111 Poster

The antidiabetic drug metformin - a novel therapeutic for HER2-positive breast carcinomas?

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A lower cancer-related mortality and cancer risk has been recently attributed to metformin consumption in diabetic patients. Intriguingly, the therapeutic application of metformin for Estrogen Receptor (ER)-negative breast cancer lesions in vivo appears to promote and increase the angiogenic phenotype and tumorigenic progression. Conversely, systemic treatment with metformin significantly increases life span in HER2 (erbB-2)-transgenic mice. Given our recent identification of a bidirectional linkage between endogenous fatty acid metabolism and HER2 in human breast cancer cells, we here envisioned that HER2 oncoprotein may represent a key cellular target involved in the anti-cancer actions of metformin. Metformin treatment decreased HER2 expression in a dose- and time-dependent manner (> 80% reduction) in three in vitro HER2-over-expressing breast cancer models. Metformin-induced suppression of HER2 occurred regardless the molecular mechanism contributing to HER2 overexpression (i.e. naturally by gene amplification in SKBR3 cells and ectopically driven by a viral promoter in MCF10A and MCF-7 cells stably transduced with the human HER2 cDNA), thus suggesting that metformin did not affect the transcriptional rate of the endogenous HER2 gene. Metformin treatment activated the fuel sensor AMP-activated protein kinase (AMPK). However, co-treatment with the AMPK inhibitor compound C incompletely prevented the ability of metformin to inhibit HER2. Moreover, AMPK activation upon treatment with 5-aminoimidazole-4-carboxamide (AICAR) failed to mimic metformin-induced inhibition of HER2. Specific inhibition of the AMPK pathway using small interfering RNA (siRNA) against the alpha1/2 catalytic subunits of AMPK did not prevent the anti-HER2 actions of metformin. Metformin treatment dose- and time-dependently abolished the activity of the ribosomal p70S6 kinase (p70S6K1), a downstream effector of the AMPK/mTOR pathway. Of note, ablation of endogenous p70S6K1 by siRNA was sufficient to completely protect breast cancer cells from the anti-HER2 effects of metformin. The expression status of HER2 remained insensitive to mTOR blockade by rapamycin. The ability of metformin to efficiently suppress HER2 overexpression through a direct (AMPK- and mTOR-independent) inhibition of p70S6K1 strongly suggests that the presence/absence of molecular hallmarks such as HER2 oncogene overexpression might dictate alternative breast cancer responses to the antidiabetic drug metformin.

112 Poster

Novel titanocene analogues induce apoptosis in prostate cancer epithelial cells by initiating a DNA damage response

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Background: Treatment options for locally advanced metastatic prostate cancer are extremely limited with docetaxel (Taxotere®) being the standard chemotherapy but only providing a three month survival advantage. The objectives of this study are to investigate novel titanocene analogues as possible alternative chemotherapies for advanced disease. The primary aims are to investigate the apoptotic effects of these novel titanocene analogues on prostate cells and to examine their mechanisms of action.

Materials and Methods: PwR-1E and PC-3 cell lines were grown in optimal conditions and treated with titanocene analogues at different doses and times. Apoptosis and viability were assessed by propidium iodide staining and flow cytometry and PARP cleavage. Cellular uptake and DNA binding of Titanium was measured by atomic absorption spectroscopy (Dr. JL Beltramo, Université de Bourgogne, France). Alkaline single cell gel electrophoresis was carried out using the Trevigen CometAssayTM kit to assess DNA damage and Replication Protein A (Ser 4/8) and p53 (ser 15) phosphorylation were assessed by western blotting to confirm a DNA damage response. Knock-down of p53 was achieved by si-RNA and assessed by western blotting.

Results: PwR-1E and PC-3 cells undergo apoptosis in a dose dependent manner following treatment with a range of titanocene analogues as determined by PI DNA staining and PARP cleavage. These compounds enter both cell lines and bind to DNA as confirmed by atomic absorption spectroscopy. These results confirm a correlation of increased Titanium-DNA binding and apoptotic responses. The differential apoptotic response between the PwR-1E and PC-3 cell lines correlates with the uptake of Titanium into the cells and consequently the level of DNA binding.

