Journal of Controlled Release xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review

Systemic tumor-specific gene delivery

Max Kullberg, Ryan McCarthy, Thomas J. Anchordoquy

Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Denver, 12850 Montview Boulevard, Aurora, 80045, USA

ARTICLE INFO

Article history:
Received 13 May 2013
Accepted 22 August 2013
Available online xxxx

Keywords:
Gene therapy
Systemic
Viral
Non-viral
Dendrimer
Delivery system

ABSTRACT

The objective of a systemically administered cancer gene therapy is to achieve gene expression that is isolated to the tumor tissue. Unfortunately, viral systems have strong affinity for the liver, and delivery from non-viral cationic systems often results in high expression in the lungs. Non-specific delivery to these organs must be overcome if tumors are to be aggressively treated with genes such as IL-12 which activates a tumor immune response, and TNF-alpha which can induce tumor cell apoptosis. Techniques which have led to specific expression in tumor tissue include receptor targeting through ligand conjugation, utilization of tumor specific promoters and viral mutation in order to take advantage of proteins overexpressed in tumor cells. This review analyzes these techniques applied to liposomal, PEI, dendrimer, stem cell and viral gene delivery systems in order to determine the techniques that are most effective in achieving tumor specific gene expression after systemic administration.

Contents

1. Introduction

The vast majority of reported viral and non-viral gene delivery systems do not preferentially target tumor tissue. Viral systems including vaccinia virus, adenovirus, and lentivirus deliver their payload primarily to the liver, while non-viral systems such as cationic liposomes and polymers principally deliver to lung tissue [1–4]. Researchers take advantage of the innate targeting of these systems, using viral and non-viral vectors to target hepatocellular carcinomas and non-small cell lung carcinomas respectively [5–8]. However, when targeting other cancers or metastases not resident in the liver and lung, accumulation in these organs hinders effective cancer therapy.

Methods for locally administering gene delivery systems include intracranial, intratumoral and intravenous injection as well as inhalation. Localized delivery techniques have been shown to be effective in treating primary tumors [9]. Intratumoral delivery of viral gene delivery systems targets the tumor tissue and while there is some distribution of the virus outside of the tumor, the toxicity and immunogenicity are tolerable. Inhalation of nanoparticles results in the payload selectively

E-mail address: max.kullberg@ucdenver.edu (M. Kullberg).

0168-3659/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jconrel.2013.08.300

reaching the lungs, while intracranial tumor injection is effective for circumventing the blood–brain barrier [10,11]. Although some of these localized methods of administration may be sufficient for primary tumor targeting, undetected metastases will need to be targeted with an intravenous injection of a delivery system that can home to tumor cells systemically. As primary tumors can often be removed surgically, metastases should be the priority when developing a delivery system for treating cancer. A tumor-specific, systemically-delivered vector would be able to capitalize on the growing number of genes that have been shown to hinder the growth of metastatic cancer cells.

Many of the genes that could be used to aggressively promote cancer cell apoptosis or stimulate an immune response to a tumor will result in unacceptable toxicity if delivered primarily to the lungs and liver [12,13]. Two cytokines that have been shown to deter metastasis and effectively treat already established metastases are IL-12 and IL-2 [14–16]. When delivered to the microenvironment of a metastatic tumor, IL-12 can polarize T helper cells towards a TH1 phenotype, which produces IFN-gamma and activates a cytotoxic T-cell response. IL-2 has been shown to illicit a response from natural killer and cytotoxic T-cells. While both of these cytokines can prevent metastatic tumor growth when delivered locally, systemic delivery that is non-specific has significant toxicity [12,17]. It is largely because of this dose-dependent toxicity that clinical trials using systemic delivery of IL-12 have had limited success.

Other attractive genes for delivery encode ligands that target death receptors leading to apoptosis, including tumor necrosis factor-alpha (TNF-alpha), CD95/FAS and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [13,18,19]. The appeal of a suicide protein being delivered to tumor cells is undeniable, but as with immune-stimulating cytokines, the non-specific systemic delivery of a suicide gene is not well tolerated [20]. Considering recent concerns of hepatotoxicity, even the relatively non-toxic TRAIL must be dosed conservatively as it moves into the clinic [21]. Herpes simplex virus thymidine kinase (HSV/tk) is another gene commonly chosen for delivery [22]. Accumulation of thymidine kinase within a target cell is toxic upon administration of a pyrimidine or purine analog drug, converting the drug to its active form in the cytoplasm [23]. As with the other genes mentioned above, non-specific delivery of HSV/tk will result in death of bystander cells. Whether specificity is incurred by targeting of tumor tissue, selective internalization into tumor cells or use of tumor-specific promoters, selective gene expression must be achieved if we are to aggressively target tumor metastases with genes that promote cell death.

