

Adeno-Associated Virus Vectors (AAV) Expressing Phenylalanine Hydroxylase (PAH)

Fenilalanin Hidroksilaz Eksprese Eden Adeno-İlişkili Vektörler

Ayşegül Akbay Yarpuzlu

Ankara University, Faculty of Medicine, Public Health, Ankara, Turkey

Abstract

Recent articles have appeared in the literature reporting use of adeno-associated virus vectors (AAV) expressing phenylalanine hydroxylase in animal trials and suggesting its use in treatment of phenylketonuria (PKU) as a form of gene therapy. However, agents used in gene therapy to deliver genes are not site-specific and DNA is may be put in the wrong place, causing damage to the organism. The adverse immunogenicity of AAVs also needs to be reconsidered. This letter is written to discuss present unreadiness for Phase I clinical trials of gene therapy of PKU. *Turk Jem 2009; 13: 18-9*

Key words: Gene therapy, phenylketonuria, adeno-associated vector, phase I clinical trial

Özet

Son zamanlarda, bazı yayınlarda adeno-ilişkili virüs ve vektörlerle (AAV) Fenilalanin Hidroksilaz geninin Fenilketonüri hastalarına klinik aktarımının yararlı olabileceğini düşündüren hayvan denemeleri yayınlanmıştır. Bu makale DNA'da bölgeye-ölgün integrasyon ve immün yan etkiler kavramlarını örnekleyerek, planlanacak Faz I klinik çalışmalar öncesi yapılması gereken diğer bazı gerekli diğer araştırmaları hatırlatmaktadır. *Turk Jem 2009; 13: 18-9*

Anahtar kelimeler: Gen tedavisi, fenilketonüri, adeno-ilişkili vektör, faz I klinik çalışma

Dear Editor,

Articles have recently appeared in the literature reporting the use of adeno-associated virus vectors (AAV) expressing phenylalanine hydroxylase (PAH) in animal trials and suggesting its use in treatment of phenylketonuria (PKU) as a form of gene therapy (1-3). However, agents used in gene therapy to deliver genes are not site-specific, and DNA is may be put in the wrong place, causing damage to the organism. Such effects have recently been observed with the phiBT1 phage during attempts to integrate the PAH gene into the human genome. It was observed that the *Streptomyces* phage phiBT1 integrase catalyzes recombination between phage attachment (att) attP and bacterial attB sites, resulting in phage DNA integration into the bacterial host genome in a unidirectional manner whereas, in the presence of multiple pseudo-attB and -attP sites in mammalian genomes, the recombination with wild-type attP and attB sequences occur on multiple sites. In fact, there were three pseudo-attP sites and two pseudo-attB sites in human cells, which are located in intergenic regions of five different chromosomes and are likely to end in non-site-specific integration (and

thus mutation) (4). Therefore, predicting preferential DNA vector insertion sites is very important to understand the implications for functional genomics and gene therapy for AAV vector systems as well (5-7). Knowing the variables that affect integration for various vectors as well as AAV subtypes will theoretically allow researchers to choose the vector with the most utility for their specific purposes.

The three principal benefits from elucidating factors that affect preferences in integration are as follows:

- in gene therapy, it allows assessment of overall risks for activating an oncogene or inactivating a tumor suppressor gene that could lead to severe adverse effects years after treatment;
- in genomic studies, it allows one to discern random from selected integration events;
- in gene therapy (as well as functional genomics), it facilitates design of vectors that are better targeted to specific sequences, which would be a significant advance in the art of transgenesis.

Thus it is advisable for the authors of animal studies attempting gene therapy of PKU by AAV that they FISH probe their AAV mediated gene integration sites most importantly on the human genome

using human cell lines, and go even further to sequence these sites to elucidate any sequential structural characteristics that will lead to specific or nonspecific integration. Otherwise, unforeseen toxicity may lead to irreversible consequences (8).

It should also be noted that there is considerable evidence about the adverse immunogenicity of AAVs. The mechanisms and means to cope with this immune side effect also need further investigation before any human pharmacological attempts.

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