RESEARCH AND DEVELOPMENT OF CANCER-TARGETING VECTORS: AN EXPLORATION ON ONCOTROPISM AND ONCOSUPPRESSION OF AUTONOMOUS PARVOVIRUSES

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ABSTRACT

Autonomous parvoviruses are single-stranded DNA viruses with a genome size of about 5 kb. They are characterized by their oncotropism and oncosuppression to the hosts. After infection the viral genes are not integrated into the chromosome of host cells. Autonomous parvoviruses could be used as potential vectors carrying heterologous therapeutic genes for the construction of recombinant autonomous parvoviruses. The specific advantages of these viral vectors to cancer gene therapy are discussed.

Key words: Recombinant autonomous parvovirus, Vector, Gene therapy

Usually, retroviruses, adenoviruses, herpes viruses and adeno-associated viruses are used as vectors in gene therapy research. These vectors transport the therapeutic gene into their target cells. Each vector has shown its own specific features.

Retroviruses are integrated randomly to the host cell chromosomes, ensuring that the exogenous genes be expressed steadily and in long-term. However, there are risks of inducing mutagenesis and carcinogenesis. When the HSV/tk gene is transduced into mouse fibroblasts by the retroviral vectors, and after the fibroblasts are injected into the human brain, the tk gene would be inserted into the dividing brain tumor cells and thus be prone to the action of ganciclovir. In contrast, the adenoviruses are not integrated to the host cell chromosomes, and are suitable for the transient expression of exogenous genes. Mutant adenovirus, lacking the E1b gene, has exhibited a selective killing action on cancers with P53 mutation. Herpes virus HSV1 is neurotropic and it can transfer and integrate larger heterologous genes into human cells even when these are in quiescent and differentiated stages.

Parvoviruses are also vectors with specific features. For more than a decade, adeno-associated viruses (AAV) have attracted much attention. They need a helper virus to complete its own life cycle, but the virus with its exogenous gene can be integrated on specific site to a host cell chromosome in the absence of the helper, and be expressed in long terms. Since AAV does not have any pathogenic effect, it is safe to be used as a vector.

Other groups of parvoviruses are autonomous, i.e., these viruses do not need a helper virus for their replication and are not integrated to the host cell chromosome. Members of this group include H1, MVM, and LuIII, etc. and are often characterized by their oncotropism and oncosupression. These vectors are most favourable for cases of cancer gene therapy where the sole requirement is the transient expression of therapeutic transgenes.

Oncotropism and Oncosuppression of H1 and MVM

H1 and MVM are two members of parvoviruses under study most intensively. Both are particles with a size of 18–26 nm in diameter and a single stranded DNA of 5 kb. Replication depends closely on the S phase and differentiated state of their host cells. The homology in DNA sequence between H1 and MVM is 77%.[1] There are two promoters – P4 and P38. The former directs the expression of non-structural proteins, NS1 and NS2, and the latter directs the expression of capsid proteins, VP1 and VP2. NS1 protein has multiple functions: regulation of viral DNA replication and expression; transactivation of P38; and cytotoxicity to various transformed and cancer cells. NS2 has synergetic effect on NS1 cytotoxicity.

Oncotropism and oncosuppression are two prominent features for MVM and H1. On the one hand, H1 and

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MVM depend on the microenvironment provided by the transformed and cancer cells for completing their life cycle; on the other hand, in concomitance with the progression of viral life cycle and the release of viral particles, the growth of transformed cells or cancer cells are suppressed and cytolysis sets in eventually.

The susceptibility of many mammalian and rodent transformed and cancer cells to H1 and MVM had been studied *in vitro*. The majority of these cells are of higher susceptibility to H1 and MVM than their normal counterparts.[2] Studies on human hepatocellular carcinoma (HCC) primary cultures have shown that H1 susceptibility of hepatoma tissue is much higher than that of parahepatoma tissue.[3] A similar result has been obtained in primary tissue culture of human breast cancer.[4] The susceptibility to H1 in most of the transformed cells is greatly increased. At the same time DNA amplification and gene expression are promoted and these cells end in cytolysis. Although uptake of the virus by normal cells is the same as in transformed cells, viral DNA amplification is limited.

