554 POSTER

Histone deacetylase inhibitors mediate pharmacological rescue and increase membrane expression of ABCG2 harboring the Q141K single nucleotide polymorphism

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ABCG2 is an ATP-binding cassette half-transporter that has garnered interest in pharmacogenomics as a modulator of oral drug absorption, drug excretion, and drug distribution through expression at the blood–brain barrier and the maternal–fetal barrier as well. A single nucleotide polymorphism (SNP) C421A in ABCG2, resulting in a glutamic acid to lysine mutation at amino acid 141 (Q141K), confers impaired protein expression and function. Pharmacogenomic studies have linked the SNP to increased exposure to substrate drugs including irinotecan, topotecan, sunitinib and gefitinib. We have studied the biology underlying altered expression and function. A large fraction of the Q141K variant is degraded before it reaches the cell surface, and variant at the cell surface has reduced transporter function. Q141K-ABCG2 is resistant to degradation by Endo H and levels are increased by the lysosome inhibitor, bafilomycin, suggesting that cell surface protein, although at reduced levels, is fully processed and folded. Proteosome inhibitors do not increase levels, arguing against retention and loss through ERAD (endoplasmic reticulum associated degradation). Pharmacological chaperones were used to evaluate impact on trafficking. Mitoxantrone (MX), a substrate previously noted to facilitate processing of ABCG2 mutants showed a small increase in Q141K-ABCG2 expression at the cell surface when imaged by confocal microscopy. Incubation with 50 μM of the dietary compounds dibenzoylmethane (DBM) and tert-butyl hydroquinone (TBHQ) increased expression of ABCG2 variant but did not improve localization to the cell surface. Romidepsin and other histone deacetylase inhibitors (HDIs), previously shown to increase levels of ABCG2 mRNA, were examined for impact on trafficking. A significant increase in total protein, surface expression, and function was seen in Q141K-ABCG2 variant when exposed to the various HDIs. Immunofluorescence analysis by confocal microscopy suggested Q141K-ABCG2 localization in the Golgi, with a dramatic shift to the plasma membrane following exposure to romidepsin and other HDIs. Results suggest that the Q141K-ABCG2 variant is trapped in the Golgi and that HDIs aid localization to the surface. Investigations into the mechanism underlying this HDI-mediated pharmacologic rescue are underway.

RNA and DNA based technologies

555 POSTER

Advancement of ALN-VSP, an experimental RNAi therapeutic for solid tumors

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Malignancies of the liver, including primary (hepatocellular carcinoma) and secondary (metastatic) tumors, represent a significant unmet medical need. We are developing the RNAi therapeutic, ALN-VSP, for solid tumors with significant liver involvement, and evaluation is currently ongoing in a Phase 1 clinical trial. ALN-VSP is comprised of lipid nanoparticle (LNP)-formulated small interfering RNAs (siRNAs) targeting VEGF and the mitotic kinesin, KSP (Eg5). Here, we report the efficacy of each siRNA component of ALN-VSP in orthotopic liver tumor models, as well as models of extra-hepatic tumors. To generate orthotopic liver tumors, human hepatoma or colorectal carcinoma cells were implanted directly into the livers of immunocompromised mice. These cell lines were also used to establish tumors at extra-hepatic sites including the lymph nodes and peritoneal cavity.
Histological analyses of tumors from mice treated with ALN-VSP demonstrated that each siRNA makes a distinct contribution to efficacy. ALN-VSP treatment leads to accumulation of aberrant mitotic figures (monoasters), a hallmark of KSP inhibition, in orthotopic liver tumors, as well as in extra-hepatic tumors of different origin. Evidence of therapeutic VEGF inhibition was shown by marked reductions in tumor microvessel density and intratumoral hemorrhage in orthotopic tumors. Similar results were obtained with a LNP formulation of the VEGF siRNA alone. Finally, we show that multi-dose administration of ALN-VSP significantly prolongs survival of mice harboring advanced orthotopic liver tumors. These studies show the contribution of both siRNAs in ALN-VSP to efficacy in tumor models, and demonstrate the therapeutic potential of ALN-VSP for the treatment of hepatic and extra-hepatic solid tumors.

