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Enhanced local delivery with reduced systemic toxicity

Delivery, delivery, and delivery

P Yu, X Wang and Y-X Fu

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Remarkable progress has been achieved in cancer gene therapy over the past few years in regard to increasing efficacy and reducing toxicity. While many gene therapy products can kill tumors or retard tumor growth, they can also non-specifically target normal tissues. Thus, successful virus-based gene therapy may very much depend on the efficacy/toxicity ratio. Systemic delivery of various cytokines and macromolecules for cancer therapy has been hampered by their toxicity at the therapeutic levels for cancer treatment and limited penetration of tumor tissues. In fact, tumor tissues have their barriers and increased interstitial fluid pressure (IFP) to exclude penetration.[1] Many studies have focused on the following area:[2] (1) development of novel vectors to improve gene delivery to the tumor; (2) modification of current gene-delivery vectors to improve selectivity, and (3) improvement of the therapeutic window to reduce the toxicity of gene therapy administered alone or in combination with conventional agents. It has been thought that intratumoral infusion may increase interstitial transport of viral vectors in tumor tissues, and possibly reduce systemic toxicity. In addition, targeting local tumor with immune modulator may enhance systemic immunity against tumor at distal site.[3] Due to very limited success in therapeutic vaccination and systemic cytokine therapy in past decade, this is an important direction to enhance host immunity without the knowledge of tumor antigens.

Intratumoral treatment becomes the most commonly used method for viral gene delivery in clinical trials. However, few showed limited therapeutic effect, partially due to the lack of efficient, specific, and safe delivery system since a macromolecule is difficult to be delivered into tumor tissues for effective distribution, interstitial penetration and cellular targeting. In contrast to early thoughts, several studies showed that such locally delivered viral particles may not stay local and some are disseminated.[4–6] Systemic dissemination from the tumor tissues often results in high toxicity and reduced expression of desired gene inside the tumors. Detections of viral dissemination has been facilitated with more sensitive system using fluorescence and luminescence reporter genes, such as enhanced green fluorescence protein (EGFP) and luciferase.[5,7,8] At the cellular and tissue level, EGFP expression was detected both in the liver and tumor tissues, whereas whole-body imaging systems, which trace and map the luciferase expression, again reveal that the liver is the major disseminating site while tumor tissues only express less than 10% of the delivered gene products. It has been estimated that the amount of disseminated viruses outside tumor can be 10-fold higher than what retained inside tumor tissues, thus reduce actual ratio of efficacy/toxicity.[5–7] Although systemic dissemination can be tumor type and vector dose dependent, dissemination can cause serious adverse effects once it occurs. Some mice could die within a few hours after intratumor injection[7] presumably due to toxicity resulted from vector dissemination. In non-human primates, it was shown that systemic delivery of high dose of adenoviral vector results in acute toxicity in baboons consistent with activation of the innate inflammatory response, the severity of which is dose dependent but is independent of viral gene expression.[9] In addition to animal models, toxicity from systemic adenoviral vector exposure is a major obstacle in clinical trials. Autopsy of a patient who soon died after adenovirus treatment also showed that liver is a major target tissue for adenovirus.[10] Dissemination causes significant toxicity, which prevents application of higher dose, decreases the amount of gene targeting tumor, and both result in reduction of desired clinical efficacy.

The mechanism of increased morbidity and mortality associated with virus-based gene therapy is not entirely clear, but it is possibly related to over-reacting immune response to virus vectors, especially innate immunity. Rapid increased pool of cytokines may lead to dysfunction and damage of multiple organs. For example, most of the disseminated viruses accumulate in the liver, resulted from rapid uptake by Kupffers cells and others, and lead to release of various cytokines, which cause liver damage.[11,12] It is possible to neutralize some key cytokines, such as TNF, to reduce toxicity, but this may not increase gene expression at tumor site. Reduction of dissemination from tumor tissues not only decreases toxicity, but also augments the expression at local site. In addition, it allows higher dose of drug and gene delivery to tumor, which may also contribute to the enhancement of antitumor activities.

