units has also been established for this response; below this dose no responses were detected. The time course experiments showed these responses to be transient, with most mRNAs returning to basal levels by day 14 post-injection into the brain. To study the mechanisms of the adaptive immune response, animals previously injected with RAd in the brain, were systemically immunized against adenovirus. In immunized animals there was an important increase in T-cell markers CD3, CD4, and CD8, while there was no increase of the monocyte marker F4/80. Expression of IFN-regulated genes was also increased. A different set of chemokines was elevated in the case of the adaptive immune response when compared to those seen in the early innate responses. These studies indicate that acute innate inflammatory responses in mice are dose dependent, transient, and mediated by an increase in interferon signaling and chemokine induction. The systemic immune response demonstrates a longer time course, and is mediated by CD4 and CD8 T-cells, and accompanied by an increase in interferon signaling, mainly due to interferons a and b, and possibly a smaller contribution of interferon g, and TNF.

TARGETING CANCER THERAPIES

1020. Development and Evaluation of a Genetically-Modified Adenovirus Vector That Specifically Targets Tumors Via the Bloodstream Following Systemic Administration

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The development of genetically modified adenovirus vectors capable of targeting tumors following systemic administration is an important goal for the treatment of disseminated cancer. Achieving this goal requires that the native adenovirus coat protein/receptor interactions are removed and replaced with new tumor-selective ligand/receptor interactions. Towards this goal, we have created vectors that are ablated for native receptor binding and that additionally contain a pseudo-receptor binding ligand or a tumor-selective peptide motif inserted into the HI loop of fiber. *In vivo* experiments showed that ablating both CAR and penton base binding is critical to reduce non-targeted tissue transduction. In addition, pharmacokinetic studies indicated that these tropism modified vectors efficiently enter and persist in the bloodstream following intraperitoneal (i.p.) administration. Interestingly, this sustained circulation was only observed after i.p. administration and not after intravenous administration. Furthermore, the combination of capsid modification and extended blood circulation could achieve receptor-mediated transduction on pseudo-receptor expressing subcutaneous tumor.

It is reported that integrin αvβ6 are dramatically upregulated in a number of tumors including lung, colon, ovarian and oral cancers. We have developed an αvβ6 targeted vector, AdL**RTD, by incorporating an RTDLXXL peptide motif with native receptor binding ablation. *In vitro* evaluation of AdL**RTD including competition analysis showed that the vector specifically transduces αvβ6 integrin expressing cells, such as NCI-H441 and SCC-25 cells. Its specificity was confirmed by the transduction on β6 integrin transfected Meth-A cells. Intraperitoneal administration of AdL**RTD reproduced the indications obtained by native binding ablated vector (AdL**), i.e., significant reduction of non-targeted tissue transduction. In addition, significantly improved transduction on αvβ6 expressing tumors was observed following systemic administration of AdL**RTD. This increased tumor gene transfer was closely correlated with the presence of the targeting peptide and the amount of vector that persisted in the bloodstream at one day post-administration. These results suggest that intraperitoneal administration of the double-ablated vector backbone may achieve targeting to metastatic tumors located throughout the body by virtue of its enhanced bloodstream persistence.

GenVec and FUSO Supporting Research

1021. VP22 Mediates Tumor Vasculature Specific Targeting

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Prostate cancer (CaP) is the most frequently diagnosed cancer in men and the second leading cause of cancer related deaths in American men. In 2003, CaP was expected to account for 220,900 cancer diagnoses and 28,900 cancer deaths. Despite recent advances in the early detection and treatment of locally advanced prostate cancer, the prognosis for patients with the advanced form of prostate cancer is grave. The explosion of research in the genetic and molecular events paralleling the development of the lethal form of the disease has lead to the identification of multiple therapeutic targets. Targeting the molecular mechanisms underlying CaP development and metastasis offers a novel approach towards the development of effective therapeutic modalities. As part of our long term goal the major focus of the current study was to integrate real-time intravital imaging of prostate tumor dynamics along with retroviral and adenoviral gene transfer approach for maximizing the therapeutic efficacy and bystander effect of apoptosis inducers by exploiting VP22-mediated tumor vasculature specific targeting. We have used intravital imaging for the real-time monitoring of tumor progression, angiogenesis and adenoviral mediated gene transfer in athymic nude mouse models of prostate cancer. The human prostate cancer cell lines, LNCaP, C4-2 and PC-3 were transduced with an enhanced green fluorescent protein expressing, gibbon ape leukemia virus (GALV) envelope pseudotyped retroviral vector MFG-EGFP-GALV. Flow cytometry revealed that the retroviral transduction efficiency is cell line dependent and was in the order PC-3 (72.46%)>LNCaP (44.37%)>C4-2 (34.47%). A fluorescent prostate tumor model was developed by subcutaneous injection of PC-3 cells in six weeks old, uncastrated, male athymic nude mice. The tumor bearing mice were injected with Dextran-Texas Red dye and subjected to sequential intravital imaging on days 17 and 24 using a dual photon Bio-Rad MRC-1024 microscope. Higly fluorescent EGFP expressing tumor cells were observed along with neo-vascularization of the tumor. Infiltration of Dextran-Texas Red dye in between the EGFP expressing tumor cells was observed thereby indicating leaky tumor vasculature. We have used this technique for the real time intravital imaging of Herpes simplex virus tegument protein VP22 mediated intercellular trafficking dynamics and novel tumor vasculature specific targeting in androgen dependent and androgen independent prostate tumor models following intra-tumoral as well as systemic delivery. For the very first time, we would like to report VP22-mediated tumor vasculature specific targeting while sparing the normal vasculature associated with hepatic and muscular tissues. Our striking and novel findings suggest that hybrid VP22-apoptosis inducers might act as a double edged sword by maximizing apoptosis induction and the associated bystander effect on one hand and selectively targeting the tumor vasculature destruction thereby potentiating the anti-angiogenic effects.