This review focuses on viral and non-viral systems that have been reported to achieve specific gene expression in tumor tissue. Unless stated otherwise, the delivery system was administered *via* tail vein injection. The type of delivery system is introduced in each section with a description of its natural targets, followed by an analysis of studies that have manipulated the system so that gene expression is greater in the tumor than in other major organs. In addition, we discuss gene delivery using stem cells which have been reported to naturally migrate to tumor tissue.

2. Non-viral systems

2.1. Lipoplex

Cationic lipoplexes are composed of a cationic lipid and a neutral lipid or cholesterol [2,24]. The negatively-charged DNA is compacted and forms a complex with the positively-charged lipid, resulting in the formation of a lipoplex. Lipoplexes interact with the cell membrane and internalize into the cell through endocytosis. It is thought that the lipid components, once internalized, lead to destabilization of the lipid complex, fusion with the endosomal membrane, and cytoplasmic delivery of the DNA. DNA can reach the nucleus when the nuclear membrane breaks down during cell division, and thus rapidly-dividing cells are generally more easily transfected. When compared with viral delivery systems, the non-viral cationic lipoplex is considered less immunogenic, and able to hold a larger payload. The drawbacks include toxicity of cationic lipids and relatively inefficient and non-specific delivery [25].

Cationic lipoplexes deliver genes primarily to the lungs and secondarily to the liver [26]. Luciferase expression is observed in the liver and \lungs with relatively low expression in flank tumors of a mouse model. Coating of the cationic lipoplex with polyethylene glycol (PEG) can be employed to increase circulation time with the hope of enhancing delivery to the leaky capillary bed of tumor tissue [1,27]. However, several groups have reported that increased levels of PEG do not improve distribution to the tumor tissue, and delivery is predominantly to the lungs and liver [1,28].

To increase the tumor specificity of systemically-delivered cationic lipoplexes, one group used both PEG shielding and integrin targeting [27,29,30]. These cationic lipoplexes have a short triethylene glycol coating and a peptide targeted to integrins, which are overexpressed in many neuroblastoma cell lines. The PEG and integrin-targeting peptides are connected to the liposome through linkages that are cleavable by endosomal furin, cathepsin B, or esterases. After binding and internalization into tumor cells, the peptides and PEG are cleaved from the liposome, allowing for destabilization and delivery of the nucleic acid. This technique has resulted in highly specific tumor targeting upon systemic administration. Tissue analysis showed that accumulation of luciferase DNA was at least two-fold greater in the tumor tissue when compared to the lung, liver and spleen, but more importantly, expression of the luciferase gene was 130-fold greater in the tumor tissue than in the other organs. When this system was used to deliver IL-2/IL-12 cytokines to subcutaneous neuroblastoma tumors in a mouse model, 1/3 of the tumors were eradicated and 2/3 of the tumors had markedly decreased growth. It is important to mention that the expression data took into account the entire tumor and organ. When measuring expression, data is usually presented as per organ, per mg protein, or per mg tissue. Since the liver certainly weighs much more than the tumor in any realistic clinical scenario, presenting data as per organ is often the least impressive and possibly the most relevant reporting method. However, data reported as “per organ” can be misleading if tumors are allowed to achieve grotesque proportions in animal models. Therefore, it is preferable to include both measurements to allow comparison with published studies.

Wang et al. were able to achieve good tumor specificity using a cationic lipid system consisting of DOTAP and cholesterol [31]. Messenger RNA for luciferase is condensed with protamine which then associates with the cationic liposome. The resulting complex is PEGylated, and anisamide is attached so that the particles will target cancer cells overexpressing the sigma receptor. This system results in at least 10-fold greater expression per mg of tumor tissue compared to liver tissue. When the suicide gene thymidine kinase from Herpes simplex virus is delivered systemically using this system, the tumors show a marked decrease in growth [31]. To assess if the delivery system was causing toxicity, serum concentrations of liver enzymes, alanine transaminase and aspartate transaminase were assayed along with blood urea nitrogen levels which can reveal kidney damage. After repeated treatment with the liposomes, all of these toxicity indicators fell within their normal range, suggesting that this therapy could be tolerable at clinical dosages.

To target prostate cancer, Ikegami et al. used a monoclonal antibody that binds to prostate-specific membrane antigen (PSMA) [32]. This membrane protein is present in prostate cancers and is not expressed in normal tissue. The antibody is coupled to polylysine, which is then used to condense DNA. The targeted polylysine is mixed with cationic liposomes and the resulting complex is used to deliver luciferase and HSV/tk to prostate tumors. With this system, luciferase expression was at least 17-fold higher per mg prostate tumor than in lung, liver and kidneys. When the HSV/tk gene was delivered to treat tumors, tumor mass was reduced by approximately 50% [32].

Please cite this article as: M. Kullberg, et al., Systemic tumor-specific gene delivery, J. Control. Release (2013), http://dx.doi.org/10.1016/j.jconrel.2013.08.300