319. Comparison of Serum-Free Media for Optimal *Ex Vivo* Transduction of Human CD34+ Cells

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Current studies involving the clinical application of genetically modified hematopoietic stem cells (HSC) require an *ex vivo* culture period during which the cells must maintain their ability to self-renew, repopulate the bone marrow, and produce the genetically corrected progeny cells. Recent advances in the formulation of optimized serum-free HSC tissue culture media led us to compare several recently commercially available culture media with the medium used in our current clinical gene therapy protocols for the transduction of HSC. We compared expansion, transduction efficiency, and differentiation of both cord blood (CB) and bone marrow (BM) derived CD34+ cells using X-Vivo15 (BioWhittaker, supplemented with 1% HSA), Cellgro® SCGM (Cellgenix), StemSpan™ H300 (Stemline Technologies), Stemline™ I (Sigma-Aldrich), and Stemline™ II (Sigma-Aldrich). We observed comparable cell viability among the various media when using BM and CB derived CD34+ cells after 4 days of culture. Both BM and CB CD34+ cells cultured in X-Vivo 15, Cellgro® SCGM, and Stemline™ II expanded to a greater extent than cells cultured in StemSpan™ H3000 or Stemline™. Higher transduction efficiency using a retrovirus containing the eGFP gene was also observed in X-Vivo 15, Cellgro® SCGM, and Stemline™ II media. A direct comparison between BM and CB CD34+ cells cultured in X-Vivo 15 or Stemline™ II resulted in 23% (BM) and 27% (CB) more transduced cells in the Stemline™ II medium. We then examined the clonogenicity of the transduced BM CD34+ cells in a Colony Forming Unit (CFU) assay. BM CD34+ cells cultured in Stemline™ II medium displayed a two-fold higher (16%) plating efficiency in the CFU assay compared to BM CD34+ cells cultured in X-Vivo 15 (8-9.5%). The number of colonies that were transduced (eGFP+) was higher in the BM CD34+ cells cultured in Stemline™ II medium compared to those cultured in X-Vivo 15 (29.9% and 10.3% respectively). From these results it is evident that BM CD34+ cells cultured in Stemline™ II medium have both a higher percentage of transduced cells and a higher percentage of these transduced cells remain capable of producing colonies in a CFU assay when compared with X-Vivo 15. From these data, Stemline™ II appears to be a more suitable medium for the *ex vivo* culturing and transduction of HSC for use in clinical therapies.

GENE EXPRESSION I

320. Modeling Human Brain Cancer in Transgenic E2F1 Mice

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Mutations disabling the retinoblastoma (Rb) pathway are among the most common in human cancers, including brain cancer. These mutations promote tumor development through deregulated control of the E2F family of transcription factors. E2F1 belongs to a class of E2F's identified as transcriptional activators and involved in the G1/S phase transition of the cell. However, E2F-1 presents with a paradox as it is considered to have membership in two gene classes, functioning as both an oncogene and a tumor suppressor. This unusual trait generates a degree of uncertainty on the role that E2F1 plays in the development or maintenance of any given tumor. Here we show that E2F1 functions as an oncogene in brain tumors through the generation of mice engineered to overexpress E2F1 specifically within glial cells and neuronal progenitors as directed by the GFAP promoter. Mice carrying the transgene develop with high penetrance a phenotype characterized by neurological deficits including paresia, ataxia, head tilt and seizures. MRI imagining of tgE2F1 mice reveal a low incidence of mild hydrocephalus, and most notably, histological analysis demonstrates that 20% of tgE2F1 mice present with the spontaneous formation of malignant brain tumors. Overall these neoplasms show histological features from a wide range of aggressive brain cancers including medulloblastoma, choroid plexus carcinoma, primary neuroectodermic tumor and malignant gliomas. Mouse age as related to tumoral type forces a comparison to the human disease with juvenile mice presenting embryonal tumors typically identified in children, and older mice revealed gliomas similar to the clinical findings in adults. In this regard, this study suggests a global role for E2F1 in the formation and maintenance of multilineage brain tumors, irrefutably establishing E2F1 as an oncogne in the brain. Further characterization of the tgE2F1 mouse should propel the development of a preclinical brain tumor model for the testing of cell-cycle or apoptosis-targetd therapies against brain cancer.

