Expression of Norovirus Virus-Like Particles in Different Systems

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Abstract. Noroviruses (NoVs) cause the great majority of epidemic nonbacterial gastroenteritis in humans. The purpose of this review is to provide the current status of NoV virus-like particles (VLPs) expression systems, including insect cell, bacteria, yeast, and plant cell expression systems. The application of NoV VLPs to vaccine development and pathogenesis studies will also be discussed.

Introduction

Noroviruses (NoVs), a member of the Caliciviridae family, cause acute non-bacterial gastroenteritis in all age groups, especially in children. Based on the amino acid sequence of the capsid protein, noroviruses are classified into five genogroups: GI to GV[1]. In spite of the genetic diversity, only a few strains, mainly genogroup II genotype 4 (GII.4), play a central role in acute gastroenteritis outbreaks in humans[2].

The NoV genome contains three open reading frames (ORFs), in which ORF2 encodes the 58-kDa major capsid protein VP1[3].The lack of an appropriate cell culture system and animal model has hampered the characterization of NoVs. Because recombinant VP1 capsid proteins self-assemble into empty virus-like particles (VLPs), which are morphologically and antigenically similar to authenticvirions[4], VLPs are considered to be used as a platform of drug delivery. In fact, VLPs have been utilized in the development of vaccines as well as in the studies of virus-cell interaction[5]. In this review, we provide several protocols for the expression of NoV VLPs, including baculovirus and other expression systems. Furthermore, the application of NoVs VLPs to vaccine development and biological studies will also be presented.

Baculovirus-insect cell expression system

Numbers of in vitro expression systems have been developed for the production of

NoVs VLPs, such as a baculovirus expression system. Balzevic[6] et al. extracted RNA of NoV GII.4 from the stool of a child with gastroenteritis in Finland. The reverse transcription polymerase chain reaction (RT-PCR) was used for cloning and amplifying a 1.6Kb DNA fragment, which contains the VP1 capsid gene ORF2. The VP1 was cloned into the pFastBac1 (Invitrogen, Carlsbad, CA) transfer vector. For generating a recombinant bacmid DNA, DH10BacTM competent E. coli cells was transformed with the transfer vector. Recombinant baculovirus (rBV) was assembled in Spodoptera frugiperda (Sf9) insect cells transfected with bacmid DNA. In order to produce and purify NoV VLPs, the infected Sf9 cells were subjected to sucrose gradient centrifugation. Many researches demonstrated antibody responses in animals against NoV VLPs. Even without using choleratoxin (CT) as an adjuvant, amarkedly high serum anti-NoV geometric mean titers (GMT):1168 was obtained at 200 μg of NoVs VLPs. When CT was utilized as an adjuvant, GMT

increased up to 6400[7]. Due to the practicability of the baculovirus-insect cell expression system, researchers from Finland produced six NoV VLPs belonging to GI and GII, respectively, through the recombinant baculovirus expression system for the development of NoV VLP-binding and blocking assays, which included human histo-blood group antigens (HBGAs) as ligands[8].

P domain expression with E. coli

Koho et al.[9] reported a different strategy for the preparation of an alternative diagnostic reagent to detect NoV infections. The NoV capsid protein, which is described earlier, contains two domains named shell (S) domain and protruding (P) domain. P domain consists of two subdomains, P1 and P2. It is assumed that the highly variable P2 subdomain contains antigenic determinants of host immunological responses[10]. As mentioned above, the preparation of NoV VLPs mostly relies on eukaryotic expression systems. Such protocol is, however, expensive and time-consuming. The easy-to-operate and economical bacterial production system has advantages in the development of vaccine as well as novel techniques in biomedical studies[9]. In a study by Koho et al., TOP10 Chemically Competent E. coli cells were transformed with pFastBac1 plasmid coding the P domain as described above. Ni-NTA affinity chromatography was used for the purification of the recombinant P domain protein. Consequently, the polyhistidine-tagged P domain proteins of GII.4 NoVs were purified at a yield of 6mg/l culture.

