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# Virosome: A novel vector to enable multi-modal strategies for cancer therapy ☆

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ABSTRACT

Despite advancements in treatments, cancer remains a life-threatening disease that is resistant to therapy. Single-modal cancer therapy is often insufficient to provide complete remission. A revolution in cancer therapy may someday be provided by vector-based gene and drug delivery systems. However, it remains difficult to achieve this aim because viral and non-viral vectors have their own advantages and limitations. To overcome these limitations, virosomes have been constructed by combining viral components with non-viral vectors or by using pseudovirions without viral genome replication. Viruses, such as influenza virus, HVJ (hemagglutinating virus of Japan; Sendai virus) and hepatitis B virus, have been used in the construction of virosomes. The HVJ-derived vector is particularly promising due to its highly efficient delivery of DNA, siRNA, proteins and anti-cancer drugs. Furthermore, the HVJ envelope (HVJ-E) vector has intrinsic anti-tumor activities including the activation of multiple anti-tumor immunities and the induction of cancer-selective apoptosis. HVJ-E is currently being clinically used for the treatment of melanoma. A promising multi-modal cancer therapy will be achieved when virosomes with intrinsic anti-tumor activities are utilized as vectors for the delivery of anti-tumor drugs and genes.



**Contents**



## 1. Development of virosomes

### 1.1. Introduction

Numerous viral or non-viral vectors for gene transfer and drug delivery have been developed [1–5]. Viral vectors, such as retroviral vectors, adenoviral vectors, adeno-associated viral vectors, herpes

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viral vectors and vaccinia viral vectors have been used in gene therapy [6–8]. Various types of non-viral (synthetic) vectors such as liposomes, polymeric micelles, and polyethylene imines have also been developed for gene and drug delivery [9–11]. Each viral and synthetic system has its own set of advantages and limitations.

Viral methods of gene delivery to cells are generally more efficient than non-viral methods. One problem with non-viral vectors is the degradation of therapeutic molecules in endosome/lysosome before they reach the cytoplasm [12–14]. The nuclear translocation of therapeutic DNA is another problem that needs to be overcome in order to deliver effective gene therapy [14]. Various techniques have been developed to solve these problems. For example, pH-sensitive liposomes with dioleoylphosphatidylethanolamine [15] or DMRIE-C (a 1:1 mixture of N-[1-(2,3-dimyristyloxy)propyl]-N,N-dimethyl-N-(2-hydroxyethyl) ammonium bromide (DMRIE) and cholesterol) [16] fuse with the endosomal membrane to escape from the endosome. Polyethylenimine has been used for drug delivery because it can disrupt the endosomal membrane by a proton-sponge effect [17]. However, viral vectors readily deliver genes to the cytoplasm due to components that can fuse with the cell membrane or disrupt endosomes [18,19]. To enhance gene expression following the nuclear import of DNA by using non-viral vectors, conjugates of an SV40-derived nuclear localization signal peptide or a non-classical nuclear localization signal peptide of heterogeneous nuclear ribonucleoprotein have been employed [20–22]. The nuclear migration of plasmid DNA might be sequence-dependent [23] because sequence-specific binding with transcription factors facilitates the nuclear migration of exogenous DNA [24]. Although the nuclear import of therapeutic DNA is not feasible when using retrovirus vectors in non-dividing cells, it is easily achieved with adenovirus and lentivirus vectors [25]. Thus, some viral vectors are naturally equipped with the functional apparatus required for efficient gene delivery to cells. Extensive modifications have been tried to impart this capability to non-viral vectors. Although this approach for vector development is attractive, more modification of the vector system makes the vector more difficult for clinical use.

Viral vectors do not permit the delivery of agents such as proteins, synthetic oligonucleotides, and low-molecular weight compounds. Non-viral vectors have been widely used for the delivery of these types of therapeutic agents [9]. Furthermore, safety is a concern with viral vectors due to the concomitant introduction of genetic elements from parent viruses, as well as leaky expression of viral genes, immunogenicity, and changes in the host genome structure, whereas non-viral vectors are less toxic and less immunogenic [5,12].

To overcome the limitations of each type of vector system, virosomes equipped with chimeric viral and non-viral vector apparatus have been developed [14]. The idea is to compensate for the limitations of one vector system with the advantages of another. This approach enables efficient drug delivery and gene expression, while reducing the cytotoxicity of various vector components. Although virosomes have disadvantages such as immunogenicity and instability in the circulation, virosomes do have unique characteristics that seem to make them suitable for cancer therapy.

