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COMMENTARY

Antibody gene therapy: an attractive approach for the treatment of cancers and other chronic diseases

Dexian Zheng[1]

[1]*National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100005, China*

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Monoclonal antibodies (mAb) have been successfully applied to the treatment of chronic diseases, such as cancer, inflammation and immune diseases. With the technical advances in antibody engineering, development of small recombinant antibody fragments as high affinity therapeutics with reduced immunogenicity has become under the spotlight. A popular format of the engineered recombinant antibody fragments is the single-chain fixed-variable (scFv) molecules, in which the VH and VL regions of the parental antibody are jointed together by a polypeptide linker. The scFv fragment retains the target specificity and antigen-binding affinity of the intact antibody, and can be genetically designed and produced in large quantity by ectopically expressing both VH and VL regions from a single cDNA in cells. Due to its smaller size, the scFv molecule shows improved pharmacokinetics in tumor penetration and is better tolerated by the host immune system. Despite these potential advantages of using scFv molecules for immunotherapy, especially for chronic diseases such as cancers, the treatment efficacy, however, is often compromised by the rapid blood clearance and insufficient concentration of the infused scFv fragments at the target site. To achieve high local concentrations in the target tissues, large amount of the recombinant scFv proteins (hundreds milligrams or more/a period of treatment) have to be administrated in the conventional antibody therapy. Thus, broad application of recombinant scFv antibody is limited by manufacturing difficulty and the high cost of production. To overcome these bottle-necks, Shi *et al*. [1] developed a promising therapeutic strategy by expressing *in vivo* scFv fragments of a mouse monoclonal antibody (AD5-10) that is directed against the TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) receptor DR5 (death receptor 5, also known as TRAIL receptor 2) [2], via recombinant adeno-associated virus (rAAV) vector-mediated antibody gene therapy.

The novel mouse anti-human DR5 monoclonal antibody, AD5-10, was developed in the same laboratory. As reported by Guo *et al*. [3], AD5-10 induces apoptosis of various tumor cell lines in the absence of cross-linking *in vitro* and exhibits a strong tumoricidal activity *in vivo*. AD5-10 does not induce cell death of human primary peripheral blood lymphocytes and normal hepatocytes, and injection with high doses of AD5-10 in mice causes no toxic reaction in liver, spleen, and kidney. AD5-10 does not compete with TRAIL for binding to DR5, and there is a synergistic effect of TRAIL and AD5-10 on their tumoricidal activity. Both TRAIL and AD5-10 activate the caspase cascade and induce a classical apoptosis response in tumor cells. However, AD5-10 kills tumor cells in the presence of Z-VAD (a pan-caspase inhibitor) while TRAIL does not, indicating that AD5-10 promotes caspase-dependent and -independent cell death pathways. Furthermore, RIP (receptor interacting protein) is essential for the caspase-independent cell death. Z-VAD inhibits the activation of p38/JNK by TRAIL but not by AD5-10. Both TRAIL and AD5-10 are capable of activating NF-κB, but there are differences between the regulation of NF-κB activity by TRAIL and AD5-10 in certain cell lines. The authors demonstrated for the first time that DR5 mediates distinct cell signals leading to apoptosis

Correspondence: Dexian Zheng
Tel: +86-10 6529 6409; Fax: +86-10 6510 5102
E-mail: zhengdx@pumc.edu.cn

