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Panning-based Virus Receptor Screening with Cellular cDNA Library

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Abstract

Identification of virus receptors has been performed by various methods. We have developed panning-based, efficient cellular cDNA library screening methods to identify virus receptors. The principles of the methods are reviewed in this article.

Introduction

Among different organisms, many similar but non-identical cellular molecules exist that show the same functions. Even in a single species, the expressed molecules are dependent on cell types. This is the case with virus receptors, which are cellular components involved in viral attachment to and invasion into cells for viral replication [1, 2]. Thus, viral infections show specificity with regards to host ranges, tissues, and cell types, indicating that the identification of virus receptors is useful for the molecular explanation of the viral tropisms [3]. The identification of virus receptor has been performed by various methods, which are roughly classified into three categories: (I) speculation based on knowledge obtained from experiments [4-7]; (II) screening of libraries based on gain-of-function or loss-of-function criteria [8-10]; and (III) identification of interactive cellular molecules by peptide sequencing or mass spectrometry [11-13].

We have established an efficient, low-cost method for identification of cell surface molecules recognized by antibodies [14], in which cellular cDNA libraries were screened by a technique called “panning”. “Panning” is a classical experimental technique for separation of a specific subpopulation from a mix population [15] but can be applied to various fields with a variety of modifications. In the review, I would like to introduce our application of the panning to identify virus receptors, which belong to the category II described above with gain-of-function criteria.

1st generation panning for virus receptor identification

We have developed an efficient, low-cost cellular cDNA library screening method using a classical panning to identify virus receptors (hereafter referred to as 1st generation panning for virus receptor identification or simply 1st generation panning) [16-18]. In 1st generation panning, cellular cDNA libraries are stably expressed in non-adherent cell lines by using a retroviral vector, which integrates genes into cellular genomes with no or low cytotoxicity and do not inhibit cell growth, and screened based on colony formation of target cells on viral particle-coated dishes. From genome of colony-forming cells, cDNAs encoding molecules which confer adhesiveness onto viral particles to non-adherent cells are recovered by polymerase chain reaction (PCR) with primers targeting retroviral vector sequences flanking to cDNA library. Therefore, interaction between viral particles and surface molecules of target cells that is strong enough to trap target cells onto viral particle-coated dishes is a prerequisite for the method. The method was also applied to identify a receptor of a protozoa *Plasmodium falciparum* [19].

2nd generation panning for virus receptor identification

For viruses for which the interaction with target cells is not strong, we have developed another efficient screening method to identify virus receptors (hereafter referred to as 2nd generation panning for virus receptor identification or simply 2nd generation panning) [20, 21]. In 2nd generation panning, same as in 1st generation panning, cellular cDNA libraries are stably expressed in non-adherent cell lines by using a retroviral vector, but two additional non- or low-cytotoxic retroviral/lentiviral vectors which bear intended viral envelope proteins and whose genome encode either a membrane spanning protein or a fluorescence protein as a reporter are used for screening. Upon inoculation of library-expressing cells with a first screening vector encoding a membrane spanning protein, target cells would be specifically infected with the vector, express the reporter stably on cell surface, and form colonies

on anti-reporter antibody-coated dishes during cell culture. Because infrequent infection of non-target cells with the screening vectors would occur and result in colony formation as authentic target cells, resultant colonies are superinfected with a second screening vector whose genome encode a fluorescence protein to eliminate false positive. Fluorescent colonies are found out under a fluorescence microscope and inserted cDNA recovered by PCR like with 1st generation panning. The ability to produce moderate to high titers of retroviral/lentiviral vectors bearing intended viral envelope proteins is a perquisite for 2nd generation panning.

3rd generation panning for virus receptor identification

We have experienced cases in which some viruses did not meet the prerequisites for the above described two methods and for which virus receptors could not be identified by our methods. One of such examples was severe fever with thrombocytopenia syndrome (SFTS) virus [22]. In the current taxonomy SFTS virus is classified as follows: species, *Dabie bandavirus*; genus, *Bandavirus*; family, *Phenuiviridae*, and order, *Bunyavirales* (https://talk.ictvonline.org/taxonomy/). In infection of SFTS virus, non-evident cytopathic effects are characteristically observed in *in vitro* short cell culture [23-26]. The genome of the genus members is composed of three negative sense RNAs of large (L), middle (M), and small (S) segments, which encode viral proteins (RNA-dependent RNA polymerase, glycoprotein [GP], and nuclear and non-structural proteins, respectively). The rescue of SFTS virus with or without mutations from cDNA (reverse genetics) has been reported [27]; in that study, five plasmids expressing three anti-genome RNAs and two viral proteins (RNA-dependent RNA polymerase and nuclear protein) were used. As an application of the reverse genetics, a virus-like particle (VLP) assay was recently reported to assess the reassortment potential of SFTS virus with its related viruses [28]. By combining our 2nd generation panning for virus receptor identification [20, 21] and the reverse genetics for SFTS virus [27, 28], we recently succeeded in performing screening of SFTS virus receptors with a cellular cDNA library [22]. In the screening, infectious VLP (iVLP), which has most of SFTS virus components but is replication-incompetent due to the replacement of GP gene with a reporter gene for selection, is used like retroviral/lentiviral vector in 2nd generation panning for virus receptor identification [22]. Similar strategies might be applicable to SFTS virus-related viruses such as Heartland virus, Uukuniemi virus, and other viruses, if a prerequisite that iVLP produced shows moderate to high titers and shows no or weak cytopathic effects upon infection is fulfilled.

Conclusion

In this review, we introduced our efficient methods for identification of virus receptor. We hope the review will be useful for various viruses, while the methods are still being improved with some modifications.

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