Advanced Research in Gastroenterology & Hepatology
ISSN: 2472-6400

Juniper Publishers
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Mini Review
Volume 9 Issue 1 – February 2018
DOI: 10.19080/ARGH.2018.09.555752

Adv Res Gastroentero Hepatol

Hepatitis E Nanoparticle: A Capsid-Based Platform for Non-Invasive Vaccine Delivery and Imaging-Guided Cancer Treatment

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Submission: February 11, 2018; **Published:** February 15, 2018

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Abstract

Through nanotechnology, progressively, drug delivery, cancer treatment, and vaccination are moving towards personalization. With personalized and precision medicine being one of the major focus points of nanotechnology and nanopharmacological studies these days, there is a great need for an efficient nanocarrier delivery system. Several nanocarrier systems have been introduced including both natural and synthetic material; however, overcoming biological barriers, targeting accuracy, and encapsulation efficiency remain a challenge. Here we summarize the latest breakthroughs of protein-capsid-based nanocarrier, HEVNP, as a platform for non-invasive mucosal delivery for vaccination and delivery of therapeutics.

Keywords: Protein capsid; Hepatitis e vlp; HEVNP; Nanoparticle; Cancer diagnosis; Cancer treatment; Site-specific delivery; Mucosal delivery; Non-invasive delivery

Abbreviations: VLP: Virus-Like Particles; FDA: US Federal Food and Drug Administration; HBV: Hepatitis B Virus; HEV: Hepatitis E Virus; HIV: Human Immunodeficiency Virus; NP:Nanoparticles; HPV: Human Papillomavirus; ORF2: Open Reading Frame 2; pORF: Plasmid of Open Reading Frame

Introduction

Today's medicine revolves around a "standards of care" to employ the best approach towards treatment and prevention for the general public. Meanwhile, a growing trend is aimed towards exploring precision medicine to meet the needs for each individual, accordingly. Current vaccine administration regimens by parenteral routes (i.e. intramuscular or subcutaneous injections) are poorly met with patient compliance. Use of needles is not convenient and may result in pain, allergic reactions, infections, and even nerve damage [1]. Additionally, non-specific delivery of drugs leads to suboptimal results and high toxicity [2]. Thus, major efforts are invested towards the development of therapeutic delivery via non-invasive routes to increase patient compliance and enhance targeting accuracy.

Recent developments in nanotechnology, particularly in nanodelivery systems has elevated the significance of personalized and precision medicine. Despite tremendous effort in design and development of effective drug delivery systems, overcoming biological barriers and achieving successful accumulation of nanotherapeutics at targeted site remains a challenge [3]. Thus, the primary focus in both pharmaceutical sciences and nanotechnology is to 1) enhance drug targeting and delivery, 2) reduce toxicity, 3) and achieve greater safety and biocompatibility [4].

Discussion

Protein-based nanoparticles for delivery, targeting, and treatment

Compared to conventional drug formulations, nanoparticle-based drug delivery systems offer solutions for overcoming pharmacokinetic limitations and are advantageous in prolonging the lifetime of circulating drugs [5,6]. Despite the success of engineered delivery vehicles, major limitations such as low encapsulation efficiency, suboptimal targeting, and undesirable degradation productions, remain a challenge [7]. A potent and effective delivery system must be equipped with following characteristics: It must be safe, biocompatible, and biodegradable; have high encapsulation efficiency and surface modification properties to enhance targeting accuracy, while

offering protection and high retention of drug composition and bioactivity. Such delivery system must be reproducible and economically feasible.

Natural biomolecules such as protein are valuable alternatives, and even more advantageous compared to synthetic polymers or natural liposome-based drug delivery systems. Protein-based nanocarrier systems can be prepared under mild conditions and without the use of toxic chemicals or organic solvents [8]. They are unique due to their molecular recognition, bioavailability, and biodegradability into amino acids. The natural protein building blocks, the primary amino acid sequence, offers possibilities for modifications such as covalent conjugation of drugs and targeting molecules (Figure 1).

