rAAV2 virus genome carrying the transgene to deliver. In comparison with mammalian viral vectors, the production of AAV/Phage is quicker, simpler and more economical. In addition, the vector is stable at 4°C for many years. The AAV/Phage vector efficiently targeted, delivered, and expressed transgene in cancer cells *in vitro*. We also proved that the vector selectively targeted gene delivery to tumors after intravenous injection in animal models of cancer. Moreover, in brain tumor models, the vector can penetrate through blood brain barrier and selectively delivers transgene expression to brain tumors. We used our vector as combination therapies with some well-known cancer drugs, such as doxorubicin, and temozolomide. Combination treatment of AAV/Phage vector carrying the *Herpes Simplex virus thymidine kinase* gene (AAV/Phage-*HSVtk*) with doxorubicin increased the targeted cancer cell killing in 2D tissue cultures and 3D tumor spheroids of rat gliosarcoma (9L) and human melanoma (M21) cells. We found that this increase in tumor cell killing was associated with a synergistic effect of doxorubicin on enhancing gene expression by AAV/Phage. We then combined AAV/Phage carrying short hairpin RNA to suppress *mTOR* gene expression (AAV/Phage-*shmTOR*) with temozolomide to treat medulloblastoma cells (DAOY). The results exhibited that treatment of medulloblastma with the vector alone efficiently suppresses the expression of *mTOR* gene, but has no effect on cell killing. Treatment of temozolomide at low dose (500 uM) did not have effect on cell killing, but combination therapies of temozolomide with AAV/Phage-*shmTOR* significantly increased cell death. Altogether, our results demonstrate that combination of AAV/Phage carrying therapeutic genes with cancer chemotherapeutic drugs is an effective strategy for cancer treatment. In future work, we plan to investigate the efficacy of AAV/Phage and cancer drug combination treatment in pre-clinical models of cancer.

673. Therapeutic Efficacy of Retroviral Replicating Vector (RRV) -Mediated Prodrug Activator Gene Therapy for Pancreatic Cancer

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Pancreatic ductal adenocarcinoma is one of the most lethal cancers, thus new therapeutic strategies for this disease are urgently needed. Retroviral replicating vectors (RRV)-mediated prodrug activator gene therapy with Toca 511 (*vocimagene amiretrorepvec*), an optimized RRV encoding yeast cytosine deaminase (yCD) which converts the prodrug 5-fluorocytosine (5-FC) to the anticancer drug 5-fluorouracil (5-FU), is showing promising clinical activity in patients with recurrent high grade glioma, and is now being evaluated in a multi-center Phase II/III clinical trial. In the present study, we evaluated the therapeutic efficacy of RRV-mediated prodrug activator gene therapy in preclinical models of pancreatic cancer. We first examined the replication kinetics of RRV expressing the GFP reporter gene (RRV-GFP) in murine (Pan02) and human (MIAPaCa-2, BxPC-3, PANC-1 and SUIT-2) pancreatic cancer cell lines by flow-cytometric analysis and quantitative PCR. In all of these pancreatic cancer lines, RRV-GFP inoculated at MOI=0.05 (~5% initial transduction levels) showed rapid viral replication subsequently resulting in high levels of transduction, with the majority of pancreatic cancer lines reaching >90% GFP-positive cells over time. Next, we tested *in vitro* cytotoxicity by MTS assay after prodrug treatment of pancreatic cancer cells (Pan02 and MIAPaCa-2) transduced with Toca 511. In RRV-transduced pancreatic cancer cells, significant (~90%) cytotoxicity was induced by exposure to 0.1-1.0 mM 5-FC prodrug for 4 days, compared to untransduced and RRV-GFP transduced controls. We then evaluated *in vivo* therapeutic efficacy of Toca 511/5-FC prodrug activator gene therapy in Pan02 pancreatic tumor models established subcutaneously in immunocompetent syngeneic C57BL/6 mice. While 5-FC treatment alone showed no obvious inhibition of tumor growth as well as the nontreated control group, the majority (n=7/8) of Toca 511-transduced tumors showed complete regression after 5-FC treatment. Notably, systemic biodistribution studies showed no detectable RRV signals in genomic DNA from normal tissues of treated mice. In orthotopic models using luciferase-marked MIAPaCa-2 tumors established in the pancreas in nude mice, significant inhibition of bioluminescent signals was observed by optical imaging after 5-FC administration in mice with Toca 511-infected tumors, as compared to untreated control tumors. Thus, RRVs are highly efficient vehicles for delivering prodrug activator genes such as yCD to pancreatic cancer cells, thereby achieving significant cell killing upon pro-drug administration. Further preclinical studies are ongoing toward translating this novel therapeutic strategy into clinical trials for patients with pancreatic cancer.