In *in vivo* study, when breast cancer cells were transplanted subcutaneously into the nude mice, and H1 injected immediately or after tumor formation, all result in tumor suppression to a certain extent.[5] When H1 infected HCC is transplanted into nude mice subcutaneously, tumor formation is completely suppressed.[6] If H1 injection is carried out in the presence of well-formed HCC, tumor growth is much faster than that of the control, and the difference between these two groups is statistically significant.[7]

H1 distribution pattern in various tissues of nude mice bearing transplanted tumor shows: the H1 specific PCR amplification band can be detected in the tumor, liver, spleen and brain at post-infection day 1, but it remains only in the tumor at post-infection day 7.[8]

When H1 of 10^{10} pfu (1 pfu means 1 infectious viral particle) is injected into the caudal vein of nude mice, not any acute toxic effect is seen within one week, and all assays on allergy, mutagenesis, teratogenesis, and pyrogenicity are negative.[9] These preliminary results show that H1 could be safe enough for future clinical use.

It must be noted, however, that if tumor suppression of H1 is not sufficient, some cancer cells would not be attacked or get killed. How to have a higher suppressive effect by exploiting further oncotropism of H1 remains a future challenge. The construction of recombinant parvovirus rather than the use of wild type virus might be a favorable option.

Research in Autonomous Parvovirus-mediated Vectors

As mentioned above, the promoter P4 in the genome of autonomous parvoviruses, regulates transcription and expression of the ns gene, and the NS protein transactivates, in turns, the promoter P38. If the vp gene could be replaced by a heterologous suppressive DNA fragment and then be packaged as a recombinant parvovirus, when the latter is introduced into tumor cells, the synergetic action of NS protein and the therapeutic gene product should strengthen the suppression on tumor growth. It is essential that whether the recombinant virus could retain the oncotropism of wild-type virus and attack tumor cells.

To date, two classes of recombinant parvoviruses have been constructed. (1) By using MVMp DNA as a vector and the vp gene replaced by the reporter gene cat or IL-2/4 cDNA, one then could co-transfect cells with plasmid containing vp gene, the recombinants could be amplified and packaged. The transformed cells are then infected by the packaged recombinant virus and the amount of CAT or IL-2/4 measured represents quantitatively the expression of the transduced genes. It is shown that the recombinant virus induces IL-2 production and releases in ras transformed rat FR3T3 cell line, and IL-2 level in SV4O transformed MRC-5V1 cells is higher than that in normal cells. The above results show that the recombinant parvovirus still retain their oncotropism;[10, 11] (2) By using LuIII DNA (its homology with H1 and MVM DNA is very high) as a vector, its coding part is replaced by the reporter gene luc but spare the terminus. Then the cells are cotransfected with plasmids containing the vp gene, or plasmid containing vp or ns gene from H1 or MVM, to package the recombinant virus particle, and the luc gene is highly expressed in the relevant host cells.[12, 13]

Therefore, it is feasible to insert the exogenous gene into parvoviral genome, and to have it transduced to tumor cells. The gene is highly expressed, and the encoded protein produces its effect.

Features of Autonomous Parvovirus as the Vector of the Therapeutic Genes

In conclusion, features of autonomous parvoviral vectors can be summarized as follows: in contrast with retrovirus, herpes virus, adeno-associated virus, the autonomous parvovirus does not integrate its DNA into host chromosome; as a vector it expresses the therapeutic gene transiently, and thus is suitable for use in cancer therapy.