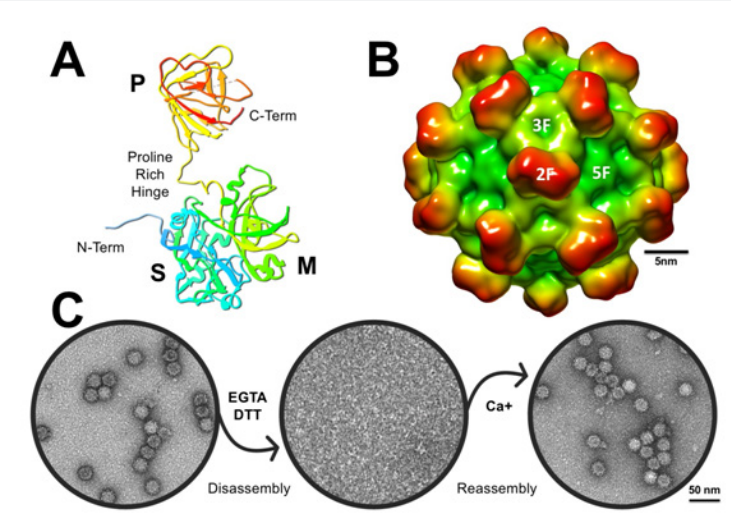

Figure 1: Hepatitis E Nanoparticle Structure, Disassembly, and Reassembly. A) HEVNP monomeric units showing the tertiary structure with S domain colored in blue, M domain in green, connected to the P domain yellow/red via the flexible proline rich hinge. In T=1 configuration, 60 identical copies of monomeric HEVNP subunits are arranged in an icosahedral lattice to form the nanocapsid. B) Surface rendering of HEVNP showing the 3-Fold (3F), 2-Fold, and 5-Fold symmetry axes. Protein capsid diameter is 27 nm. C) Process of disassembly and reassembly using EGTA (or EDTA) and DTT, and replenishment of Calcium to achieve reassembly.

Virus-Like Particles (VLP) have shown great promise in vaccination studies, as well as cancer diagnosis, targeting, and treatment [9-11]. The use of VLP-based vaccination as its own immunogen has been well-established in two FDA-approved vaccines against HBV in 1996, and HPV in 2006 [9]. In recent years, the research advancement of Hepatitis E viral nanoparticles (HEVNP) has shown great promise in encapsulation of both organic and inorganic material, as well as surface functionalization. Such genome-free capsids have been exploited as advanced drug delivery systems due to their highly symmetrical structure, bioavailability, and stability.

Hepatitis E nanoparticles

The virion-sized HEV capsid has a diameter of ~45 nm (PDB ID: 3IYO), assembled into an icosahedral cage in an RNA dependent manner, with 180 protruding arms [12]. Genomic modifications to ORF2 plasmid (pORF2; nucleotides 5145-7125), include a 111 amino acid truncation at the N-terminal and a 52 amino acid truncation at the C-terminal, which formulate an RNA independent self-assembled HEVNP with 60 protruding arms. The diameter of HEVNP is approximately 27nm [13,14]. The structure of HEVNP was resolved by x-ray crystallography and cryo-electron microscopy (PDB ID: 2ZZQ and 2ZTN) [15] (Figure 1).

The non-infectious and highly stable HEVNP, is comprised of three domains: S (shell domain; amino acids 118-317), M (middle domain; amino acids 318-451), and P (protrusion domain; amino acids 452-606) [12,16]. The S domain is the most conserved regions among HEV genotypes and along with M domain is responsible for the formation of HEV capsid. Moreover, the M domain interacts strongly with the P domain through a long proline-rich hinge. The P domain serves as the primary binding site for both cellular receptor and neutralizing antibodies. 60 repeated copies of the P domain on the surface of HEVNP, provide high accessibility for surface modulations that may include imaging molecules, tracking nanoparticles, targeting ligands, and immunogenic peptides [11].

Surface modulation: vaccine delivery and cancer targeting

Recently, HIV vaccine-encapsulated HEVNP was successful in mucosal delivery and immune response in mice [17], where Jariyapong and co-workers showed that the insertion of a short 15 amino acid peptide from the V3 loop of HIV-1 gp120 (p18) into the surface of HEVNP significantly lowered detection immune system response against HEVNP [18]. Moreover, the p18-HEVNP retained its icosahedral arrangement, suggesting that intermolecular forces between the recombinant nucleocapsid

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were not disrupted by the p18 insertion. The insertion was made in the antibody-binding site of the HEVNP, in the middle regions of pORF2, after the residue Tyr485. Spatial configuration of antigenic domains of HEVNP have been well characterized [19]. The chimeric HEVNP triggered an HIV-1-specific cytotoxic T-lymphocyte (CTL) response [18]. The reactivity of p18-HEVNP was tested by two antibodies, 447-52D and 224, which specifically target the V3 loop of HIV-1 gp120, and conformational epitope of WT HEV, respectively (Figure 2A).

With the success of p18 peptide insertion into the P domain of HEVNP, chemical activation of functionalized HEVNP for cancer targeting was explored as a more feasible tool for site-specific drug delivery. In 2014 Chen and colleagues carried out site-directed mutagenesis on the P domain Five residues were tested: Y485, T489, S533, N573 and T586C. These sites were selected based on their 3-dimensional location as well as the feasibility of sequence mutation to minimize potential distortion of the HEVNP assembly. N573C did not disrupt HEVNP formation and showed high accessibility [20]. Subsequently, a breast cancer targeting ligand LXY30, a cyclic peptide, with selective binding to α3 integrin, was chemically conjugated to the HEVNP-573C and administered into female SPF BLAB/c mice [20,21]. Real-time in vivo fluorescence microscopy confirmed that the chemically activated HEVNP-573C-LYX30 was delivered to the breast cancer tissue successfully. HEVNP-573C-LXY30 showed higher binding to cancer cell lines as well as tumor tissue compared to WT HEVNP [11,20] (Figure 2B).

