pathways in the tumor microenvironment show infiltration dependent alterations in gene expression levels, suggesting infiltrating cells as possible targets for new anticancer therapies. To evaluate this option further we use liposome-encapsulated clodronate (Clodrolip) to specifically deplete macrophages *in vivo*. This tool allows us to study the development of tumors in the presence and absence of TAMs. In this study murine teratocarcinoma tumors (F9 cells) were grown s.c. in Sv129S1 mice. Two groups were studied over the course of three weeks, one control group and one group receiving Clodrolip. Upon immunohistochemical confirmation of macrophage depletion in Clodrolip treated mice, total RNA was extracted from the isolated tumors and subjected to a gene expression analysis. Data obtained from this study confirmed down regulation of macrophage specific markers as well as proinflammatory signaling and proangiogenic factors in TAM-depleted tumors. Moreover, the data indicates TAM-dependent modulations of the extra cellular matrix, cell proliferation, cell adhesion and migratory activity, all of which have been confirmed biochemically. Migration and invasion assays *in vitro* have further confirmed a TAM dependent stimulation of F9 cell aggressiveness. Together these data suggests TAMs to affect basic features of tumorigenesis, supporting the establishment of solid tumors via high tumor cells proliferation activity, highly organized tumor structure and tumor cell migration and extravasation. We are now investigating the molecular mechanisms, through which TAMs may regulate these pro-tumorigenic events. Results obtained from this study will contribute to the understanding and evaluation of TAMs as a possible target for new anti-cancer therapies.

360 POSTER

Ror2 in renal cell carcinoma: evaluating its role in RCC tumorigenesis

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Sporadic renal cell carcinoma (RCC), a notoriously hard to treat solid tumor malignancy, has minimal sensitivity to traditional chemotherapy and immune system modulation. The most current and effective therapies for renal cell carcinoma involve the use of kinase-specific inhibitors targeted against receptor tyrosine kinases (RTK) and ligands specific to angiogenesis signaling. However, though these agents have shown promise, they remain to be fully optimized. Currently, there are no reported cancer cell specific kinases expressed on RCC identified as amenable targets for tumor cell directed therapy. Using a phospho specific RTK screen in renal carcinoma cells, we identified Ror2, an orphan receptor tyrosine kinase previously unknown in renal carcinoma cells. Ror2 is normally expressed in the heart, brain and lungs of developing mice and has also been implicated in the Wnt/β-catenin signaling pathway. Activated kinase expression was observed in RCC cell lines, with Ror2 expressed in a manner dependent on the inactivation of the von Hippel-Lindau (VHL) tumor suppressor and subsequent stabilization of the hypoxia inducible factor (HIF) family of transcription factors. In addition, transcripts of Ror2 were detected in more than 55% of a set of 19 archival human RCC tumor specimens. Among these archival RCC tumor specimens, Ror2 expression also clustered with genes involved in the epithelial to mesenchymal transition (EMT). Additionally, inhibition of Ror2 expression by RNAi not only limited RCC growth in soft agar, a surrogate for invasive cellular growth potential, but also produced fewer tumors in xenografts. Therefore, we hypothesize that we may have identified a kinase involved in key aspects of the transformation process in this malignancy. Further analysis is currently underway to delineate the importance of Ror2 for RCC tumorigenesis and regulation as it represents a potentially important molecular target for RCC.

361 POSTER

FGFR4 Y367C: Contributes to a constitutively active FGFR4 and tumour aggressiveness in breast cancer cell line

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Background: FGFR4, member of the fibroblast growth factor receptor family, is an emerging tyrosine kinase cancer target. A polymorphism in the transmembrane domain of the FGFR4 is linked to the progression and worsened prognosis of melanoma, sarcoma and breast-, prostate-, colon- and head and neck carcinoma. Recently, we detected a mutation, FGFR4 Y367C, in the breast cancer cell line MDA-MB-453. Remarkably, a homologous cysteine substitution in FGFR1−3 causes various osteogenic disorders through enhanced dimerization and constitutive kinase activation; and contributes to tumour progression. Hence we set out to determine the role of the FGFR4 Y367C variant in cancer cells and the phenotypic consequences of its inhibition.

Methods: We examined FGFR4 protein expression and activity by western blot analysis and transcript levels by real-time PCR. Full-length FGFR4 was cloned and transfected into Hek293. Cells were treated with cycloheximide (10 μg/mL) for 24 hours, PD173074 (0.1 μM-10 μM) and FGFR4 siRNA for 72 hours.

