Sunday 27 June 2010 09:45–17:30

Poster Session

Experimental/Molecular Therapeutics, Pharmacogenomics

198 Exploring a new therapy for neuroblastoma: silencing of doublecortin-like kinase using RNA-interference

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Neuroblastoma is one of the most common childhood cancers. Microtubule-destabilizing agents are used in the treatment of these tumours. However, resistance to chemotherapeutic agents and systemic toxicity make neuroblastoma a difficult drug target.
In our previous work, we found that doublecortin-like kinase (DCLK) gene transcripts are crucial microtubule-associated proteins for correct proliferation and differentiation of neuroprogenitor cells. Gene expression profiling revealed a high expression of these transcripts in neuroblastoma patients. Furthermore, these transcripts are endogenously expressed specifically in neuroblasts but are not found in other cell types. Suppression of DCLK by short interfering RNA (siRNA) disrupted the mitotic spindles in neuroblastoma cells and gene expression profiling revealed numerous differentially expressed genes indicating apoptosis. Apoptotic cell death of neuroblastoma cells by DCLK knockdown was further confirmed by several assays. Interestingly, mitochondria were the most affected cell components after DCLK-long knockdown. We also found in human neuroblastomas a significant correlation between DCLK expression and genes related with mitochondria activity. Furthermore, we showed a successful delivery of siRNA targeting DCLK to neuroblastoma cells by using specific peptide-siRNA conjugates.
In conclusion, silencing of the DCLK gene by siRNA interference is a novel potential therapeutic approach for neuroblastoma with the promise of combining high specificity with fewer side effects. Peptide-siRNA conjugates might be the tool needed for specific neuroblastoma delivery.

199 IGFBP-3 promoter methylation activates the PI3K/Akt intracellular signaling pathway in CDDP resistant cell lines

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The purpose of this study is to investigate the mechanisms regulated by the *IGFBP-3* promoter methylation that induce tumour cell proliferation and loss of sensitivity to CDDP in non-small cell lung cancer (NSCLC). We have recently reported that reduction of *IGFBP-3* expression by promoter methylation is involved in the CDDP acquired resistance process in 3 matched CDDP sensitive/resistant human cancer cell lines and in a human cohort of 36 NSCLC patients, probably because CDDP also induces DNA methylation *de novo.* The biological significance of *IGFBP-3* is of great importance in controlling cell growth, transformation and survival as IGF-I binds to *IGFBP3* with stronger affinity than to its own receptor (IGFIR), blocking their interaction and abolishing the mitogenic and antiapoptotic actions. IGF-I is also able to activate EGFR receptor. Those tyrosine kinases receptors (IGFIR and EGFR), signal through the PI3K/Akt pathway, that plays a crucial role in cell growth, proliferation, and survival and is commonly upregulated during tumourigenesis, including NSCLC; although the precise mechanism is not well defined.
The present study is based on a panel of three-paired CDDP resistant/sensitive NSCLC (H23 and H460) and ovarian (41M) cancer cell lines, with different *IGFBP-3* promoter methylation status. We have studied the relation between (1) *IGFBP-3* gene expression levels and promoter methylation status measured by RT-PCR, and by bisulfite sequencing and methylation specific PCR (2) the activation of EGFR and IGFRI biological pathways through the analysis of the PTEN, AKT, pAKT, pEGFR, EGFR, pIGFRI and IGFR protein levels by Western-Blot and (3) response to CDDP by crystal violet survival curves. Our results suggest that loss of *IGFBP-3* expression by promoter methylation in tumour cells treated with CDDP may activate the PI3K/AKT pathway through the derepression of the IGFIR signaling, inducing a resistant phenotype to CDDP and increasing cell growth. This study provides information regarding first, *de novo* promoter hypermethylation of *IGFBP-3* and the development of CDDP resistance, through the activation of the cell survival pathway PI3K/AKT, and second, the potential use of *IGFBP-3* and PI3K/AKT members as biomarkers and targets enabling the diagnosis and a personalized chemotherapy treatment of NSCLC.
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200 Response of cancer cell lines to chemotherapeutic drugs: DNA repair phenotyping as an early cell- and drug-specific exposure marker

