEL/AML1-positive) and a validation set B of ten samples (7 TEL/AML1-positive patients of whom 6 CD9-negative and one CD9-positive, and 3 no rearranged CD9-positive ones). A TaqMan ®MicroRNA Arrays approach has been applied to the training set A to detect and quantify up to 760 miRNAs. U6 RNA was used as internal control for RQ values and one and the other TEL/AML1-positive CD9-positive patient as calibrator for normalizing the arrays. To select miRNA that were differentially expressed in CD9-positive and CD9-negative patients, we performed two class unpaired Significance Analysis of Microarray (SAM), with mean-centred and linear & lowest normalized RQ values. Only values present on at least 70% of patients, with a q value above 0.05 have been retained. Results have been validated using the set B of samples with real-time-RT-PCR.
Results: SAM analyses identified 34 distinct miRNAs. In the oher hand, the questioning of miRNA databases (miRanda, MirBase, PicTar, TargetScan) revealed 85 different miRNAs predicting to target CD9. The combined results of both approaches revealed 8 miRNAs in common. Five miRNAs have been selected for further validation because they were either differentially expressed whatever calibrator is chosen or expressed with a 1.5-fold minimum difference between CD9-positive and CD9-negative patients.
Conclusion: We have shown that low level of CD9 could result from the presence of miRNAs. We now, ought to validate in vivo whether or not thoses sequences specifically target CD9 to conclude on the regulatory mechanism leading to the low level of CD9 which characterizes TEL/AML1-positive leukemia.

PP129

Molecular biomarker analysis of clinical prostate biopsy specimens: Tissue print techniques simplify development of DNA methylation marker tests while preserving the FFPE specimen for histology

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Background: Many studies support the use of tumor-associated changes in DNA methylation (DNA-M) as biomarkers for prostate cancer (PrCa). In addition, we and others have shown that changes in DNA-M patterns also occur in histo-benign tissues adjacent to PrCa. Clinical prostate biopsy procedures often yield tissues suspicious for cancer but lacking the criteria for a definitive diagnosis. An assay to test histo-benign prostate biopsies for molecular biomarkers associated with the presence of nearby cancer could help guide patient management when there is concern about a false-negative diagnosis. Tumor-associated changes in DNA-M in histo-benign tissue adjacent to PrCa are attractive as a chemically stabile class of "field effect" biomarkers for such an assay. It is possible to perform DNA-M analysis using formalin fixed paraffin embedded (FFPE) prostate biopsy tissues. However FFPE biopsy specimens offer limited amounts of tissue and the priority use for these samples is histological diagnosis.
Materials and Methods: As an alternative to using FFPE tissues for molecular biomarker analysis, we have developed a set of tissue printing techniques that allow us to obtain micropeel samples of needle biopsy tissues without compromising the specimen for pathology diagnosis. Briefly, we obtain a microscopic layer of cellular material on a nitrocellulose membrane as the biopsy core is transferred from the cutting needle to the fixative jar. The diagnostic tissue cores are processed as usual for pathology review; the tissue prints are snap-frozen. Later in the lab the biomaterial that was transferred from tissue to nitrocellulose is extracted and its components purified as separate protein, RNA and DNA fractions.
Results: Prostate biopsy tissue prints routinely yield 200–400 ng of high quality RNA and ~1000 ng of high quality DNA. We used quantitative

Methylation-Specific-PCR (qMSP) to characterize potential "field effect" markers in DNA samples from tissue prints obtained from diagnostic prostate biopsies and confirmed the technical validity of the assay design. Biopsy tissue print techniques allowed us to design DNA-M marker panels that include up to 6 candidate field effect markers. Tissue prints also simplify the development of tests that include both DNA and RNA based assays.
Conclusion: By getting the most from the least tissue, a tissue print "field effect" biomarker test might be used with prostate biopsies to predict the presence of an adjacent cancer while reserving the FFPE specimens for histology.

PP121

High coexpression of both the epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor-1 (IGF-1R) correlates with a poor patient prognosis in resected non-small cell lung cancer (NSCLC).

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Background: Following the success of the EGFR inhibitors a renewed interest in IGF-1R inhibitors has emerged. IGF-1R overexpression has been identified in several tumour types and protects cancer cells from apoptosis. Currently, several different approaches are being investigated for targeting the IGF-1R, including small-molecule kinase inhibitors, IGF1R monoclonal antibodies, antisense oligonucleotides and RNA interference. To date, it is not clear what factors influence sensitivity to IGF-1R blockade but it is likely that tumours that respond well to treatment will be those where IGF-1R overexpression results in a poor patient prognosis. Initial data show that tumour type may also determine response to therapy with squamous non-small cell lung cancers responding well to a combination of a IGF-1R monoclonal antibody and chemotherapy. The aim of this study was to elucidate the EGFR and IGF-1R expression profile in a cohort of NSCLC patients and correlate the results to patient clinico-pathological data and prognosis.
Materials and Methods: EGFR and IGF-1R expression were evaluated in 197 NSCLC patients (92 − squamous, 87 − adenocarcinoma, 18 − others) using immunohistochemistry analysis and the results were scored by a pathologist as follows: 0 (negative), 1+ (weak), 2+ (moderate) and 3+ (strong). Expression of EGFR and IGF-1R were also examined in a panel of cell lines (SKMES1, A549, HCC827, H1819, H1299) and patient samples (10 squamous and 10 adenocarcinomas) using Western Blot analysis.
Results: The panel of 6 NSCLC cell lines examined showed variability in IGF-1R expression. In the fresh frozen resected NSCLC tumours IGF-1R was overexpressed relative to matched normal tissues. Furthermore squamous cell carcinomas had higher levels of expression than adeno-carcinomas. Immunohistochemistry analysis demonstrated that squamous cell tumours have higher IGF-1R expression levels than adenocarcinomas (3+/2+ Squamous [70/197] versus 3+/2+ Adenocarcinoma 27/197)] $p < 0.0001$). Patients with squamous cell carcinoma also had higher EGFR expression than those with adenocarcinoma ($p = 0.002$). Patients with EGFR and IGF-1R overexpression had a poorer survival ($p = 0.043$).
Conclusion: Our findings indicate that while EGFR and IGF-1R expression alone are not independent prognostic markers of survival in NSCLC. Taken together overexpression of both proteins correlates to a poor survival. This subset of patients may benefit from a combination of TKIs/monoclonal antibodies and chemotherapy.