Expression of NoV Capsid Protein in Yeast

A relatively cost-effective yeast expression system was utilized by Xia et al, which provided self-assembled NoV VLPs[11]. As in the case for the production of VLPs through the baculovirus-insect cell system, a 1.6Kb DNA fragment coding the capsid protein was amplified by PCR using a cDNA clone as a template. The DNA fragment was cloned into a pPICZ-A vector. Yeast Pichia strains X-33, KM71H and GS115 were transformed with the vector plasmid using an electroporator. Cells were harvested by pelleting and purified by means of sucrose gradient centrifugation, and examined for the expression of the protein. Consequently, a vector (pPICZ-A) containing only NoV ORF2 was high expressed in yeast cells. It was noteworthy that significant immune responses were observed when either intramuscular or oral administration of the yeast-expressed NoV VLPs was subjected to mice.

More recently, a similar strategy was developed for the production and purification of the novel canine NoV VLPs[12]. In this research, Pereira et al. tested different S. cerevisiae strains, namely the diploid By4735 and the haploids CG379, for optimizing the production of recombinant VP1. The yeast cells were transformed through the standard lithium acetate protocol. A yield of 1.6-1.8 mg/L of VP1 capsid protein of canine norovirus was harvested, followed by the application to the equilibrated column. The VP1 sample was assessed by transmission electron microscopy and in-house ELISA assay, the results suggested that the VLPs morphologically and antigenically resemble authentic virus particles, which could be applied to the detection of antibodies against the novel canine norovirus.

Plants expression system

The expression of proteins using plants as highly efficient vehicles has been actively explored since 1989 for the production of pharmaceuticals. For instance, a functional monoclonal antibody was produced in transgenic tobacco[13]. The primary benefit of the use of plants for the production of NoV VLPs is that the tissues are edible. Thus, the strategy makes oral delivery of NoV VLPs possible without purification.

A plant-optimized norovirus capsid protein gene was introduced into tomato fruit using the plasmid pNV110 with the help of an electroporator, resulting in high yields of the protein, as high as 100 μg per gram of the freeze-dried fruit. Anti-NoV intestinal IgA was detected in all tomato-fed mice[14]. Subsequently, a novel transient expression system have been developed for producing the

NoV capsid protein exhibiting its immunogenicity[15]. TMV-derived system in N. benthamiana leaves provided 0.8 g of the assembled NoV capsid protein per kg leaf, when engineered plant viruses were utilized[16] (Figure 1).

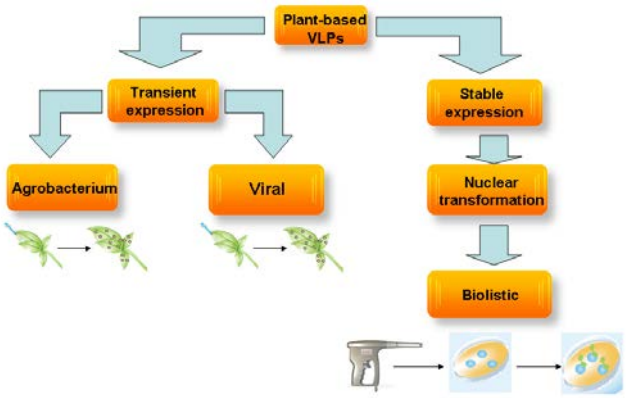

Figure 1. A visual summary of the different modes of protein expression in plants.

Conclusion

NoVs have an extremely low infectious dose and are highly stable in environment. In addition, the lack of convenient animal models has hampered the development of efficient therapeutics for NoV infections. Furthermore, there has been no appropriate in vitro assay systems to cultivate NoVs and assess NoV vaccines

Because NoV VLPs induce potent systemic and mucosal antibody responses and can be harnessed as versatile vectors for vaccination, we reviewed several protocols for the production of NoV VLPs, including baculovirus-insect cell, E.coli, yeast, and plant expression sytems.

Although the baculovirus-insect cell expression system appears to be the most efficient eukaryotic expression system, it is expensive to produce NoV VLPs at commercial scale. Bacterial expression system is generally simple and inexpensive. The bacterial NoV VLPs, however, fail to self-assemble into the naturally occurring structure[11]. The yeast expression system seems an alternative for the production of NoV VLPs, because it is relatively inexpensive, can form VLPs, and yields a relatively high level of protein production. As for the plant expression system, the biggest advantage is the possibility to develop oral delivery systems, because the proteins are expressed in plant tissues, which are easily edible.

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