321. Sea Urchin sns Chromatin Insulator Prevents Silencing and Positional Effect Variegation of Oncoretroviral Vectors Transgene Expression in Murine Erythroid Cell Line

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Silencing and position effect are considered significant obstacles to obtain a consistent level of transgene expression in viral gene therapy. Furthermore recent studies had shown that retroviruses tend to land on active genes with the potential consequence of insertional mutagenesis. The inclusion of elements, such as chromatin insulators, capable to insulate a gene from the surrounding chromatin effects at the integration site should improve both efficacy and safety of gene therapy vectors. We have previously characterized a 265 bp insulator element, termed sns, localized at the 3' end of the early histone H2A gene of the sea urchin Paracentrotus lividus. This sequence contains three cis-acting elements (Box A, Box B, and Box C+T) all needed for the enhancer blocking activity in both sea urchin and human cells. By colony assays, in human (K562) and mouse (Mel) erythroid cell lines, we have recently demonstrated that the sns insulator displays directional enhancer-blocking activity in that it interferes with the communication between the human beta-globin enhancer (LCR) and the gamma-globin promoter. By electrophoretic mobility shift assays (EMSA) we found bindings of sns insulator with the erythroid specific GATA1 and the ubiquitous Oct1, and Sp1 transcription factors.

Here we report that sns-5 sequence, a fragment that contain sns insulator, cloned in the 3' LTR of MSC virus-based vector, does not effect vector titer and stability of provirus. Sns-5 is capable to reduce the influence of the chromatin environment on the reporter (GFP) gene in mouse erythroid cell line (Mel) in that: (i) Mel transduced clones from limiting diluition have shown that the presence of sns5 increases the fraction of cells expressing the reporter gene (86% versus 26%); (ii) the expression of the reporter gene in insulated vectors is copy-number dependent; (iii) flow-cytometric analysis of GFP (+) sorted cells have shown a reduction of transgene extintion.

These results may have significant implications for the conservation of insulator function in evolutionary distant organisms and may prove to be of practical benefit in gene transfer applications for erythroid disorders such as hemoglobinopathies and thalassemias.

322. Syngeneic Cotton Rat Cancer Model for Replicating Adenoviral Vectors

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Background: Oncolytic adenoviruses are under study as a potential anticancer treatment. Their preclinical antitumor activity; however, has not been effectively translated to the clinic. A contributing factor may be the clinical relevance of immunodeficient mouse tumor xenograft models used to study these vectors. While these demonstrate viral replication in the xenograft, they are limited by the lack of natural host immune responses to the virus, associated toxicity, dissemination and systemic viral replication. Cotton rats (*Sigmodon hispidus*) are semi-permissive for the replication of human adenovirus and have been used to study these vectors. Until recently this model lacked a transplantable tumor to assess oncolytic activity. We characterized three syngeneic transplantable cotton rat tumor cells lines that support adenovirus replication and oncolysis in this immunocompetent rodent model.

Methods: Three tumor cell lines: CCRT, LCRT and VCRT were derived from a spontaneously arising osteosarcoma, and fibrosarcomas of the breast and jaw in animals from an inbred cotton rat colony. The ability of the cells to be infected with adenovirus was examined using an adenovirus expressing green fluorescent protein (Ad.GFP). Viral burst assays were used to determine titers of infective adenovirus produced by each cell line at 4 and 72 hours following infection with wild type adenovirus type-5 (Ad5). Transmission electron microscopy (TEM) was used to visualize the relative number of virions at each time point. We also evaluated replication and oncolysis of the cell lines *in vivo*.

