Biohelikon: Cell Biology, 2013 1:3

Review Article

Recombinant adenovirus, a powerful vector to transfer gene

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Abstract

Recombinant adenovirus has been used extensively to express foreign genes because of its high infection efficiency and its ability to infect a broad spectrum of cell-types. In this review, we describe these advantages and how it works by using a recombinant adenovirus to study the NS2 of Hepatitis C virus (HCV) and focal adhesion kinase(FAK)- related non-kinase(FRNK). Taken the research we have done together recombinant adenovirus demonstrates a very useful vector for transferring genes of interest from the outside.

Keywords
Recombinant adenovirus, NS2, FRNK

Recombinant adenovirus has been used extensively for transferring foreign genes because of several unique advantages. For example, it can infect most types of cells. High titer of virus and high expression level of foreign genes were easy to be obtained [1] . The most commonly used adenovirus is serotype 5 human adenovirus with a linear and double stranded DNA genome [1] . Currently the engineered adenoviral vectors have made the expression of foreign genes much more convenient as compared to the previous adenoviral vectors. The most critical improvement is that the recombination process happens in *E.coli* instead of mammalian cells, which have low recombination efficiency and needs plaque purification [1] . The engineered adenoviral vectors include a GFP marker that can easy identify the efficiency of transfection and infection. These unique advantages of newly engineered adenovirus have been applied to most of our previous research.

An important non-structural gene of HCV genome is *ns2*. NS2 was shown to inhibit several promoters in hepatic and nonhepatic cell lines, such as promoters that control the expression of CMV and SV40 [2] .NS2 can block apoptosis by inhibiting the release of cytochrome C [3] . In order to study the function of NS2 in the HCV life cycle, a system that can express a high level of NS2 in hepatic cell lines, such as HepG2, would be needed. From our previous experience, the level of expression of foreign genes in HepG2 cells is usually low with traditional methods, such as lipofectin transfection. Instead we constructed a recombinant adenovirus that can express *ns2* with a GFP marker. The titer of this recombinant adenovirus can be high up to 10^9 PFU/ml after propagation in 293 cells [4] . The infection efficiency of this recombinant adenovirus in HepG2 cells can be up to 90% [4] . This high infection efficiency contributes to the high expression of *ns2* in HepG2 cells [4] . The GFP marker carried by recombinant adenovirus also make it easy to observe the antiviral effect of natural product such as dioscin [5] .

FAK (focal adhesion kinase), also referred to as cell adhesion kinase (CAK)-B, related adhesion focal tyrosine kinase (RAFTK) or calcium-dependent protein tyrosine kinase (CADTK) [6] , belongs to the members of the FAK family of non-receptor protein tyrosine kinases. FAK has been shown to be closely related with cell adhesion, migration and growth regulation. Increased FAK expression has been correlated with increased cancer cell motility, invasiveness and proliferation [6] . FRNK is located at the C-terminal region of FAK. It doesn't have kinase activity or the autophosphorylation site Tyr-397 that is seen in FAK. FRNK is a dominant-negative inhibitor of FAK by competitive inhibition [7] . Recently, Cao et al., not only constructed a recombinant adenovirus that expresses murine FRNK [8] that can inhibit tumor cell motility and invasion but also a recombinant adenovirus that expresses human FRNK(hFRNK) [9] . The titer of these two recombinant adenovirus can be high up to 10^{12} PFU/ml, which facilitates the following research on the study of the function of FRNK. Colo320, a colonic carcinoma cell line, is difficult to undergo transfection with lipofectin. Cao et al., used a constructed adenovirus that expresses FRNK to infect the colo320 and studied the effect of FRNK on the phosphorylated 190RhoGAP and RhoA Activity in colo320WT colorectal cells. Cao et al., found that hFRNK can inhibit expression of phosphorylated p190RhoGAP and enhance RhoA activity in the cells stimulated with Gastrin17 [10] , which activates beta-catenin/Tcf-4 signaling in Colo320WT cells, thereby leading to an over-expression of c-myc and cyclin D1. This mechanism probably works with hFRNK blocking FAK phosphorylation and the FAK

pathway [11] . Cao et al., also found that adenovirus carrying hFRNK can inhibit abnormal distribution of E-cadherin and beta-catenin in the gastrin17-stimulated cells. The mechanism probably works similarly with hFRNK dephosphorylating FAK and blocking the FAK pathway [12] .

Generally, recombinant adenovirus is an efficient system that is characterized by high efficiency infection and high expression level of foreign genes. Furthermore, the important and critical advantage is that the high efficiency infection is cell independent, which enables us to study the effect of interested genes in most of type of cells without the previous constraint. The high expression level of foreign genes also can allow us to save cost on the carrier that transfers the foreign DNA into cells such as lipofectin.

Acknowledgement

We thank Liu Lisa for critically reading the manuscript. This work was supported by a grant from the health department of Hubei province (JX1B027) of China.

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