Bacteria are also experimentally used as carriers of drugs and genes. Non-pathogenic bacteria strains such as *Bifidobacterium longum* and *Clostridium* have been genetically engineered for use as gene delivery vectors [26–28]. The cytosine deaminase gene has been successfully delivered to cancer cells to achieve suicide gene therapy after administration of the non-toxic prodrug 5-fluorocytosine. Cancer tissues are targeted because the anaerobic bacteria accumulate in hypoxic tissues even after systemic administration. Based on a similar concept as virosomes, bacteria ghosts have also been developed. Empty bacterial shells of gram-negative bacteria can be produced by protein E-mediated lysis, which causes the fusion of the inner and outer membranes of the bacterial cells to construct intermembrane tunnels. Through the tunnels, all cytoplasmic contents of the bacteria are lost, but the inner and outer membrane structures remain intact. Doxorubicin is incorporated into the bacteria ghosts and delivered to cancer cells. Methods for the efficient incorporation of DNA into the ghosts are being developed. Although bacteria are attractive vectors, especially due to their targeting ability, bacterial systems for cancer therapy have not yet been well-established. Therefore, in this section, I focus on virosomes.

### *1.2. HVJ-liposomes*

Viral components are useful tools for the delivery of therapeutic molecules. Viral envelope proteins with membrane fusion ability have been utilized to increase the efficiency of drug delivery to cells. A fusogenic viral liposome with a fusogenic envelope derived from the hemagglutinating virus of Japan (HVJ; Sendai virus) was constructed [29]. HVJ is a mouse parainfluenza virus and is not a human pathogen. A hallmark of HVJ is its ability to induce fusion with the cell membrane. The HN (hemagglutinating) and F (fusion) proteins of the virus contribute to cell fusion [30]. HN binds to acetyl-type sialic acid bound to the terminal galactose residue at the α-2,3 linkage and degrades the sugar chain with its neuraminidase activity. Then, F associates with lipids, such as cholesterol, within the cell membrane to induce cell fusion. The F glycoprotein is first synthesized as inactive F0 in HVJ-infected cells and then cleaved by a host protease to produce the active F1 and F2 forms. F1 contains hydrophobic peptides of approximately 25 amino acids that induce cell fusion. Since acidic pH is not necessary for the fusion of HVJ with the cell membrane, the fusion occurs at the cell surface at a neutral pH. For fusion-mediated gene transfer, DNA-loaded liposomes were fused with ultraviolet (UV)-inactivated HVJ to form a fusogenic viral-liposome, HVJ-liposome, which is 400–500 nm in diameter.

Molecules of interest might be protected from degradation within endosomes and lysosomes by fusion-mediated delivery. When FITC-labeled antisense oligodeoxynucleotides (AS-ODN) against the decorin gene were introduced into human fibroblasts by using either HVJ-liposomes or lipoplex (Lipofectin), only the delivery by HVJ-liposomes suppressed the decorin expression. To elucidate the mechanism, the fluorescence resonance energy transfer system was used. More than 85% of ODN, which was labeled with two different fluorescent dyes at the 5′ and 3′ ends, remained intact within the nucleus following delivery by HVJ-liposomes, as compared to only 30% following Lipofectin-mediated delivery [31].

HVJ-liposomes have been widely used in animal studies of cancer treatment. Melanoma-associated antigen gene or RNA delivered by HVJ-liposomes injected into skeletal muscle or the spleen successfully evoked tumor-immunity to prevent melanoma growth [32]. Radio-inducible herpes simplex virus thymidine kinase gene driven by the early growth response-1 promoter enhanced the effects of cancer radiotherapy on hepatocellular carcinoma when delivered by HVJ-liposomes [33].

Reconstituted HVJ-liposomes can also be constructed. The HVJ virion is completely lysed with detergent. Then, the lysate is mixed with a DNA solution and various lipids. By removing the detergent with dialysis or column filtration, reconstituted HVJ particles containing DNA can be constructed [34–38]. Instead of using the entire HVJ virion, fusion proteins (F and HN) isolated from the virion can be added to the lipid/DNA mixture in the presence or absence of detergent. Since F protein is recognized by the asialoglycoprotein receptor on hepatocytes, reconstituted HVJ particles containing only F protein have been constructed to specifically target hepatocytes *in vivo* [36]. In another approach, envelope proteins, F and HN, have been purified from the HVJ virion and liposomes containing F and HN were constructed by the detergent-lysis-dialysis method [35,37,38].

Reconstituted fusion liposomes are as effective as conventional HVJ-liposomes, which contain fully intact HVJ virions, in terms of the delivery of FITC-ODN or the luciferase gene into cultured cells [38]. The LacZ gene can also be directly transferred into mouse skeletal