In general, the parvoviral vector carrying the therapeutic gene is defective; that is, it does not have its vp gene (sometimes not even the ns gene). Therefore, it is essential to provide a helper plasmid containing the vp gene in order to obtain the recombinant parvovirus by cotransfection. As shown in the MVM/IL-2 recombinant case above it has been shown by experiments that the vp gene plays a decisive role in the oncotropism of

parvoviruses and the recombinant virus shows generally a tropism specifically instructed by its vp gene. It is feasible to use various parvoviral vp genes in packaging recombinant virus, e.g., MVM or H1 VP is used for packaging the recombinant LuIII, or H1 VP to package recombinant MVM DNA and so on. By these means, the host cell spectrum is extended and the killing effect to tumor cells is much strengthened.

The gene transduction efficiency depends on the promoters P4 and P38, and P38 depends on the P4 and NS protein transactivation. P4 and P38 both have effects on human fibroblast cells, epithelial cells and the haemopoietic cells. The NS protein of parvovirus is the main suppressive agent, if the ns gene remained in recombinant parvovirus, it is anticipated that NS protein and the exogenous therapeutic gene both produce synergic and dual suppressive effects on tumors.

Prospects and the Interesting Projects

As virus-mediated vectors for cancer gene therapy, the autonomous parvoviruses have a very promising potential in clinics.

Chemotherapeutics usually exhibit high side effects owing to their nonselectivity in killing tumor cells. In contrast, parvoviruses are selective in their action on tumor cells and do not harm normal cells and tissues; thus they can be used as vectors in carrying therapeutic genes into tumor cells. Since these gene products could be a cytotoxic agent or an enzyme capable of activating prodrug, they kill cancer cells directly, or activating as inducer for prodrugs. It has been observed, as in recombinant retroviruses and adenoviruses, the recombinant parvovirus *in vitro* carrying the drug-activating gene (a suicide gene) HSV/tk targeted into cancer cells, can phosphorylate the transduced prodrug and converts the latter into a toxic compound to cancer cells.

The products of transgenes could elicit the defense mechanism of host cells against tumors, and then transmit the antitumor effect to non-target cancer cells. The overall efficacy could be much higher than the local direct killing effect of wild type parvovirus.

By transferring cytokine-encoding transgenes into tumor cells the autonomous parvoviruses can contain cytokine (e.g. IL-2) production locally, and affect only very close neighboring cells. It could, through this means, avoid the toxic side effect produced by the systematic injection of IL-2.

Besides H1, MVM and LuIII, other members in parvovirus could also be used as vectors. B19 is the only known member pathogenic to humans that exhibits a tropism to erythropoietic progenitors. A new hybrid parvovirus could be constructed using an AAV2 promotor to replace the B19 promotor. The hybrid virus replicates only in erythropoietic progenitors and there is no need for a helper virus.[14, 15] It is not pathogenic as B19; and thus might be promising in gene therapy for diseases of the erythropoietic system.

A couple of theoretical and technological problems have to be solved before clinical application of parvovirus-mediated vectors. In *in vitro* system, it has been demonstrated that all cells transformed by various oncogenes, viruses, physical or chemical agents exhibit an enhanced susceptibility to parvovirus and increased virus amplification. The underlying mechanism should be studied further. Moreover, the oncotropism of parvovirus should be studied in *in vivo* system, and its susceptibility to the virus assayed using various human tumor models, or short-term cultures from the human tumor samples. The aim is to confirm the most sensitive tumor targets to the virus. One intriguing problem is: concurrent with packaging of the recombinant parvovirus, there is a recombination between recombinant virus and helper virus that leads to the production of a wild type virus. It happens even when the titer of wild type virus is much higher than the recombinant parvovirus and thus severely affects the yield of recombinants. Two proposals have been put up for its solution: one is to select a constuitively defective plasmid for cotransfection with the recombinants, and reduce the probability of recombination with wild type parvovirus; the second is to integrate a vp gene directly into cells and to establish packaging cells expressing the VP. Nevertheless, there is still a long way to go before the above problems are solved.

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