Toward more effective gene delivery
Hannah H Chen, Ryan Cawood and Leonard W Seymour

Address: Oxford Institute for Cancer Medicine, University of Oxford, Old Road Campus, Oxford, OX3 7BN, UK.
Correspondence: Leonard W Seymour. Email: Len.Seymour@clinpharm.ox.ac.uk

Published: 18 January 2008

Genome Biology 2008, 9:301 (doi:10.1186/gb-2008-9-1-301)

A report on the symposium 'In vivo barriers to gene delivery', Cold Spring Harbor, USA, 26-29 November 2007.

Many different obstacles impede the in vivo efficiency of both viral and non-viral agents for gene delivery. A recent symposium at the Cold Spring Harbor Laboratory, New York, addressed the latest approaches to overcoming these barriers. Topics encompassed diverse technologies for preventing vector neutralization, enhancing the persistence of transgene expression and the use of modified measles coat proteins to allow receptor specific HIV-1 pseudotyping.

Mislocalization, sequestration and targeting

Successful gene delivery depends on selective and efficient localization to target tissues. However, many current approaches are frustrated both by mislocalization and by sequestration in non-target sites. Approaches presented at the meeting that aim to circumvent these barriers include alterations to viral coat proteins, the coating of viruses with polymers and the tissue-specific repression of viral replication by endogenous microRNAs.

Genetic modification of viruses can endow tumor-selective replication, controlled by specific components of the tumor phenotype. However, site-specific targeting of these ‘oncolytic’ viruses to tumor cells often depends on an increased density of the virus receptor on the cancer cell surface relative to other cells. This means that such viruses are selective for tumor cells rather than absolutely specific, and an important aim of work in this area is to increase selectivity. Stephen Russell (Mayo Foundation, Rochester, USA) described work using a measles virus that efficiently enters and fuses cells via the cell-surface receptor CD46. He showed that viral entry increases linearly with CD46 density but that cell fusion is dependent on a threshold level of the receptor. Moreover, by fusing measles virus H coat proteins to ligands for different cell surface receptors, including the epidermal growth factor receptor (EGFR) and the CD38 cell-surface protein, it was demonstrated that this phenomenon is not receptor specific. Russell showed that by fusing measles H protein with different single-chain antibodies to Her2, another member of the EGFR family, to increase the affinity of the virus for the receptor, fusion could occur at lower receptor densities. This finding may prove important for many viral retargeting strategies, in which the affinity of a retargeting ligand could be correlated with receptor density on the cancer cell surface in order to mediate selective intercellular fusion.

The use of pseudotyped viruses, expressing exogenous viral envelope proteins, permits modified tropism while retaining desirable viral properties. Sabrina Funke (Paul-Ehrlich Institute, Langen, Germany) showed that truncation of the cytoplasmic tails of measles virus H and F proteins was necessary to allow pseudotyping of a lentiviral vector (HIV-1). By inserting single chain variable fragments of a CD20 ligand in the ectodomain of modified measles virus H protein, both successful targeting and transduction of CD20 positive cells was confirmed by the modified HIV-1 vector. This CD20-retargeted HIV vector successfully transduced >70% of primary B cells in vitro, compared with an established CD20-retargeted HIV vector (based on the Vesicular Stomatitis virus glycoprotein) which gave only 18% transduction. These results show a promising step towards stable B cell-specific gene delivery.

Mislocalization and sequestration by non-target tissues are major hurdles in the systemic use of tumor-destroying viruses. The intravenous administration of adenovirus is currently hindered by significant loss (99% of injected dose) of viral particles to multiple non-target sites, including hepatocytes and Kupffer cells (hepatic macrophages). The consequence of such sequestration is a significant reduction in circulating infectious particles available for infection of tumor cells. One of us (LWS) described how cloaking
Adenovirus with reactive multivalent polymers based on poly[N-(2-hydroxypropyl)methacrylamide] decreased both infection of hepatocytes and virus phagocytosis by macrophages, increasing the percentage of injected dose in the circulation from 4% to 48% 30 minutes after an intravenous injection in mice. Moreover, the depletion of macrophages using clodronate liposomes together with polymer cloaking extended the circulating half-life of viral particles from 3 minutes to 120 minutes. This significant improvement in the amount of circulating adenovirus may increase tumor infection by allowing an extended period for passive and tumor-specific targeting.

Vector neutralization

Viral gene-delivery vectors, in particular those administered intravenously, face an array of challenges from the innate and adaptive immune systems, which rapidly diminish the availability of vectors for delivery to target sites. Robert Carlisle (Oxford University, UK) described how following addition of human adenovirus type 5 (Ad5) to fresh whole human blood, virus particles associated almost exclusively with the cell fraction. Further investigation revealed that binding to erythrocytes accounted for most (more than 90%) of this interaction and that heat inactivation of plasma abrogated this binding. Carlisle showed that complement protein C3 and the complement receptor 1 (CR1) on human erythrocytes are instrumental in this binding, revealing these as potentially important targets for the inhibition of Ad5-erythrocyte association.

These findings were complemented by a study presented by Zhili Xu (Food and Drug Administration, Bethesda, USA) elucidating the molecular mechanisms underlying the nonspecific uptake of adenovirus by murine liver Kupffer cells - a major pathway for clearing circulating virus particles. Administration of scavenger receptor antagonists, such as polyinosinic acid, was shown to decrease Kupffer cell capture. This study also showed the binding of Ad5 to complement proteins C3 and C4 and the likely involvement of this interaction for the recognition of Ad5 by Kupffer cells. Notably, the authors demonstrated that serum from naïve mice contained IgM capable of binding virus, and this raised the possibility that ‘natural’ antibodies might play a role in complement fixation and Kupffer cell clearance. This was reinforced by the observation that antibody-deficient RAG1-knockout mice showed decreased Kupffer cell capture.