674. Insertional Mutagenesis to Identify Mechanisms of Cetuximab Resistance in Colorectal Cancer

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Anti-cancer drugs designed to target specific molecular pathways have shown an excellent therapeutic potential but also very poor long-term durability of tumor responses, mainly due to the outbreak of resistant clones among the residual neoplastic cell population. For that reason, understanding the molecular mechanisms underlying the onset of anti-cancer drug resistance (ACDR) is one of the major goals of clinical research. ACDR has been widely studied by DNA/RNA sequencing of primary human samples and several culprits identified. We have previously developed an approach based on lentiviral vector (LV)-induced insertional mutagenesis that allowed to identify the genes involved in lapatinib and erlotinib resistance on HER2+ human breast cancer cell lines and EGFR+ pancreatic cell line respectively. Here we took advantage of this platform to investigate ACDR genes in colorectal cancer (CRC). Cetuximab, anti-EGFR monoclonal antibody, is used as first line therapy in metastatic CRC, which results in prolonged survival of treated patients. However, nearly all patients relapse due to ACDR. We thus selected CRC cells sensitive to cetuximab deriving either from five microsatellite stable cell lines or from eight Patient Derived Xenografts (PDX), primary human CRC cells implanted subcutaneously into immunodeficient mice (NSG). To induce insertional mutagenesis we generated a luciferase-expressing LV harboring the SFFV enhancer/promoter in the long terminal repeats able to perturb the expression of genes nearby the integration site. As control, we used a non-genotoxic SIN-LV. We set up a collagenase IV-based disaggregation protocol that allows single-cell suspension and a serum-free culture condition to maintain the stemness of *in vitro* cultured cells. This protocol allowed to efficiently disaggregate and expand CRC cells *in vitro* as well as reach a LV copy number per cell ranging from 0.25 to 5.6. Luciferase gene expression was stable and allowed live-animal monitoring for up to 30 weeks after transplant. CRC-0069 and -0077 PDXs and NCI-H508 and HDC82 cell lines were transduced ex vivo and kept in vitro and/or

transplanted in NSG mice. After in vitro or in vivo expansion of the transduced CRCs cetuximab treatment was applied. After an initial shrinking of the tumor mass in mice we observed ACDR in 3 out of 10 mice transplanted with NCI-H508 cells transduced with SFFV-LV and in none of the controls. Genomic DNA from resistant cells is being used for insertion site (IS) analysis to identify common IS, ACDR gene candidates. IS obtained from SIN-LV groups will be used to filter LV integration biases, whereas IS from SFFV-LV transduced cells but not treated with cetuximab will be used to filter mutations that provide a proliferative advantage unrelated to cetuximab treatment. We will validate the most promising candidates by LV-mediated overexpression and knockdown techniques. This approach could pave the way to perform insertional mutagenesis-based forward genetics studies on primary human samples.

675. Exploring the Effects of Stroma-Derived PPARB Signaling in Colorectal Tumorigenesis

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Peroxisome Proliferator Activated Receptors (PPARs) are members of the superfamily of nuclear receptor transcription factor. While the roles of isotypes PPARα and PPARγ in colorectal cancer have been well studied, the role of PPARβ/δ still remains controversial. The reason for the discrepancies remains unclear and several explanations have been put forth. One explanation for the contrary view is the heterogeneous tumor microenvironment. A tumor is made up of the cancerous epithelia, as well as stromal elements such as cancer-associated fibroblasts (CAFs). The role of PPARβ/δ in CAFs is not well understood, and concomitantly on tumor progression is not easily determined. We first examined mRNA expression of PPARβ/δ in murine intestinal tumor stroma. Laser-capture microdissected tumor stroma showed a statistical significant change in stromal PPARB expression upon tumor formation. Mouse carrying a stromal-specific deletion of PPARβ/δ showed that these mutant mice demonstrated increased cancer survivability, with reduced tumor size and numbers compared to their wild-type littermates, confirming the importance of stromal-derived PPARβ/δ in colorectal tumorigenesis. Preliminary observations also suggest that stromal PPARβ/δ may be a potential target for adjunctive anti-tumor treatment.