Several distinct approaches to reduce liver toxicity have been explored. Genetic replacement of the adenovirus shaft fiber reduces liver tropism, and modification of the knob allows increased efficiency to transduce tumor cells.[13] However, both classes of modified adenovial vectors still faces the problem of vector dissemination after intratumoral injection. To reduce the dissemination, Yuan's group first mixed adenovirus expressing target gene with a viscous alginate solution, which could reduce virus dissemination but not necessarily increase gene expression in tumor.[5] His group has further developed a novel method based on a biocompatible polymer, poloxamer 407, which has been used for wound healing and drug delivery, and tissue engineering.[8] They demonstrated that intratumor injection of such mixture significantly increased the viscosity of virus suspension when the temperature was changed from 4 to 37°C. With this method, they not only reduced virus dissemination, but also increased local gene expression in solid tumors after intratumoral infusion of adenoviral vectors. Consequently, the poloxamer solution increased the tumor/liver ratio of gene expressions by 12- to 275-fold. The

mechanism of reduction in dissemination was likely to be that the viscous poloxamer solution blocked convection of virus in the interstitial space and the lumen of microvessels in the vicinity of the infusion site, thus preventing virus from entering systemic circulation via tumor microcirculation system.

There are several issues to be addressed: (1) Can poloxamer solution interfere with the ability of adenoviral vectors to transfect cells? Yuan's group mixed Ad-EGFP with a diluted poloxamer solution and showed no reduction of expression. *In vivo*, much higher expression of gene products was evident, suggesting the interference is minimal. (2) Can microclots formed by poloxamer solution escape from tumor causing clots in critical organs? It is unlikely since the clots may detach and accumulate in the lungs, but no such evidence of clots was detected there. It is anticipated that such insignificant detached clots pose minimal threat to the lung, which can rapidly by dissolves clots. In fact, Poloxamer 407 has been used as a temporary vascular occlusion agent and much larger clot is formed.[14] (3) Pre-existing immunity to adenovirus in most patients may complicate the outcome of adenovirus gene delivery since the adenovirus can be rapidly neutralized in the circulation. It is possible that such immunity does not prevent tumor regression following intra tumoral administration, but inhibits virus dissemination to liver.[6] But another study showed pre-existing immunity to adenovirus in rhesus monkeys fails to prevent vector-induced toxicity.[12] It is important to report and summarize whether and how human patients will respond to the adenovirus, which will likely depend on the level of pre-existing immunity against adenovirus, serotypes of adenovirus, as well as host health condition at the time of treatment. Protective treatment may be required to prevent and reduce such toxicity. Clinical monitoring of pre-existing immunity to different types of adenovirus may be necessary. If the gene is targeting liver cancer, it is possible that pre-existing immunity may prevent efficacy of systemic infusion, while naïve patients or mice with liver cancer may have favorable response against tumor. To increase local delivery and reduce leakiness of viral gene delivery, more work is needed to design and test different type of polymers. Yuan's study opens new avenue to the field.

It is possible that reduced dissemination and increased expression of gene products by this new mixture will increase efficacy/toxic ratio leading to limited tumor growth and/or increase local immunity against tumor depending on the gene used. It will be important to test whether much higher dose of adenovirus can now be used safely and whether increased antitumor activity can be better demonstrated using such mixtures in comparison with adenovirus alone. It is also important to demonstrate the effectiveness of Yuan's approach in an expanded list of tumor types. It is possible that no single delivery method or vector can meet all the requirements for various cancers. It remains to be determined whether such kind of polymer can be extended to other delivery systems, including other viruses, DNA, and proteins where prolonged local effect is desired.

In summary, the new delivery method could significantly increase gene expression in the tumor and decrease dissemination leading to dramatically increase of efficacy/toxic ratio. Such improvement is urgently needed for enhancing the efficacy/toxicity ratio in viral gene therapy critical for clinical use. Although this method was tested only for adenoviral vectors, it could also be used to deliver other therapeutic agents in solid tumors because the mechanisms of passive transport are the same for all agents. ■

Y-X Fu and P Yu are at the Department of Pathology and Committee on Immunology, The University of Chicago, 5841 S. Maryland, MC3083, Chicago, IL 60637, USA.
E-mail: yfu@uchicago.edu and X Wang is at the Biogen Idec Inc., 14 Cambridge Center, Cambridge, MA, USA.
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