Next, we used HUVEC cells as endothelial cell model to evaluate the transfection efficiency of DNA encapsulated NPs. The transfected cells were analyzed by flow cytometry after transfection using NPs carrying p2X-GFP plasmid. GFP expression was detected in ~26% of the cells, indicating NPs/DNA can successfully transfect HUVEC cells. Next, we evaluated the efficacy of different sgRNAs for *in vivo* gene editing of mutant FVIII gene by hydrodynamic injection of Cas9/sgRNA plasmid into HemA mice. An immunodeficient hemophilia A (NSG HA) mice that contained premature stop codon in exon 1 of FVIII were used as the HemA mouse model. Two different sgRNAs that can edit wild type FVIII sequence (mF8sgRNA) or mutant FVIII exon 1 sequence (NSGHAsgRNA) were designed, respectively. Efficiency of gene editing was estimated *in vitro* by T7E1 assay using mouse embryonic fibroblast NIH3T3 cells. The results showed that mF8sgRNA can specifically induce double-strand breaks in wild type FVIII gene of NIH3T3 cells. The Cas9/sgRNA plasmids were hydrodynamically injected to NSG HA mice and the FVIII expression was examined by aPTT assay after one week. Both FVIII-targeting sgRNAs can promote the recovery of FVIII expression in NSG HA mice, suggesting the successful gene editing in mutant FVIII. After injection of Cas9/NSGHA sgRNA expression plasmid, FVIII activities maintained at least one month after treatment. Our data showed high transfection efficiency of DNA encapsulated NPs in HUVEC cells. Furthermore, we investigated *in vivo* gene editing using CRISPR/Cas9 technology to correct the mutated FVIII gene and regain the expression of FVIII protein in NSG HA mice. Our future goal is to use NPs that carry Cas9/sgRNA plasmid to correct the mutant FVIII gene in NSG HA mice.

Targeted Gene and Cell Therapy for Cancer

113. Gene-Based Immune Reprogramming Overcomes the Immunosuppressive Microenvironment of Liver Metastases and Enables Protective T Cell Responses

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The liver hosts an immune suppressive environment, which favors metastatic seeding and proliferation of cancer cells. Current pharmacological treatments, including most recent immunotherapies, fail in the presence of liver metastases (LMS). Therefore, identifying new interventional tools to break tumor tolerance and unleash immune responses in patients with LMS is of pivotal importance. Our group has shown that vesicular stomatitis virus G protein-pseudotyped lentiviral vectors (LVs) delivered systemically to mice and non-human primates efficiently target and transduce liver cells, including liver resident macrophages termed Kupffer cells (KCs). Building on these findings, we developed a novel LV-based platform, termed KC-LV, to selectively engineer KCs in vivo with the goal of delivering therapeutic molecules specifically to LMS. To this aim, the KC-LV design exploits a reconstituted mannose receptor c type 1 (MRC1) promoter, active in macrophages, including KCs. To further fine-tune KC specificity, the KC-LV also includes microRNA target (miRT) sequences that prohibit LV transgene expression in liver sinusoidal endothelial cells and hepatocytes. We observed that systemic delivery to mice of a KC-LV driving the fluorescent marker GFP resulted in transgene product expression in KCs, but not in other liver cell types or organs, such as brain, gut, lung, lymph nodes and bone marrow. In mice bearing LMS, GFP expression was enriched in the tissue rim surrounding LMS. To leverage KC-LV as a therapeutic platform we included a sequence encoding for interferon a (IFNα), a cytokine with pleotropic immune function. Long term analysis of mice treated systemically with IFNα KC-LV showed a rapidly established, vector dose-dependent and sustained IFNα expression, with no signs of hepatotoxicity, neutropenia or macroscopic skin reactions. IFNα KC-LV systemically delivered to mice challenged with colorectal cancer LMS, either obtained from cancer cell lines or organoids, significantly delayed tumor growth and achieved, in some mice, a complete response. Furthermore, mice that completely cleared LMS were refractory to rechallenge with matched cancer cells indicating persisting adaptive immune protection. Single cell RNA sequencing analysis of LMS revealed upregulation of IFN-responsive genes and altered activation/polarization profile in tumor infiltrating host cells in mice treated with IFNα KC-LV indicating robust immune reprogramming of the LMS microenvironment. In particular, IFNα KC-LV promoted macrophage skewing from a protumoral (M2-like) to an antitumoral (M1-like) polarization state and expansion of LMS specific CD8 T cells. In summary, we have developed an innovative gene-based platform that upon a single well-tolerated intravenous LV infusion can rapidly establish a protective response against mouse LMS through promotion of macrophage reprograming and adaptive immune activation.

114. Inducible Tumor-Targeted Interferon-α Gene Therapy Inhibits Glioblastoma Multiforme in Mouse Model without Adverse Systemic Effects

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Background: The pleiotropic immunostimulatory effects of cytokines such as interferon alpha (IFN-α) have been extensively investigated in cancer. However, systemic administration leads to sharp oscillations in cytokine plasma levels and OFF-target toxic effects, which strongly