in "leaky" virus replication, we hypothesize that the incorporation of multiple miRTs could overcome this limitation and result in an even safer vector. Thus, this strategy has the potential to provide a basis for clinical application of VSV vectors for the treatment of primary and metastatic liver disease, as well as other cancers, in the future.

558 POSTER

Systematic functional analysis of microRNAs by transfection of 1129 miRNAs into prostate cancer cells

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The molecular mechanisms behind prostate cancer development, progression and therapy failure are incompletely understood, but the androgen receptor (AR) is almost invariably involved. Altered microRNA (miRNA) expression profiles have recently been demonstrated in a large number of cancer types, including prostate cancer. These short RNA molecules of ~21 bases regulate mRNA stability and translation by base-pairing mainly to the 3′-untranslated region (3′UTR) of their targets.
In this study, we analyzed the functional impact of miRNA gain-of-function on prostate cancer cell growth and survival as well as on the expression of AR protein. Seven prostate cell lines (LNCaP, LAPC-4, MDA-PCa-2b, 22Rv1, CWR-1R, RWPE-1, and EP156T) were screened with 1129 miRNA mimics (Ambion and Dharmacon), double-stranded oligonucleotides designed to mimic the function of endogenous mature miRNAs. Cell viability was measured with CellTiter-Glo® (Promega) whereas AR, cell proliferation (Ki67) and apoptosis (cPARP) were detected with specific antibodies using protein lysate microarrays as described in our recent publication on estrogen receptor targeting miRNAs (Leivonen *et al*., Oncogene 2009). When transfected to the prostate cancer cell lines, 68 of the miRNAs had anti-proliferative and 52 had pro-apoptotic functions. In addition, 52 miRNAs down-regulated the levels of the AR protein in all five AR-positive prostate cancer cell lines. Western blots were used to verify knock-down, and the direct functional role of the miRNAs was clarified by 3′UTR luciferase assays and by analyses of down-stream consequences of the knock-downs.
In summary, we have systematically defined those miRNAs that are critical for prostate cancer cell growth, survival and the expression of the androgen receptor. This integrated, multi-parametric profiling provides new systematic evidence on the role of miRNAs in prostate cancer as well as novel clues for therapeutic targeting by manipulating key pathways and cell survival pathways by miRNA gain-of-function.

559 POSTER

Novel approaches to enable molecular analysis of clinical samples with limited amount of tissue

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Molecular marker analysis of tumor biopsies is vital for understanding the molecular mechanisms underlying oncogenesis and for studying the molecular basis of response or resistance to targeted therapies. Molecular marker data gathered from such clinical trial samples offer great potential for predictive or prognostic diagnostics, and for identifying new targets or pathways to target for future drug development. Since archival tumor biopsies commonly exist as formalin-fixed and paraffin-embedded (FFPE), the ability to obtain high quality nucleic acids is highly compromised. In addition, the amount of tissue obtained from fine needle aspirates (FNA), core biopsies and certain tumor types such as lung can be extremely limited, further limiting the ability to evaluate biomarkers. In Oncology Biomarker Development Group at Genentech, we have focused our efforts over the last few years in optimizing approaches and methodologies for molecular analysis of such challenging samples, with the objective of (a) increasing the sensitivity of nucleic acid analysis from FFPE tissues, (b) minimizing the RNA/DNA input requirements for assays, and (c) maximizing the number of analytes assayed from a single tissue section, while ensuring robustness and quality of data obtained. We will discuss several technologies and methodologies, review strengths and weaknesses, and show validation data generated using clinical samples.

560 POSTER

In vivo pharmaceutical targets screening using lentiviral inducible-knockdown shRNA system

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Background: Small interfering RNAs (shRNAs) are able to suppress essentially any gene expression through the endogenous cellular process of RNA interference pathway and have been used in many studies to screen for therapeutic targets in various pathological conditions. In this project, we aimed to develop an efficient *in vivo* cancer target validation method using a lentiviral inducible-knockdown shRNA. To verify our approach, we used an essential cell-cycle protein polo-like kinase 1 (PLK1) as a proof of concept target.
Methods: An inducible shRNA construct targeting PLK1 or a control construct was transfected into colon carcinoma cell line SW620. PLK1 knock-down was quantified by real-time PCR. Inducible shRNA expression (marked by Turbo RFP) was monitored by fluorescence microscopy and flow cytometry. Xenografts were established by subcutaneous or orthotopic injection of PLK1 inducible-knockdown cells into nude mice. Therapeutic effect was assessed by calliper measurement of tumour size.
Results: Following doxycycline induction, PLK1 inducible-knockdown cells showed dose- and time-dependent PLK1 down-regulation, which was consistent with induction of shRNA expression. There was about 60% gene knock-down and 40% protein knock-down 72 hours post-induction. In the *in vivo* model, there was a decline in tumour growth rate in the PLK1 knockdown group compared with the control group. *Ex vivo* analysis showed significantly lower PLK1 gene and protein expression in the doxycycline-treated group compared with the control group.
Conclusion: The results support the anti-tumour effects of PLK1 down-regulation and confirm an efficient methodology for cancer target screening using a lentiviral inducible-knockdown shRNA system.

561 POSTER

miR-221 as potential therapeutic target for childhood hepatoblastoma

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Background: We aimed to elucidate the expression level of miR-221 in a large contemporary cohort of hepatoblastomas and to assess its potential as a therapeutic target. MiR-221 has been shown to be upregulated in other cancers but not in hepatoblastomas, which are rare hepatic malignancies of childhood with limited therapeutic options that may derive from hepatic progenitor cells. Overexpression of miR-221 in a mouse model of liver cancer stimulated growth of tumorigenic murine hepatic progenitor cells. MiR-221 targets the CDK inhibitor p27 and enhances cells growth in vitro.
Material and Methods: miR-221 expression was analyzed by real-time quantitative PCR in 12 human formalin-fixed paraffin-embedded hepatoblastoma resection or explantation specimens and matched non-tumorous liver samples of the same patients from the archives of the Department of Pathology at the University of North Carolina at Chapel Hill. Patient characteristics were as follows: Gender: 5 females and 7 males; mean age: 33 months (range 3–144 months). In two patients, subsequent lung metastases were also analyzed and compared with the primary tumor. Assays were performed in triplicate using Taqman MiRNA methodology (Applied Biosystems). RNU66 was used for normalization and the fold-expression changes of miR-221 were calculated by the $2^{-\Delta\Delta Ct}$ method.
Results: MiR-221 is markedly upregulated in hepatoblastomas compared to matched non-tumorous liver parenchyma. Hepatoblastomas that metastasize to the lungs have even higher miR-221 levels than those that do not metastasize. Suppression of miR-221 by antagomirs may be a novel therapeutic strategy in primary and metastatic childhood hepatoblastomas.
Conclusions: MiR-221 is an oncomir that stimulates the onset of tumors when compared with vector-infected cells, possibly through p27 and/or DDIT4 downregulation. In this study of hepatoblastomas, miR-221 is upregulated in tumor samples and promotes tumor progression and metastasis. The use of synthetic inhibitors such as antagomirs of miR-221 may prove to be a promising novel approach to treatment of hepatoblastomas.