

Approaches to the Study of the Pathogenesis of HIV Infection

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Abstract: Since its discovery over twodecades ago, much has been learned about HIV type 1 virus and molecular biology of viral replication cycle. This knowledge has been used to develop lentivirus based system wich have advantage over conventional retroviral replication systems [1]. Advances in our undestanding of the molecular mechanisms of viral replication cycle decide current approaches in selection of treatment strategy of the disease and cellular targets for anti-retroviral therapeutics [2]. Here we describe basic features of the HIV-1 rmodels. We also provide detailed information on propagation of HIV-1 derived lentiviral vectors, cell transduction protocol andmethod for evaluation of the titer of a lentivirus [3].

Key words: HIV % Lentivirus % Pathogenesis

INTRODUCTION

HIV: General Information: HIV-1 belongs to the genus Lentivirus, family Retroviridae. It is believed that HIV was introduced into human population about 70 years ago as a result of human bushmeat activity [2]. Of all HIV circulating in animals, HIV-1 is closest to simian immunodeficiency virus detected in chimpanzees (SIVcpz). It has been shown that some chimpanzee populations canserve as a reservoir for this virus. HIV-2 is less pathogenic and it, closely related to HIV-1 strain circulating in humans [2]. It is believed that HIV-2 is derived from the simian immunodeficiency virus because, genetically, this virus closely related to simian immunodeficiency virus type collar zone Mangobo (SIVsmm) [4]. There are many characteristics which differ HIV-1 and HIV-2 [5]. It has been shown that HIV-2 infections more often remain untreated as compared to HIV-1. Several serological, biochemical and molecular methods are used to detect HIV-1. However, these methods are specific for detection of HIV-1 and cannot be used to reveal HIV-2. Non-nucleoside reverse transcriptase inhibitors are effective to treat HIV-1, however, these drugs fail to block the reverse transcriptase of HIV-2 and therefore they are not effective for treatment of HIV-2 caused infection [5].

Study of the pathogenesis of HIV virus for example models.

***In vitro* Models:** Several groups of researchers developed mammalian cell based model to utilize lentivirus based vectors to grow HIV. This systems are not consider to be dangerous to staff and does not require special protection to work with. This model allows studying mechanisms of virus entry and integration of viral genetic material into the host genome. It has been demonstrated that this system is a good model for virus reproduction. Additionally, this model allows studying early stages of the pathogenesis of HIV infection such as cell entry, reverse transcription, chromosomal integration, etc. Also, this method can be used to evaluate activation of antiviral mechanisms in infected cells and study antiviral mechanisms of antiviral therapeutics [6-7].

Recently, a new method was developed for cloning of HIV fragments or complete HIV genome using yeast based system. Over 38 different genes and open reading framescan be inserted into the base vector pREC_nfl_ different HIV-1 by the yeast recombination technology. A total of 500 genes of HIV were cloned and inserted into chimeric viruses containing genes. These genes code for viral env, gag and pol genes. In this method, human cell lines are transfected with proviral DNA vector containing nonspecific gene and viral DNA PCR amplified from HIV-1 infected patient. Homologous recombination inside transfected cells generates HIV-1 genome and ultimately assembly the virus. Although this

method is relatively simple, there are few downsides hampering effective eukaryotic recombination: the low efficacy recovering the cloned gene, long time required for recombination to take place [8,9].

In vivo models. Animal model is used to study HIV pathogenesis. The best model so far involves anthropoid monkeys. However, the use of chimpanzees to study HIV is expensive; also, using these animals is controversial from the ethical point of view. Therefore, a "humanized mouse" model was developed to overcome these obstacles. "Humanized" mice immediately became accepted as a model for the preclinical testing of experimental drugs. The first "humanized mice" have been generated by crossing mice with congenital immunodeficiency. There is virtually no immune system in these mice and they could not reject human tissue. Additionally, a group of scientists successfully generated mice with humanized immune system. In these mice, human liver, thymus and bone marrow cells were transplanted from human embryos [10,11]. This model allows to carry out HIV studies previously not possible using other animal systems. [11]. Over the last decade several strains of immunodeficient mice have been developed where cells of the human immune system were engrafted, thus making these animals susceptible to HIV infection. In 2009, a team of scientists led by Dr. Garcia-Martinez demonstrated that antiretroviral drugs are taken before exposure to the virus may prevent vaginal transmission of HIV 2 in humanized mice. This suggests that if women have an increased risk of HIV transmission, they can take daily medication against the virus for protection from infection. This method of prevention is very promising as these drugs have been approved for the treatment of people with HIV and their safety has been well established [10,11].

In silico Models: The National Cancer Institute (NCI) maintains a database of chemical compounds, many of which are available as actual samples for testing in test tubes. There are some 230,000 compounds in total. Somewhere in this giant haystack of molecules, there might be one or more "lead" molecules for new HIV protease inhibitors. A "lead" molecule is one that is similar to a final drug, but must first be modified to make it less toxic or more soluble in water. Stage 1 of preclinical trial involved virtual screening of the 2,000 compounds in the NCI Diversity Set against 270 wild type and mutant HIV proteases searching for potential new leads and ultimately new drugs.

Recombinant Lentiviruses Biosafety Model: The data on the life cycle of HIV have been used to develop lentiviral vector systems which have several advantages before conventional retroviral vector systems. Replication incompetent lentiviral vector systems were developed therefore minimizing the risks associated with using replication competent virus. However, some experiments require utilization of replication competent HIV viruses. For example, HIV replication competent viruses are utilized for generation of conditionally replicative strains of HIV-1 as a future safe live attenuated vaccines and gene therapy. Also, these viruses could be used for designing mini-HIV variants as selective tumor viruses for viral therapy (virotherapy) against leukemia [2]. Several Genn vectors have been developed and used in experiments using different animal models [1].

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