Results: We found several breast carcinomas highly expressing FGFR4. MDA-MB-453 has a much higher FGFR4 protein expression despite comparable mRNA levels with HuH-7, a cell line with the highest FGFR4 expression among the NCI-60 panel. This high FGFR4 expression is likely due to increased protein stability contributed by Y367C, based on both Hek293 overexpression system and comparing MDA-MB-453 (367C) with HuH-7 (WT). FGFR4 in MDA-MB-453 is also constitutively active. In addition, FGFR4 is the major FGFR isoform in MDA-MB-453, making this cell line an appropriate system to utilise PD173074 (a potent FGFR family inhibitor), as a probe for FGFR4 function in cancer. FGFR4 inhibition with PD173074 and FGFR4 siRNA blocked MDA-MB-453 proliferation. Furthermore, the reduction on downstream MAPK signalling with FGFR4 siRNA indicates the mechanism of FGFR4's proliferative potential in breast cancer.

Conclusion: The novel FGFR4 Y367C variant may confer protein stability that contributes to MDA-MB-453 specific FGFR4 overexpression and highly invasive phenotype. The high expression of FGFR4 in breast carcinoma and anti-proliferative effect of PD173074 and FGFR4 siRNA provide compelling evidence to consider FGFR4 as a therapeutic target against breast cancer.

362 POSTER

STAT3 protein binding to supercoiled DNA

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Background: Signal transducer and activator of transcription 3 (STAT3) proteins modulate various physiological functions including cell-cycle regulation, apoptosis and cell survival by regulating gene expression. Upregulation of phospho-STAT3 activity or increases of unphosphorylated STAT3 levels have been observed in cancers. Both forms of STAT3 are able to regulate gene transcription and contribute to tumor progression. The phosphorylated STAT3 forms a dimer and binds to IFNγ-activated sequences (GAS) on DNA. It is not clear yet whether unphosphorylated STAT3 binds to its own DNA site or regulates gene expression through interaction with other transcription factors. Atomic Force Microscopy (AFM) allows visualization of structural relationships in the interaction between protein and DNA. In this study we used AFM to analyze binding of unphosphorylated STAT3 to DNA to better understand the mechanism of STAT3-dependent transcriptional regulation. In addition, we studied a truncated STAT3 isoform, generated by proteolytic processing at the C-terminus, to elucidate whether distinct transcriptional activities of the different STAT3 isoforms are due to differences in DNA binding.

Materials and Methods: Recombinant STAT3 protein was mixed with supercoiled GAS-containing plasmid and topoisomers of plasmid DNA (pUC8F14) in 50 mM Tris-HCl, 10 mM NaCl buffer with pH 8.0. Freshly cleaved mica was incubated in a mixture of a 1-(3-aminopropyl)silatrane (APS) solution for 30 min to prepare APS-mica. The protein-DNA complexes were deposited on APS-mica for 2 min, then washed with deionized water, and dried with nitrogen gas. The AFM images were obtained using the NanoScope IIIa instrument equipped with an E-scanner (Digital Instruments, Santa Barbara, CA) and analyzed by the accompanying software in the imaging module.

Results:

1. Binding to GAS full sized STAT3 dimer
2. Binding to hairpin full sized STAT3, and 67.5 kDa

We observed that the 67.5 kDa STAT3 fragment binds to the ends of the hairpin arms of the cruciform structure on DNA containing 4 unpaired nucleotides. Full length STAT3 proteins bind to the four-way junction region in the cruciform structure. Statistical analyses of the volume distributions of STAT3 molecules in DNA-STAT3 complexes are interpreted to show that the 67.5 kDa STAT3 fragments form predominantly monomers or dimers with cruciform structures. The STAT3 proteins form predominantly dimers or tetramers with the cruciform structures of the plasmid DNA.

Conclusion: Secondary DNA structures, such as cruciforms, can create new protein binding sites and potentially block the movement of the transcription-elongation complex. The affinities of the truncated forms and full length unphosphorylated forms of STAT3 to secondary structures of DNA provide additional insight into mechanisms that may underly STAT3 protein effect on gene regulation. Accordingly, inhibition of STAT3 DNA binding may offer a novel therapeutic target for gene regulation.