The titanocene compounds induce DNA damage in both cell lines as shown by the formation of 'comet tails' of DNA fragmentation upon single cell gel electrophoresis and the phosphorylation of Replication Protein A and p53. However induction of apoptosis by the titanocene compounds is not p53 dependent as demonstrated by knock-down of p53 by si-RNA in the PwR-1E cell line and no expression in the PC-3 cells.

Conclusion: These pre-clinical studies demonstrate for the first time that these novel titanocene analogues induce apoptosis in prostate cancer cell lines. Further evaluating the mechanism of action will indicate their appropriate clinical use in different stages of prostate cancer development.

113 Poster

Retinoids, in combination with Histone Deacetylase (HDAC) inhibitors, as a potential therapy for pancreatic cancer

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Background: The aim of this study was to investigate the role of retinoids in combination with pharmacological agents that reverse epigenetic silencing, as potential therapies for pancreatic cancer (PC). Retinoid-based treatments, which have been used successfully in some leukaemias, have had disappointing results in other cancers. We hypothesised that this may be in part due to aberrant retinoic acid (RA) signalling. Cellular Retinoid Binding Protein 1 (CRBP1) plays a key role in RA signalling by presenting retinoids to their metabolising enzymes. In other cancers such as breast cancer, CRBP1 is frequently silenced due to epigenetic modification. Downregulation of CRBP1 expression leads to localised retinoid deficiency that could result in the downregulation of RA receptors, primarily RARβ. Restoring CRBP1 expression may restore RA signalling, enabling retinoid-based therapy to be developed in PC.

Methods: CRBP1 expression in human PC and normal samples, and PC cells lines was determined using immunohistochemistry and quantitative real time PCR (QRTPCR) respectively. Methylation status of CRBP1 promoter was investigated using methylation specific PCR. MiaPaCa2 (MP2) cells demonstrated methylation of the CRBP1 promoter and complete loss of CRBP1 expression. MP2 cells were then treated with 300μM of demethylating agent 5-aza-2'-deoxycytidine (5-AZA) and 100nM of HDAC inhibitor Trichostatin A (TSA). The expression of CRBP1 after drug treatment was measured using QRTPCR.

Results: The majority of human PC (70%) demonstrated loss or downregulation of CRBP1 expression (n=90). This was also evident in early PC precursor lesion, pancreatic intraepithelial neoplasia (PanIN), suggesting that loss of CRBP1 expression was an early event in the development of PC. Methylation of the CRBP1 promoter was identified in 28% of PC samples with loss or downregulation of CRBP1 expression (n=32), but not in normal samples (n=5). Treatment of MP2 cells with 5-AZA and TSA resulted in detectable expression of CRBP1 mRNA using QRTPCR.

Conclusion: The loss or downregulation of CRBP1 expression occurs in a significant proportion of human PC and PanIN. The loss of CRBP1 expression in the PC cell line MP2 was associated with epigenetic silencing and could be reversed pharmacologically, resulting in detectable mRNA expression. These results suggest that demethylating agents and HDAC inhibitors, in combination with retinoids, may have a therapeutic role in PC.

114 Poster

Mechanisms of EGFR-TKI induced cell death and resistance in EGFR mutant non-small cell lung cancer

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Although large clinical trials have shown that EGFR tyrosine kinase inhibitors (TKI) prolong survival of lung cancer patients, the precise mechanism of EGFR-TKI drug action leading to apoptotic cell death in EGFR dependent lung cancer hasn't been clearly elucidated. Moreover, patients who initially respond to TKI therapy invariably develop acquired resistance to the drugs, necessitating alternative approaches to EGFR TKI monotherapy in non-small cell lung cancer (NSCLC).

A series of in vitro and in vivo experiments were performed to better understand the cell death and resistance mechanisms induced by EGFR-TKI. In EGFR tyrosine kinase domain mutant NSCLC cells, which undergo rapid apoptosis upon treatment with EGFR-TKI, alterations of EGFR downstream signaling pathways were probed in response to TKI. Part of this cell death program is initiated by BH3 only protein, Bim. The induction of Bim and the initiation of apoptosis are mediated by a couple of EGFR downstream signaling pathways, including ERK dependent Bim phosphorylation and its inhibition. In addition to Bim induction, it was found