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