676. An Engineered CAR T Cell Platform for Allogeneic Combination Immunotherapy

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The adoptive transfer of CAR T cell represents a highly promising strategy to fight against multiple cancers. The clinical outcome of such therapies is intimately linked to the ability of effector cells to engraft, proliferate and specifically kill tumor cells within patients. When allogeneic CAR T cell infusion is considered, host versus graft and graft versus host reactions must be avoided to prevent rejection of adoptively transferred cells, host tissue damages and to elicit significant antitumoral outcome. This work proposes to address these three requirements through the development of multidrug resistant TCR-deficient CAR T cells. We demonstrate *in vitro* and in an *in vivo* xenograft mice model, that these engineered T cells displayed efficient antitumor activity and proliferated in the presence of single or muliple nucleotide analogues, currently used in clinic as preconditioning lymphodepleting regimens and antineoplastic agents. The absence of TCR at their cell surface along with their nucleotide analogues-resistance properties could prevent their alloreactivity and enable them to resist to lymphodepleting regimens that may be required to avoid their ablation via HvG reaction. By providing a basic frame work to develop a universal T cell compatible with allogeneic adoptive transfer, this work is laying the foundation stone of the large scale utilization of CAR T cell immunotherapies.

677. Evaluation of Tumor Specific Promoters for Use in Conditionally Replicating Adenovirus Mediated Virotherapy of Canine Lymphoma

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Conditionally Replicating Adenoviruses (CRAds) are genetically modified therapeutic viruses that incorporates transcriptional targeting of replication to promote selective killing of tumor cells. Transcriptional targeting utilizes tissue/tumor-specific promoters driving the expression of genes in a tissue- or tumor-specific manner to allow replication of the virus in tumor cells while sparing normal cells. Selection of appropriate intermediate animal models is a basic-requirement for successful cancer virotherapy. The dog is an outstanding animal model of cancer and other complex human diseases. The outbred nature of the dog, the heterogeneity of their tumors, their genomic similarity to humans and similar disease causation, progression and pathology support the use of this animal model. Previous studies have shown high levels of expression of several promoters, including human telomerase reverse transcriptase (hTERT), survivin, chemokine receptor 4 (CXCR4) and progression elevated gene 3 (PEG3) in a variety of human cancers and murine models. The exogenous promoter PEG3 from rats has not only shown tumor-specificity in the human model, but has also shown pan-tumor properties with active transcription occurring in almost all tumor cells. None of these promoters have been tested for their potential as a transcriptional targeting tool for canine cancers. Non-Hodgkin lymphoma accounts for 83% of all hematopoietic cancer and 6 % of all malignancies in the dog. Resistance to current treatments has emerged as a critical challenge for lymphoma treatment due to the presence of genetic diversity among tumor cells. These studies explore tumor-specific activity of these promoters with the goal of identifying a suitable canine lymphoma specific promoter to generate transcriptionally targeted CRAds facilitating viral replication in canine lymphoma, but not in normal cells. In this regard, a GFP reporter gene driven by the rat PEG3 promoter was evaluated for activity after transfection into canine lymphoma cells as well as normal canine cells. The activity of the endogenous canine promoters CXCR4, cTERT, and cSurvivin were examined using quantitative reverse transcriptase PCR. Results showed negligible expression differences between normal and lymphoma cells for cTERT and PEG3 whereas cSurvivin and cCXCR4 showed markedly higher expression in tumor cells when compared with most normal cells and tissues. However, cCXCR4 also showed a high level of expression in normal peripheral blood mononuclear cells (PBMC) cells. In contrast, cSurvivin showed increased expression in canine lymphoma cells, along with other canine tumors, with reduced expression in normal canine cells/tissues and canine PBMCs. These findings will be used to generate a canine lymphoma specific CRAd.