556 POSTER

Therapeutic siRNA delivery against PKN3 improves the antineoplastic efficacy of gemcitabine in an orthotopic pancreatic cancer model

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Background: Since therapy of human pancreatic cancer remains a great challenge, the development of novel molecular targeted therapies is required that may improve the antineoplastic potential of chemotherapeutic agents. Particularly antiangiogenic approaches are of continued interest in this context. Recently, siRNA-lipoplex directed against protein kinase N3 (PKN3) has proven antiangiogenic properties in experimental tumor models. For this study we hypothesized that therapeutic application of PKN3-siRNA-lipoplexes may improve the antineoplastic efficacy of gemcitabine in an orthotopic pancreatic cancer model.
Materials/Methods: An orthotopic tumor model was employed using the human pancreatic cancer cell line L3.6pl. Animals (Balb/c nu/nu) received either PKN3-siRNA-lipoplexes (3x/week; 2.8 mg/kg siRNA) (ATU027; Silence Therapeutics, Berlin), or gemcitabine (50 mg/kg, 2x/week), or a combination of both per tail vein injections. Control animals received carrier (sucrose). Treatment started on day 11 post tumor cell implantation and the experiment was terminated on day 33.
Results: Single agent therapy with either gemcitabine, or PKN3-siRNA-lipoplexes both significantly reduced orthotopic tumor growth (tumor weight) ($p < 0.05$). Importantly, combination therapy further reduced pancreatic tumor growth ($p < 0.001$), and substantially diminished the occurrence of either lymph node or liver metastases. In vivo PKN3 knock-down was proven in lung tissues from mice receiving combinational therapy. Signaling analyses of tumor tissues and staining analyses are pending.
Conclusion: Therapeutic PKN3-siRNA delivery reduces growth and metastasis of human pancreatic cancer and improves the antineoplastic efficacy of gemcitabine. Thus, PKN3-Lipoplex-siRNA may be an effective approach for treating pancreatic cancer.

557 POSTER

Generation of microRNA-sensitive VSV vectors to reduce neurotoxic side effects

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Vesicular stomatitis virus (VSV) is an oncolytic virus which shows promise as a therapeutic agent for cancer, however, toxic collateral effects on the brain and the liver occur when it is administered at high doses. We hypothesized that by employing a novel strategy, termed microRNA-engineering, we could eliminate off-target replication and improve the therapeutic index. This approach involves the incorporation of target sequences of a specific miRNA (miRTs) into the viral genome, resulting in degradation of viral gene transcripts in cells in which this miRNA is highly expressed, without interfering with virus replication in cells not expressing the relevant miRNA.
We screened a panel of candidate miRNAs to select for those with high expression levels in the brain and/or liver and low levels in primary human HCC and CRC and cell lines. We then confirmed the miRNA-mediated regulation of the candidates in an *in vitro* reporter gene assay. Four tandem repeats of perfect complementary miRTs were cloned into the 3′-UTR of a luciferase reporter gene, and these plasmids were transfected into a panel of normal and tumor cells. We then selected only those miRTs which resulted in a reduction of luciferase expression in primary hepatocytes and neurons but not in tumor cells. As a final control, we tested the candidate miRNA expression levels in the presence vs. absence of VSV infection. Based on these preliminary experiments, we chose three miRTs which best fit our selection criteria, and created recombinant VSV vectors containing four tandem repeats of the miRTs in the 3′-UTRs of crucial endogenous viral genes. After characterization of these vectors in tumor and non-tumor cells *in vitro*, thorough toxicity and efficacy studies will be performed in mouse models of HCC and CRC. We ultimately aim to engineer next-generation viruses with one or more viral gene regulated by multiple miRTs. Because copy number of endogenous miRNAs is critical in restricting viral replication and could easily become saturated in the target tissues resulting