Results: Using Ad.GFP, the cell lines were readily infected and demonstrated GFP expression comparable to human A549 cells. All cell lines showed significant higher ($P<0.05$) intracellular titers of adenovirus and in supernatants 72 hours after infection compared to 4 hours after infection with Ad5 indicating viral replication and increased number of functional virions. In addition, all the cell lines exhibited greater expression of adenoviral hexon protein at 72 hours than at 4 hours after infection, indicating late transcriptional unit gene activation. The cotton rat cell lines formed tumors in cotton rats when injected subcutaneously. On days 5 and 8, PCR demonstrated 2 to 3-log greater number of Ad5 genome copies in tumors injected with Ad5 compared to those injected with a non-replicating adenovirus (Ad.null). This was consistent for CCRT, LCRT and VCRT. Ad5 slowed tumor growth in cotton rats subcutaneously injected with CCRT and VCRT cells. Despite *in vitro* oncolysis, no anti-tumor effect was seen in the LCRT tumors with the virus rapidly cleared from the tumor.

Conclusions: CCRT, LCRT and VCRT, three novel transplantable cotton rat tumor-derived cell lines demonstrate replication of Ad5 both *in vitro* and *in vivo*. In addition, CCRT and VCRT demonstrated *in vivo* oncolysis. The varied antitumor effect in this model mirrors the results of human clinical trials highlighting the potential relevance of these cotton rat tumor models for assessing replication competent adenovirus vectors.

323. *In Vivo* Regulation of rAAV-Mediated GDNF Expression in the Rat Striatum

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A number of tetracycline-regulated systems constructed in rAAV vectors were evaluated to control *in vivo* expression of glial cell line-derived neurotrophic factor (GDNF) in the rat neostriatum. The first system involved two vectors: rAAV construct contained tetracycline (tet) -inducible promoter driving expression of GDNF (tet-GDNF), and the other was a bicistronic vector encoding the reverse transactivator and silencer for the tet-regulated system (rtTA/tTS). The second vector system incorporated all the elements required for the regulated expression of GDNF in one construct. This arrangement contained a mutated rtTA (rtTA2^s-M2), a bidirectional tet-induceable autoregulatory promoter controlling the expression of rtTA2^s-M2 and GDNF, in AAV2 inverted terminal repeats (TR2:M2-GDNF). The third construct system was identical to the later vector but contained AAV5 TR (TR5:M2-GDNF). As much as 5.4 fold induction of GDNF over un-induced levels could be achieved *in vivo*, using the dual vector system. However, measurable background expression was observed in the un-induced state. Un-induced GDNF expression could be reduced to near background levels by increasing the ratio of the reverse transactivator-repressor vector to the tet-GDNF. On the other hand the bidirectional constructs showed high expression of GDNF in the uninduced state, but the TR5 seemed to reduce the expression observed in the uninduced state with the TR2 bidirectional construct. We conclude that a dual vector system might be more efficient at regulating gene expression in the context of rAAV vectors. Based on these results we are developing improved versions of the two vector system to limit uninduced transgene expression and to increase the levels of induction in the "on" state.

Dr. Muzyczka is an inventor on patents related to recombinant Adeno-associated virus technology and owns equity in a gene therapy company that is commercializing AAV for gene therapy applications. Dr. Mandel and Dr. Burger are inventors on patents related to recombinant Adeno-associated virus technology.

324. Development of Bioluminescence Tomography for Three-Dimensional Real Time Imaging of Gene Expression

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Introduction. Non-invasive monitoring of gene expression in living animals is an active area of molecular imaging and has proven useful to identify real time gene transfer. Improvements in optical imaging techniques have lead to the mounting luciferase reporter systems. Standard 2D bioluminescence limits the interpretation of gene expression in vivo. Bioluminescence tomography (BLT), the 3D extension of bioluminescence imaging, offers a new tomographic method for molecular imaging. BLT captures the spatial distribution of luciferase light signal in three dimensions, which provides a powerful tool for studies such as cancer imaging, cell trafficking and protein function.

Methods. 22-gram, male BALB-C mice were used for in vivo experiments. Mice tail veins were injected with 1 ml and 2 ml of 10ug/ml luciferase DNA expression plasmid in PBS. Twenty-four hours after injections, animals were anesthetized and administered