PP81

18F-FDG PET/CT for early detection of relapse in head and neck squamous cell carcinoma

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Background: A key prognostic factor in head and neck squamous cell carcinoma (HNSCC) is the loco-regional control of the disease. Early detection of relapse by selected imaging modalities is therefore of upmost importance. We evaluated the diagnostic accuracy of 18F-FDG PET/CT and MRI for the assessment of HNSCC relapse. Since early treatment might anticipate on inoperable relapse, we also evaluated if early 18F-FDG PET/CT might help in residual tumor detection despite treatment-related changes.
Materials and Methods: The study was prospectively performed on 32 patients with 36 primary HNSCC who underwent 18F-FDG PET/CT and MRI before treatment and at 4 and 12 mo after treatment completion. 18F-FDG PET/CT was also performed 2 weeks after the end of radiotherapy. All images were blindly and independently interpreted and graded on a 5-point scale. Histopathology or a minimum of 18 mo follow-up were used as gold standard.
Results: Before treatment 18F-FDG PET/CT and MRI detected all primary tumors except for 2 limited vocal fold lesions (sensitivity: 94%). MRI was more sensitive than 18F-FDG PET/CT for the detection of precise local extension sites (sensitivity: 75% versus 58%, $P < 0.05$) but at the cost of a higher rate of false positive results (positive predictive value: 74% versus 86%, $P < 0.05$). For relapse detection at 4 mo, sensitivity was significantly higher for 18F-FDG PET/CT (92%) than for MRI (73%) ($P < 0.05$), but the diagnostic performances were not significantly different at 12 mo post-treatment. For the detection of residual malignant tissue at 2 weeks post-radiotherapy, sensitivity and specificity of 18F-FDG PET/CT were respectively 86% and 85%, when using an SUV cut-off value of 5.8.
Conclusion: This study demonstrates that 18F-FDG PET/CT is effective in the differentiation between residual tumor and radiation-induced changes, as early as 2 weeks after treatment of a primary HNSCC. For follow-up, accuracy of 18F-FDG PET/CT and MRI are similar except for a higher sensitivity of 18F-FDG PET/CT at 4 mo.

PP14

RRM1 expression in muscle invasive, locally advanced urothelial cancer is associated with survival in younger patients

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Background: RRM1, the regulatory subunit of ribonucleotide reductase, plays an important role in DNA repair after chemotherapy damage and in regulation of tumor progression. Prior studies demonstrated a survival benefit to high expression in resected early stage lung cancer and a trend to longer time to progression in patients with low expression in unresectable advanced bladder cancer treated with gemcitabine-cisplatin therapy. We undertook this study to assess whether patients with resected locally advanced (T2−4NxM0) urothelial carcinoma (UC) whose tumors had higher RRM1 expression would have longer overall survival (OS).
Materials and Methods: 84 radical cystectomy specimens with muscle invasive UC were identified from existing tissue microarrays. The medical records of these patients were retrospectively reviewed to confirm pathology and stage. Specimens were analyzed for RRM1 expression using automated quantitative analysis (AQUA). The median value of RRM1 was established a priori as the cutoff for high and low expression. Older patients were defined as having an age $\geqslant$70 years.
Results: Median age was 69.3 years. 43 patients were <70 years; 41 were $\geqslant$70 years. There was near equal distribution of stages: 30%, 38%, and 32% for stage II, III, and IV respectively. The majority were high grade (99%) with no nodal involvement (69%). Median OS was 2.0 years (0−13.1). Tumoral RRM1 expression levels did not correlate with OS. However, when adjusted for age, high tumoral RRM1 expression in younger patients (<70 years) correlated with increased survival. Younger patients with high RRM1 had a median OS of 10.6 years compared to 1.6 years in older patients ($p = 0.0013$). No difference in survival was seen among low RRM1 expressors: 2.3 vs. 1.6 years in younger and older patients respectively, ($p = 0.215$). 40% of younger patients were high expressors. 32% of younger patients had nodal involvement compared to 29% of the older subset. In terms of T stage, 33% of younger patients had T3 disease compared to 54% of older patients and 33% of younger patients had T4 disease compared to only 15% of older patients.
Conclusion: Our results suggest that high RRM1 expression may be prognostic for improved survival in locally advanced UC patients less than 70 years old. This novel finding suggests that the biology of bladder cancer in "younger" patients is inherently different than their older cohort such that RRM1 gene expression should be the target of a larger investigation in this subset of patients.

PP16

Metronomic weekly use of zoledronic acid for breast cancer with bone metastases has more potent antitumor and bone-preserving effects than conventional zoledronic acid given every-four-weeks

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Background: Zoledronic acid (ZOL) has direct and indirect antitumor effects, however, the pharmacokinetics of the drug in breast cancer patients remain to be elucidated and optimized. The main study objectives were