

RESULTS: In all mice, GFP expression was present from birth and confined to hepatocytes. At 12 weeks, liver copper was decreased and histology was preserved in injected knockout mice compared with controls. Immunohistochemistry and Western blot confirmed presence of ATP7B in the hepatocytes of injected knockout mice. Western blot confirmed presence of copper-bound ceruloplasmin in serum.

CONCLUSIONS: Efficient gene transfer to hepatocytes can be achieved with early gestational intravascular injection of lentiviral vector. With this approach, we observed phenotypic improvement in a mouse model of Wilson disease and demonstrated proof in principle for in utero gene therapy.

Predictive markers of KIT inhibition in gastrointestinal stromal tumors (GIST)

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INTRODUCTION: GISTs are GI mesenchymal tumors characterized by gain-of-function mutations in KIT and PDGF-alpha receptors. Imatinib and sunitinib are tyrosine kinase inhibitors currently approved for the treatment of GIST. We have previously identified 7 genes associated with imatinib response. We sought to validate those genes as reliable markers of KIT inhibition with sunitinib therapy.

METHODS: GIST882 and GIST-T1 cells were utilized to assess the inhibitory effects of sunitinib. A panel of imatinib-responsive genes and KIT downstream signaling were analyzed by RT-PCR and immunoblotting in both lines treated with sunitinib at various therapeutic concentrations.

RESULTS: Constitutive KIT activation along with activation of the downstream proteins AKT and ERK1/2 was effectively inhibited in both GIST882 and GIST-T1 cell lines following sunitinib treatment. Compared with imatinib, sunitinib had a greater inhibitory effect on GIST-T1 cell proliferation. Prolonged treatment with higher concentrations of sunitinib was necessary to achieve a similar degree of proliferation inhibition in GIST882 cells. A panel of 7 previously identified imatinib-responsive genes was analyzed in the context of sunitinib therapy. In both cell lines, expression of 4 genes was associated with sunitinib-induced proliferation arrest. Within 6 hours of sunitinib treatment, expression of Sprouty4A, Frizzled8, and PDE2A transcripts was downregulated, while MAFbx mRNA levels were increased.

CONCLUSIONS: We have identified 4 genes associated with KIT inhibition by imatinib and sunitinib. These markers can be reliably detected in GIST specimens shortly after treatment initiation. This method could provide a cost-effective alternative to PET scan in assessing the therapeutic efficacy of tyrosine kinase inhibitors in GIST.

Modulation of the effectors of radiation fibrosis: A targeted gene therapy approach

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INTRODUCTION: Radiation therapy frequently induces fibrotic processes in the skin, including accumulation of extracellular matrix (ECM). Smad3 plays an essential role in ECM gene expression, and Smad3 knockout mice are highly resistant to radiation skin fibrosis. Our study characterized the expression of genes involved during early radiation-induced fibrosis. Additionally, we performed Smad3 gene silencing in an attempt to abrogate the effects of radiation.

METHODS: C57 wild-type murine dermal fibroblasts were irradiated with 20Gy, RNA isolated (0, 6, 12, 24, 48, and 72 hours post-irradiation), and mRNA analyzed (RT-PCR) for known mediators of fibrosis: Smad3, IL-13, TNF-alpha, COL1A1, TGF-beta, MMP-1, MMP-2, and TIMP-1. SMAD3 gene silencing was performed with siRNA in an effort to restore an unirradiated gene profile.

RESULTS: Following irradiation, there was a steady increase in mRNA expression of Smad3, IL-13, TGF-beta, Col1A1, MMP-2, TIMP-1, and, with peak expression at 12 to 24 hours and subsequent decline by 72 hours. TNF-alpha expression remained elevated throughout. Inhibition of Smad3 significantly decreased expression of Col1A1, TGF-beta, MMP-2, and TIMP-1. IL-13 and TNF-alpha expression was not affected by Smad3 silencing.

CONCLUSIONS: We have characterized the early-phase expression profiles of the major mediators of radiation-induced fibrosis. Furthermore, SMAD3 siRNA is effective at abrogating the elevation of Col1A1, TGF-beta, TIMP-1, and MMP-2, with preirradiation treatment most effective. As expected, IL-13 and TNF-alpha are unaffected by SMAD3 silencing but appear to be minor regulators of the mediators of fibrosis, in contrast to SMAD3. These findings suggest a therapeutic rationale for SMAD3 silencing in vivo.

Smad3 silencing prevents dermal fibroblast-mediated collagen gel contraction

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INTRODUCTION: Smad3 is a key signaling intermediate in radiation-induced fibrosis. Smad3 knockout mice have been shown to be resistant to radiation-induced cutaneous injury. Our objective was to determine if Smad3 suppression using small interfering RNA (siRNA) prior to high-dose external beam radiation injury affects murine dermal fibroblast contraction of collagen gel matrices.