730. T Cell Responses to AAV Vector Capsid in Normal Donors and Subjects Who Have Undergone Liver-Directed AAV-Mediated Gene Transfer

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In a Phase I/II study of gene transfer for hemophilia B, an adeno-associated viral vector serotype 2 (AAV-2) was introduced into the liver of human subjects and therapeutic levels of transgene expression (up to 11% of normal) were reached. However, the duration of gene expression was limited; four weeks after infusion, levels of circulating factor IX (F.IX) began to decline, and returned to baseline by week 10. This phenomenon was accompanied by a mild, self-limited, increase in liver enzymes. After a similar phenomenon was observed in another patient, the clinical trial was halted. We hypothesized that T cells directed towards AAV capsid antigens harbored by transduced hepatocytes were activated and these mediated destruction of the transduced hepaotocytes, thereby causing loss of transgene expression and a transient transaminitis. We used IFNγ ELISpot analysis of peripheral blood mononuclear cells (PBMCs) from a subject in the clinical study, combined with bioinformatics tools for the prediction of MHC class I binders, to define an MHC Class I-restricted T cell epitope for an HLA type common in the general population (B*0702). We then designed pentamers in which five peptide-loaded MHC class I molecules are linked to a fluorochrome to detect and study AAV-specific B*0702-restricted CD8+ T cells. Indeed, in the HLA B*0702 subject who developed transaminitis after vector infusion, AAV-specific CD8+ T cells were detected by pentamer staining of PBMCs up to two years later. In contrast, we have not detected AAV-specific CD8+ T cells in the peripheral blood of normal donors, though after several rounds of ex vivo stimulation with AAV capsid or peptide epitopes, we have been able to enrich a population of AAV-specific T cells, suggesting that AAV-specific T cells exist in the T cell memory pool. Functional assays on expanded AAV-specific T cells have demonstrated that these T cells specifically produced IFNγ upon detection of AAV antigen and specifically lyse target cells presenting antigen in the context of the HLA B*0702. These data provide direct evidence that humans mount a cytotoxic immune response to AAV capsid proteins, and that a contracted pool of AAV-specific T cells can remain as memory T cells in normal donors. Thus, re-activation of an AAV-specific T cell response may account for the limited duration of transgene expression that we observed in our clinical study. Moreover, the use of alternate serotypes may not easily avoid this immune response, as T cells from subjects infused with AAV-2 showed functional responses to AAV-1 and AAV-8-derived peptides by both cytokine production and killing assays, and T cells from normal donors that were expanded with an AAV-2 derived epitope also cross-react with the homologous epitope from AAV-5 by pentamer staining. We conclude that the use of immunomodulatory therapy may be a better approach to achieving durable transgene expression in the setting of AAV-mediated gene therapy.

731. Hematopoietic Stem Cell Gene Transfer and Integration Site Analysis in Tumor-Prone Mice Uncovers Low Genotoxicity of Lentiviral Vector Integration

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Insertional mutagenesis represents a major hurdle to successful gene therapy and mandates for sensitive pre-clinical assays of genotoxicity. Cdkn2a$^{-/-}$ mice are defective for p53 and Rb pathways, and are susceptible to a broad range of cancer-triggering genetic lesions. We exploited the sensitivity of these tumor-prone mice to develop an *in-vivo* genotoxicity assay, based on transplantation of Cdkn2a$^{-/-}$ hematopoietic stem cells (HSC), treated or not with prototypical retroviral (RV) and lentiviral (LV) vectors. In our rationale if RV or LV treatment is genotoxic, then transplanted mice will show a significantly earlier tumor onset. The sensitivity of the model was shown by the ability to detect a vector dose-dependent acceleration in tumor onset in mice transplanted with RV-treated cells. Such acceleration, as in previous studies, is consequent to genetic lesions, produced by vector integration, that cooperate with the germ-line mutation, and is contingent on LTR activity.

We then compared the integration site selection in pre-transplant cells and tumors from the transplanted mice. RV showed a strong bias for genes previously described as common integration sites (CIS) in the Retroviral Tagged Cancer Gene Database, transcription factors and kinases. Moreover, RV insertions at CIS and cell cycle genes were further enriched in early-onset tumors, suggesting cooperation in tumorigenesis. Indeed, retrospective survival analysis of mice stratified according to the presence or absence of tumor integrations targeting each given gene class demonstrated that RV integrations close to CIS or cell cycle genes were associated with a significantly earlier tumor onset. These data showed that the RV integrations which were enriched in the tumors were non-neutral and likely triggered early tumor onset.

Remarkably, LV, tested in the same conditions, did not show any tumor acceleration, despite high integration loads and robust transgene expression in all hematopoietic lineages. LV targeted CIS much less frequently than RV in pre-transplant cells and tumors, and did not show selection for integrations at any specific gene class in tumors. We are now studying whether incorporating strong LTR-like promoters in LV results in a similar genotoxicity as the prototypical RV we tested. Similarly, we are comparing Self-INactivating RV and LV carrying cellular promoters. This assay provides a validated strategy to conduct such inquiries.

Our results provide the first direct evidence that prototypic LV have low oncogenic potential, and encourage their application to HSC gene therapy.