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Background: Resistance of tumours to cytotoxic agents is a major concern of cancer therapy. There is strong evidence that modifications of DNA repair pathways participate to therapeutic failure. However underlying mechanisms are poorly understood. In this study we investigated the earliness of the DNA repair response using a set of colorectal cancer cell lines treated by sub-toxic and toxic concentrations of different chemotherapeutic drugs. DNA repair is a complex network of several intricate pathways; in order to better address the DNA repair response, we used a comprehensive functional approach on dedicated biochips that allowed investigating Nucleotide and Base Excision Repair as well as Intra-Strand Cross-Links Repair simultaneously.
Material and Methods: Three colon cancer cell lines were treated for 48h with chemotherapeutic drugs at IC20, IC20/10 and IC20/100 (MTT test). Nuclear extracts were prepared and the DNA repair phenotype was established using an *in vitro* multiplexed DNA synthesis/excision repair assay on miniaturized support.
Two different parameters were investigated regarding DNA repair: the global DNA repair response and the contribution of each of the measured DNA repair pathways (toward photoproducts, 8oxoguanine, alkylated basses, cisplatin adducts, abasic sites and glycols) to the global response.
Results: Each cell line exhibited a specific repair phenotype at basal state (without treatment). Cytotoxicity and DNA repair response were not necessarily correlated. For almost all the cell lines and the treatments tested, the treatment impacted the DNA repair phenotype before any detectable cytotoxic effect. The precocity, the extent and the nature (inhibition or stimulation) of the repair response (modification with respect to basal state) were highly cell type- and drug-dependent.
Conclusion: Genetic background of the cell lines is heterogeneous and mutations in some important genes might drive the repair response. Our aim is now to combine view on DNA repair response, genotypes and cytotoxicity in the purpose of identifying predictive biomarkers of chemotherapy response. In addition, considering the complexity of the DNA repair mechanism responses, the use of a comprehensive approach is the most efficient strategy to decipher the relationship between DNA repair and drug response.
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201 The roles of STAT transcription Factors in imatinib resistance and sensitivity in BCR/ABL positive chronic myeloid leukemia cells

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Background: STAT proteins have important roles in carrying the information coming from extracellular signals to inside of the nucleus. Chronic myeloid leukemia (CML) is a hematological cancer resulting from the reciprocal translocation between 9th and 22nd chromosomes bringing the *BCR* and *ABL* genes together to form BCR/ABL protein. Imatinib binds to the ATP binding domain of the fusion protein and prevents the subsequent phosphorylation of the target proteins. Despite the survival periods of CML patients are prolonged by means of imatinib, in most of the cases, leukemia gains resistance and eventually, chemotherapy remains ineffective.
Aims: In this study, we aimed to identify the roles of STAT proteins in imatinib resistance and sensitivity in K562 cells, and revealing the effects of STAT siRNA suppression on cellular growth and apoptotic induction.
Methods: Expressions of *STAT* genes were assessed by quantitative real-time PCR (Q-PCR). For silencing the gene in both sensitive and 3 μM imatinib-resistant K562 cells (K562/IMA-3), HiPerFect Transfection Reagent was used. Cell proliferation was detected by XTT cell proliferation assay, and apoptosis was evaluated by changes in caspase-3 enzyme activity.
Results: Q-PCR analysis revealed that the *STAT5a* has the most significantly changing expression level among the others in K562-IMA-3 cells as compared to sensitive K562 cells. *STAT5a* expression increased by 67%, where *STAT5b* showed 56%, and *STAT3* showed 4% increases, respectively, as compared to sensitive K562 cells. The results of this study has also demonstrated that that silencing of *STAT5a* sensitized both sensitive and K562/IMA3 cells to imatinib. Transfected K562/IMA3 cells became almost 4.5-times more sensitive than the non-transfected counterparts while transfected sensitive cells showed approximately 1.12-fold increased sensitivity. These results