The inflammatory effects triggered by the detection of viral antigens by the immune system tend to suppress viral infection, thus posing another substantial obstacle to the success of viral vectors. Michael Roberts (Wellstat Biologics Corporation, Gaithersburg, USA) has investigated the effects of different dosing regimes and infusion rates on humans in an attempt to desensitize the immune system to the introduced virus and thus reduce the release of pro-inflammatory cytokines. He found that slower rates of infusion (typically infused over four hours) enhanced tolerance to intravenously administered oncolytic Newcastle disease virus. These observations may inspire further endeavors aimed at managing the immunological challenges confronting systemic delivery of therapeutic viruses.

John Bell (Ottawa Health Research Institute, Montreal, Canada) described a mutant strain of oncolytic vesicular stomatitis virus (VSV) that has a preference for productive infection of tumor cells. This strain (VSVΔ1) has a mutation in a protein necessary for the intracellular transport of the host-cell mRNA for the antiviral protein interferon-β. Bell also described the screening of a variety of histone deacetylase inhibitors (HDACIs) as novel pharmacological agents to downregulate interferon production, and the finding that some synergized dramatically with the oncolytic VSV strain, increasing its growth in monolayers and tumor explants as well as in vivo in a highly tumor-specific manner. Down-regulation of interferon production by HDACIs is a potentially valuable approach to augment the oncolytic potency of viruses, in particular those with an attenuated ability to suppress host interferon responses.

Persistence of recombinant viral vectors

The durability of transgene expression from viral vectors is often limited by activation of immune components. Recombinant adeno-associated virus (AAV) vectors have received much attention recently as they are maintained episomally to achieve sustained therapeutic gene expression. An emerging problem preventing the persistence of AAV-introduced genes is a cytotoxic CD8 T-cell response that is elicited against AAV capsid antigens in humans. Federico Mingozzi (Children’s Hospital of Philadelphia, USA) reported the observation of T-cell activation following intramuscular injection of an AAV-1 vector carrying a lipoprotein lipase transgene, which was correlated with the loss of therapeutic efficacy. Such immune stimulation was not induced in response to transgene-derived antigens, but was generated specifically against the AAV-1 capsid. Furthermore, activated CD8 T cells were shown to cross-react with other AAV serotypes. Similar effects were reported by Katherine High (Children’s Hospital of Philadelphia, Pennsylvania, USA) in a clinical trial using AAV vectors for the genetic treatment of hemophilia, where patients developed treatment-limiting anti-AAV capsid responses that had not been observed in mouse studies. This capsid-directed immunity is clearly a setback for the clinical development of this vector.

In regard to responses to AAV in mice, however, Hildegund Ertl (Wistar Institute, Philadelphia, USA) described a study in mice in which a recombinant AAV vector expressing the
HIV-1 Gag protein was found to stimulate the generation of transgene product-specific CD8 T cells that were impaired in their ability to proliferate and in their cytotoxic function. This phenomenon, termed T-cell exhaustion, was shown to be a consequence of continual exposure to the antigen and was reversed if the CD8 T cells were removed from the presence of the antigen.

These different observations may reflect differences in the previous exposure of human patients and laboratory animals to AAV. Prior exposure to AAV in the environment could have resulted in a pool of memory CD8 T cells in the human patients, whereas naive animals would be expected to respond differently to the virus. Together, these findings suggest the importance of conducting studies in animals that have been previously exposed to such vectors.

Preventing premature termination of transgene expression is also important in cancer, particularly where treatment is intended to act over an extended period. Retroviruses cannot penetrate an intact nuclear membrane and so exclusively infect actively dividing cells; this property enables them to be used to selectively target proliferating tumor cells. Noriyuki Kasahara (University of California, Los Angeles, USA) described replication-competent retroviruses (RCR; based on murine leukemia virus) armed with genes promoting cell suicide (apoptosis). These exhibited high anti-tumor efficacy in vivo, in mice, which was partly attributable to persistent expression of the transgene from residual infected tumor cells, increasing the effectiveness of repeated administration of a prodrug. This strategy may offer an efficient means of controlling tumor regrowth and metastasis. Kasahara described how the technology is currently being augmented by tumor-specific transcriptional targeting and the development of RCR vectors with the capacity to suppress cellular innate immune responses. He also described an innovative approach using adenovirus-retrovirus hybrid vectors consisting of an adenovirus encoding all the components required for the production of a retrovirus upon target-cell infection. This allows desirable features such as high adenoviral transduction efficiency and stable retroviral integration into the genome to be combined.

Advancement in gene-delivery technologies has been largely hampered by difficulties in overcoming the multitude of physical, metabolic and immunological barriers that prevent potentially powerful exogenous genetic material from exerting its full therapeutic benefit. Deeper insights into and understanding of these in vivo obstacles will aid in the development of technologies for vector design and delivery, with the ultimate aim of efficient, safe and efficacious gene therapy.

Acknowledgements
We thank Robert Carlisle for critical review of this report. We are grateful to Cancer Research UK and The New Zealand Tertiary Education Commission for financial support.