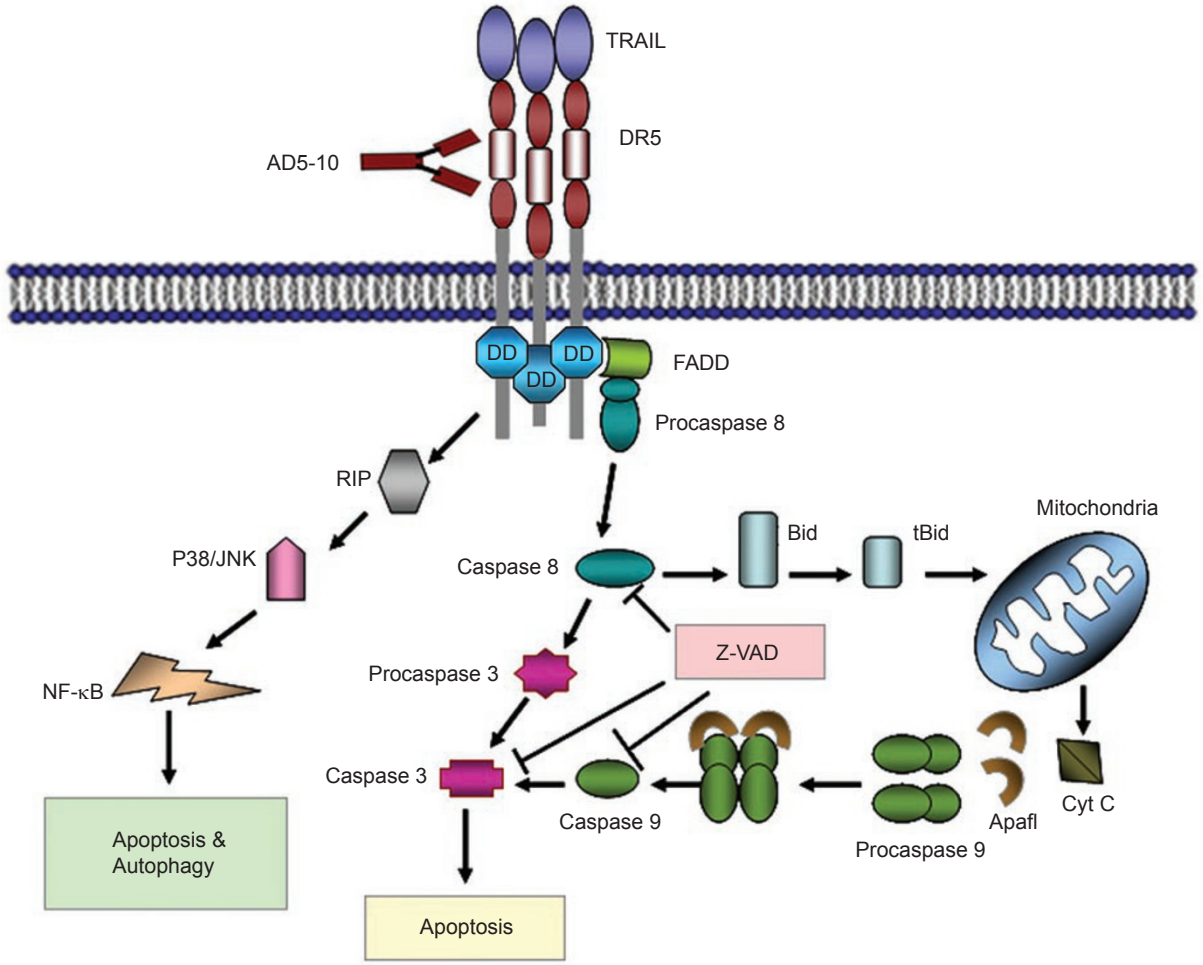

Figure 1 DR5 mediates distinct cell signals when interacting with different extracellular proteins. Both TRAIL and AD5-10 activate the caspase cascade and induce a classical apoptosis response in tumor cells. In the presence of Z-VAD, AD5-10 could also promote caspase-independent cell death (apoptosis and autophagy), in which the activations of RIP, JNK/p38, and NF-κB are essential.

and autophagy when interacting with different extracellular proteins in the presence or absence of Z-VAD (Figure 1). Therefore, AD5-10 is an important functional antibody for further studies on both the underlying mechanisms and potential clinical applications.

The great shortcoming of using mouse monoclonal antibody (mAb) in human, however, is the associated human anti-mouse antibody (HAMA) reaction, which makes long-term use of the mouse mAb therapy infeasible in patients with chronic progressive conditions such as cancers. The immunogenicity of rodent mAbs sometimes can be successfully reduced, but in a laborious and time-consuming way, by antibody chimerization and humanization using gene-engineering technologies. In addition, due to their large size, it is difficult for intact antibodies to penetrate into solid tumors, further limiting their applications in cancer therapy [4]. Since AAV-mediated gene therapy enables long-term transgene expression *in vivo* and shows low immunogenicity, Shi *et al*. [1] adopted this system to express scFv fragments of AD5-10 in mouse models and achieved a significant therapeutic efficacy against tumor xenografts.