Lately, Stark et al. [22] successfully conjugated nanogold clusters (AuNC) onto HEVNP-573C nanoparticles. The study used Au102 surrounded with 44 molecules of pMBA (here after Au102pMBA44) with a of diameter of ~2.5nm [23]. AuNCs were directly conjugated to the exposed 573C through maleimide-thiol ligand exchange. Computer enhanced molecular simulations and cryo-EM single particle analysis revealed that surface conjugated Au102pMBA44-C6 nanoclusters tend to concentrate at the axial center of the 5-fold [22] (Figure 2C). Such efficient conjugation design offers insight for future ligand designs towards theranostic applications in cancer treatment.

Encapsulation: delivery of nanotheranostics towards tumor treatment

Significant effort has been invested in demonstration of HEVNP's ability to encapsulate drugs, DNA, RNA, proteins, and even inorganic beads, such as ferrite oxide nanoparticles. This is achieved by disassembling the HEVNP, adding the cargo, and reassembling the protein capsid. Removal of calcium ions is the key factor to achieve disassembly by disrupting calcium bridges. Re-supplementation of calcium to the disrupted HEVNP constituents, and removal of reducing and/or chelating agents leads to reassembly of HEVNP [12,14].

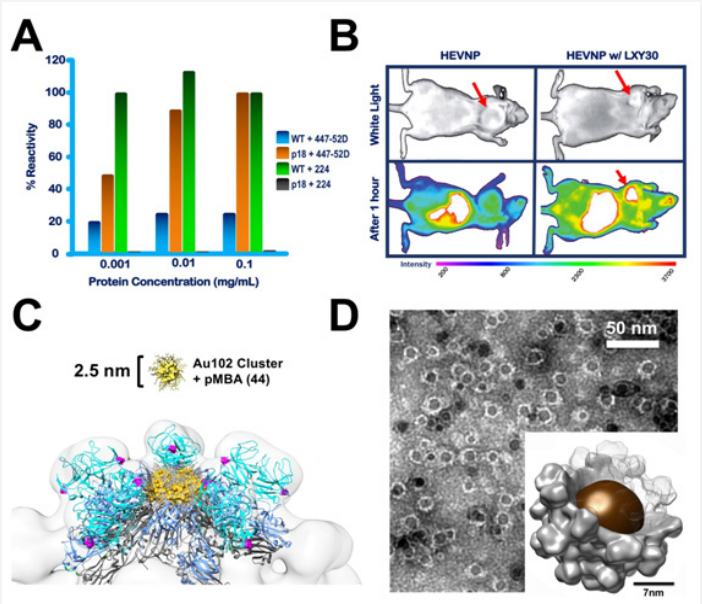

Figure 2: Recent achievements of HEVNP in vaccine delivery, tumor targeting, and encapsulation of nanotheranostics A) Reactivity of chimeric HEVNP with p18 insertion. Upon insertion of p18, HEVNP shows no reactivity to HEV-specific antibody 224. Additionally, HIV-1-specific antibody shows high reactivity to HEVNP-p18. B) In vivo imaging of breast cancer tumor targeting in mice. Without surface functionalization of HEVNP with targeting molecules, LXY30, HEVNP remain in circulation but does not accumulate at tumor site. With LXY30 conjugated to the surface of HEVNP, the nanocapsid accumulates at the tumor site. C) Surface functionalization of HEVNP with nanogold clusters, Au102. D) Encapsulation of ferrite oxide nanoparticles in HEVNP with average diameter of 30 nm.

In 2017, Chen and colleagues illustrated successful encapsulation of ferrite oxide particles with averaged diameter of ~15 nm, in HEVNP [24] (Figure 2D). This achievement is a milestone in development of tumor targeting strategies through hyperthermia treatment. Demonstration of encapsulation properties and highly modulatable surface properties make

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HEVNP an ideal candidate for vaccine delivery, tumor diagnosis, targeting, and treatment. Moreover, since Hepatitis E virus is feco-orally transmitted, by nature, its non-infectious counterpart, HEVNP is resistant to enzymatic and pH degradation and can be delivered at mucosally [10,24].

Conclusion

HEV is a non-enveloped, feco-orally transmitted RNA virus. The genetically modified, non-infectious HEVNPs retain the natural structural stability, antigenicity, and cell binding capabilities of HEV. Biochemical engineering allows for surface modifications to functionalize the capsid-platform. The controlled disassembly and reassembly properties of HEVNP opens avenues for encapsulation of various therapeutic agents, such as DNA or RNA for gene therapy, or magnetic particles for enhancing tracking signals as well as hyperthermia cancer treatment, and proteins or peptides for metabolic disease treatment.

Acknowledgement

The authors acknowledge the sponsorship of the funding to RHC by NIH grant numbers: A1095382, EB021230, CA198880, National Institute of Food and Agriculture, as well as Finland Distinguished Professor Program.

Conflict of Interest

The authors had no conflict of interest in this review article.

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