Production of Virus-Like Particles

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Virus-like particles based on retroviruses could be a potential envelope for safe and efficient drug formulations. Human endogenous retroviruses would make it possible to overcome the host immune response and deliver drugs to the desired target. PEG10 is a promising candidate that can bind to mRNA because it is secreted like an enveloped virus-like extracellular vesicle. *PEG10* is a retrotransposon-derived gene that has been domesticated.

Keywords: retroviruses ; virus-like particles ; drug delivery ; precise therapy

1. Introduction

Advances in the understanding of molecular disease mechanisms, biochemical processes, and molecular pharmacology have led to the understandable need to target specific cells or tissues and signaling pathways involved in disease progression. In line with this, advances in research and technology of drug development are paving the way to target specific cells or tissues and signaling pathways involved in disease progression and to develop tailored, advanced, and innovative drug formulations that can affect the specific site. High drug concentrations at the target site or receptor are achieved, side effects are minimized, and therapeutic efficacy in treating disease is dramatically increased. This precise therapy has advantages over conventional drug formulations, e.g., tablets or capsules [1]. Conventional dosage forms are associated with a number of issues such as low bioavailability, frequent use, side effects, or lack of patient compliance [2]. The use of nanoparticles, liposomes, or other available forms for formulations of drugs could help to overcome these issues. Nanoparticles could be engineered to encapsulate and deliver drugs to specific targets. The use of current nanotechnologies has enabled the development of nanoparticles that can release drugs in response to specific stimuli such as light, magnetism, or pH changes [3]. Precision medicine and pharmacogenomics will undoubtedly play a role in the discovery of tissue-, organ- or cell-specific drug formulations and in the development of customized therapies. Therapies will be tailored to minimize adverse drug effects but maximize therapeutic activity in individual patients according to their genetic information, family history, lifestyle, metabolic data, etc. [4].

Virus-like Particles (VLPs) as a Strategy for Targeting Specific Tissues and Cells

Drugs can often target non-specific areas and cause both desired and undesired pharmacological effects. This makes the discovery of new molecules a very complicated, costly, and unpredictable process [5]. For example, cytotoxic drugs usually do not distinguish between normal (blood marrow cells, skin, scalp, and stomach cells) and cancer cells that grow quickly. Thus, drugs have a toxic effect on healthy cells [6]. A large number of drugs are converted by hepatic enzymes into active compounds that can act on target receptors. Most of them are metabolized by the cytochromes P450 [7]. Mammals produce cytochromes P450 (CYP450) in various cells, tissues, and organs (liver, kidney, brain, heart, adrenal gland, skin, etc.). The cytochromes are membrane-bound enzymes with some exceptions [8]. Normally, the expression of CYP450s is localized in certain cells and associated with certain physiological functions of tissues and organs or cells, or they may be localized in different subcellular compartments. The endoplasmic reticulum and the mitochondria are the places where CYP450s can be found most frequently. CYP450s can also be detected at the outer nuclear membrane, various Golgi compartments, peroxisomes, and plasma membranes [8]. Targeting diseased cells could be a challenge. Healthy cells may have higher CYP450 activity, which means that drugs that target fast-growing diseased cells (such as cancer cells) may have an even higher effect on normal cells as well [9].

Direct delivery of the target-specific therapy by using virus-like particles (VLPs) is a promising strategy for precise therapy. There are various strategies for making treatment more efficient and avoiding adverse effects of drug therapy. The introduction of genes coding for drug-activating enzymes—GDEPT (Gene-Directed Enzyme Pro-Drug Therapy), the introduction of transgenes using viruses (vectors), called VDEPT (virus-directed enzyme prodrug therapy), and ADEPT (antibody-directed enzyme prodrug therapy), in which antibodies conjugated with an enzyme are used against target cells [10][11].

2. Viral Nanoparticles

Viral nanoparticles (VNPs) or non-infectious virus-like particles (VLPs) are being explored as self-assembling, efficient, and adaptable drug delivery systems used in various applications [12][13]. VNPs are nanomaterials that are derived from viruses (human, bacterial, plant). VLPs are genome-free structures of viruses [14]. VNPs are used for the encapsulation of drugs and their delivery through biological barriers to the target site where they are needed. However, host immunogenicity may remain an obstacle to the clinical use of certain VNPs. Recognition of viral components can trigger an immune response that leads to clearance of them from the organism [13].

Virosomes are a kind of nanoparticles. They were first produced after the inclusion of viral spike proteins in a liposome [15]. In 2018, Donaldson and colleagues defined enveloped VLPs as nanoparticles that lack the capsid protein [16]. Virosomes are usually obtained from viruses. Influenza viruses could be the most common source among them, due to the specific characteristics of interaction with human tissues and the immune system. It is known that these virosomes can induce human immune activity [17][18]. Therefore, the use of various viral vectors may be very practical for the development of drug delivery systems (formulations). Viral nanomaterials surpass the properties of synthetic nanoparticles used for medical applications. They possess an ability to cross biological barriers, interact with target cells and receptors, and evade the immune system more efficiently than synthetic biomaterials. However, to date, there is still no significant improvement in biomedical nanomaterials or formulations which are based on viral traits. Synthetic nanoparticles, compared to viral nanoparticles, are safer and more flexible for use [19].

3. Production of VLPs

Different platforms can be used for the production of VLPs, such as yeast [20] bacteria, insects, plants, mammalian cells, or even cell-free expression systems. The quaternary viral capsid proteins can be individualized according to the parameters of the expression system [21][22]. Chimeric VLPs can contain structural proteins from different viruses [23].

The most important steps in the production of VLPs are production (upstream processing), purification (downstream processing), and formulation [24][25][26]. The concept is based on the production of a clone of viral structural proteins and the expression of these proteins in self-assembling format in expression systems (bacteria, plants, mammalian cells). Cultivation and lysis (or mechanical disruption) of the cells is followed by clarification (filtration or precipitation), purification (main step, usually chromatography is used), and polishing of the target VLPs [27][28].

Bacterial cell cultures (such as *Escherichia coli*, *E. coli*) were investigated as a platform for VLP production [29][30]. Bacterial and yeast systems, however, may produce VLPs that can be contaminated with residual elements of the host cells such as nucleic acids, lipids, or proteins. These contaminants may stimulate a response in the human organism when treated [31]. The most suitable environment for correct VLP assembly is mammalian cell cultures. Mammalian cells are used to produce large proteins, but the introduction of the desired gene could be expensive and time consuming [32] [33]. CHO (Chinese hamster ovary cells) are preferred over human cells for the production of VLPs because they are not susceptible to human viral infections, but high contamination with fetal albumin could be another disadvantage [34][35]. Plant-based environments are suitable for the production of a large numbers of VLPs and cheap [36] and may enable one to avoid infection [37].

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