Shi *et al*. [1] demonstrated that viral transduction using the rAAV encoding scFv of AD5-10 led to stable expression and apoptosis-inducing activity in lung SCLC, liver Hep 3B and colon HCT116 carcinoma cell lines *in vitro*, which could be inhibited by recombinant DR5 specifically. The virally expressed scFv fragments in the media of infected HEK293 cells and the sera of transduced mice maintained its affinity for its antigen ($Kd = 3.3\times10^{-9}$ M in the cell culture media and $Kd = 4\times10^{-9}$ M in the mouse sera, respectively) comparing with that of the parental intact an-

tibody (Kd =10^{-10} M). It is interesting that primary human hepatocytes (PHH) did not express detectable DR5 receptor, and therefore, were resistant to the killing by the scFv; and they remained resistant despite forced over-expression of DR5 in those cells, suggesting that PHH cells lack the functioned DR5-mediated death-signaling machinery. The resistance of PHH cells to scFv-mediated cytotoxicity provided initial data for the safety of using rAAV-scFc/AD5-10 in human cancer gene therapy. *In vivo* administration via a single intramuscular injection of 10^{11} genome equivalents of the rAAV-scFc/AD5-10 particles into nude mice showed that scFv protein expression in the serum was maintained at about 100-120 μg/ml for more than 240 days; apoptotic cell death and significantly reduced growth of previously established subcutaneous human lung SCLC and liver Hep3B tumor xenografts were observed.

However, the apoptotic effect of the scFv antibody fragments on tumor cells *in vivo* and *in vitro*, can only be described as moderate even though the concentrations of the soluble scFv protein secreted into the culture media and sera were relatively high. The possible explanations might be the relatively low affinity of the scFv fragment comparing to the parental intact antibody, and the insufficient concentration of the scFv in the sera. Combination of the rAAV-scFv virus-mediated antibody gene therapy with the conventional therapeutics, such as chemotherapy and irradiation, may increase the curative efficacy.

Systemic gene delivery carries the risk of delivery to other tissues such as liver. Therefore, it is highly desirable to achieve tissue-specific gene transfer and expression. In this study, the authors used the rAAV2/1 vector system, which has been shown to be more selective and safe for muscle transduction via intramuscular administration. Therefore, the high muscle-specific expression of scFv achieved *in vivo* via rAAV2/1-mediated gene transfer would make this vector potentially suitable for clinical application.

This study took advantages of the specific tumor killing activity of the anti-DR5 mAb. The combination of the scFv fragment technology with rAAV-mediated gene transfer to achieve tumor suppression in mice provides new evidence that therapeutic antibody gene delivery of scFv is an attractive strategy, which can generate antibodies at therapeutic levels *in vivo* and overcome the bottle-necks of conventional antibody therapy.

In the same year of 2006, two laboratories reported that intracranial AAV-mediated delivery of anti-Aβ [5] or anti-pan Aβ [6] mAb scFv fragments in AD (Alzheimer's disease) mouse models led to significantly reduced amyloid deposits at the injection sites compared to animals injected with PBS. As pointed out by Levites *et al*. [6], the ability to achieve widespread, apparently permanent expression of genes delivered intracerebroventricularly by AAV1 to the mice establishes a novel cost- and time-effective paradigm for validating therapeutic targets or strategies in mouse models. Such data extend previous studies whereby AAV1-mediated delivery of a transgene is used to attenuate pathology in mouse models of lysosomal storage disease [7].

Antibody gene therapy could be traced back to the year of 2002. Ye *et al*. [8] firstly reported that infection of K1735 melanoma cells with the retroviral vector pLNCX encoding the cell-bound anti-4-1BB scFv fragment induced a strong type I T-helper cell response, and the vaccinated mice rejected established wild-type K1735 tumor growing as subcutaneous nodules or in the lung, suggesting that such antibody gene therapy might be effective against micrometastases in human patients. In 2005, Fang *et al*. [9] developed an efficient mAb delivery system that allows for continuous production of a full-length antibody at high-concentration *in vivo* after gene transfer by using AAV8 vector. The mAb is expressed from a single open reading frame by linking the heavy and light chains with a 2A self-processing peptide derived from the foot-and-mouth disease virus. Using this technology, they generated a recombinant adeno-associated virus vector encoding the VEGFR2 (vascular endothelial growth factor receptor 2)-neutralizing mAb DC101 (rAAV8-DC101). A single dose of rAAV8-DC101 resulted in long-term expression of >1 000 μg/ml of DC101 in the mouse serum up to 4 months after the vector administration, leading to a significant anti-tumor efficacy. This technology may facilitate both laboratory research and clinical application of antibody gene therapy for the treatment of cancers or other chronic diseases.

It is worthwhile to point out that although antibody gene therapy might be effective for the treatment of human cancers or other chronic diseases, we, however, are just at the beginning point of the raceway. It will, therefore, be beneficial to investigate this approach in various tumor models, to evaluate the safety in non-human primates, and to test if it can be improved in combination with other therapeutics, such as chemotherapy and radiotherapy before a potential advance to clinical trial in human patients.

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