363 POSTER

Fatty acid synthase is a potential therapeutic target in Micro-satellite-instable colorectal cancers

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Background: Many human epithelial cancers, particularly those with a poor prognosis express high levels of fatty acid synthase (FASN), a key metabolic enzyme linked to synthesis of membrane phospholipids in cancer cells. Over-expression of FASN is linked with activation of phosphatidylinositol-3′-kinase (PI3K)/AKT pathway. In this study, we investigated the role of FASN and its relationship with PI3K/AKT activation in a large series of colorectal carcinoma (CRC) tissues in a tissue micro array (TMA) format followed by in vitro and in vivo studies using CRC cell lines and NUDE mice.
Materials and Methods: Analysis of apoptosis and cell cycle was evaluated by flow cytometry and DNA fragmentation assays. FASN and phospho-AKT protein expression were determined by IHC and Western blotting.
Results: Correlation of FASN with various clinico-pathological parameters on 448 CRC samples was assessed. Activated AKT was found in 283/400 (68.8%) of CRC and was associated with FASN over-expression. FASN over-expression was observed in 109/403 (27.1%) and was significantly more common in Micro-satellite-instable (MSI) than Micro-satellite-stable (MSS) tumors ($p < 0.01$). In addition, our in vitro data using HCT-15, an MSI CRC cell line showed a better apoptotic response following inhibition of FASN activity as compared to Colo-320, an MSS CRC cell line. Finally, treatment of HCT-15 cell line xenografts with C-75 resulted in growth inhibition of tumors in NUDE mice via down-regulation of FASN and AKT activity.
Conclusions: These data identify FASN as a potential biomarker and a novel therapeutic target in distinct molecular subtypes of CRC.

364 POSTER

Obatoclax in SCLC: preclincal evaluation of a BH3 mimetic

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Lung cancer is the leading cause of cancer death with small cell lung cancer (SCLC) representing approximately 15% of cases. Despite combination chemotherapy with platinum/etoposide survival rates remain dismal warranting the development of novel therapeutic strategies. The Bcl-2 family of proteins are key regulators of apoptosis, with most apoptotic stimuli converging at the mitochondrial surface where interactions between Bcl-2 family members determine cell fate. The observation that Bcl-2 is present in 75% of SCLC clinical specimens and that over-expression of anti-apoptotic Bcl-2 family members confers resistance to chemo-radiation in vitro has promoted clinical development of Bcl-2 targeted therapies in SCLC.
Obatoclax, a novel BH3 mimetic, is thought to bind inclusively to the BH3-binding groove of anti-apoptotic Bcl-2 family proteins. Using a short-term viability assay (MTS) for suspension cells, the IC50 values for obatoclax were calculated as 0.07–1.04 μM in a panel of eight SCLC cell lines in vitro. Induction of apoptosis (PARP cleavage) in response to obatoclax was both time- and concentration-dependent. Chou-Talalay combinational index studies showed synergy over 96 hours with the clinically relevant chemotherapeutics cisplatin and etoposide. The synergy was schedule-dependent, with exposure of cells to 48 h obatoclax prior to cisplatin and etoposide resulting in greater synergy than either the reverse sequence or 96 h concomitant treatment, in all eight cell lines. Experiments determining the timing of apoptosis in relation to obatoclax sensitivity as a single agent and in combination with cytotoxics will be reported.

365 POSTER

Effects of PPARgamma agonists on adrenocortical carcinoma in a murine xenograft model

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Objective: The purpose of this study was to evaluate effect of rosiglitazone (RGZ), a synthetic high-affinity ligand for PPARgamma, in a mouse model of human adrenocortical carcinoma. Previous studies in vitro demonstrated that treatment of human adrenocortical cancer cell line with PPARgamma agonists reduced malignant potential (Ferruzzi P, et al. J Clin Endocrinol Metab, 2005).
Material and Methods: Tumour xenograft was obtained by subcutaneous injection of 7×10^6 H295R cells, a human adrenocortical cancer cell line, in the right flank of nude Balb/c mice. When the tumour size reached a mean of 5 mm, the animals were randomly allocated to 2 groups of 9 mice treated with: (1) RGZ 5 mg/kg in oral administration (gavage) 6 days a week; (2) water, same schedule (control group). Tumour volume was evaluated twice a week for 31 days by the formula: tumour volume = length x width2/2 and tumour response was estimated versus initial volume. Once mice were sacrificed, tumours were removed, fixed and stained with haematoxylin and eosin.
Results: We observed a significant reduction of tumour growth in the RGZ group ($p = 0.007$). At the analysis of tumour specimens, in the control group tumour presented characteristics of invasiveness, richness in small vessels and numerous mitotic figures. In the RGZ group tumour presented expanding and not infiltrating borders, there were an evident lack of vessel and numerous apoptotic bodies.
Conclusions: This study supports a role of RGZ in adrenocortical carcinoma therapy. Further investigations are underway to improve our knowledge on RGZ molecular mechanisms in tumour and to define the optimal RGZ dose for anticancer effect.

366 POSTER

NAV3 gene aberrations in colorectal cancer target signalling pathways associated with inflammation and the progression of cancer

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We have previously shown that chromosome 12q21 aberrations, specifically allelic loss of the neuron navigator 3 (NAV3) gene, associate with Cutaneous T-cell lymphomas. Since loss of the chromosomal region 12q is reported to associate with a poor prognosis in several cancers of epithelial origin, we looked for eventual NAV3 gene aberrations in colorectal cancers (CRC) as well as in intestinal adenomas. As a result, copy number changes, in the form of allelic loss of NAV3 but also in amplification of this gene, were found.
To mimic the in vivo gene deletion and to shed light on the function on the NAV3 gene in CRC carcinogenesis, we successfully silenced the expression on NAV3 using commercially available pooled oligonucleotides in the established colorectal cell lines CRL1539 and CRL 1541 (ATCC, Manassas, VA, USA). Post-transfection RNA-samples from different time points were processed for Agilent 4*44K microarray analysis. With thoroughly preprocessed microarray data across all samples, we identified 39 differentially expressed genes (DEGs) using fold changes as the selection criterion. These genes were further analyzed with Gene Ontology and pathway analysis tools to reveal their possible functional roles. With annotation methods we identified genes that are most likely drug targets (e.g., genes that are already associated with cancer and code membrane proteins).
Altogether, 16% of the up-regulated genes were membrane proteins and thus potential targets for antibody-based therapy. Likewise, 16% of the up-regulated genes were genes known to be associated with different types of cancer, including carcinomas of ventricle, breast, pancreas, lung and prostate as well as tumours of neural and lymphoid origin (both T- and B-cell lymphomas). In pathway analysis, two of the NAV3-regulated membrane proteins significantly target signalling pathways contributing to oncogenesis.
We conclude that NAV3 aberrations affect signalling pathways associated with cancer-related inflammation and the progression of cancer. Thus, the