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Truncated Hepatitis B virus like nanoparticles: A novel drug delivery platform for cancer therapy

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ABSTRACT: Nowadays, Nano-sized drug delivery systems have been studied extensively for their potential in cancer therapy. Various drug nanocarriers are being developed including liposomes, micelles, and Virus like nanoparticles (VLNPs). VLNPs offer many advantages for developing smart drug delivery systems due to their precise and repeated structures and relatively large cargo capacities. Truncated Hepatitis B core antigen (tHBcAg) self-assembles into VLNPs which have been used widely as a nanocarrier for the development of multi-component vaccines. However, the potential of these VLNPs as a nanocarrier for developing a smart drug delivery system has not been studied for years. tHBcAg VLNPs are easily expressed in bacteria and formed VLNP from multiple copies of the same protein, as a result they are highly uniform. Recent studies have shown the capability of these nanoparticles for conjugation or/and packaging of the anti-cancer drug, and targeting to the cancer cells, suggesting the potentials of the tHBcAg VLNPs as novel promising nanocarriers for targeted and controlled drug delivery systems.

Keywords: *Cancer therapy, Controlled drug delivery systems, Nanocarriers, Virus like nanoparticles.*

INTRODUCTION

Nanotechnology has contributed specificity to the development of nanoscale materials and nanoparticles (NPs) with broad applications in life sciences as well as in the emerging field of nanomedicine. Recently NPs for the delivery of therapeutic/imaging agents have received considerable attention as a novel nanotechnology platform for cancer treatments [1,2]. The unique properties of these NPs promise to deliver targeted therapies more efficiently than today's medicines and with fewer adverse effects. They also show the promise to deliver a new generation of diagnostic reagents with higher signal-to-noise ratios than current imaging modalities [3]. To date, several classes of nanomaterials have been developed for cancer drug delivery, including liposomes, dendrimers, micelles, and hydrogels [2,4,5].

Recently, virus capsids, namely virus-like nanoparticles (VLNPs), have been recognized as a potential new

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class of bio-nanoparticles for biomedical nanotechnology applications and drug delivery system [6,7]. VLNPs consist of protein subunits that self-assemble to form a symmetrical nanostructure with defined exterior and interior surfaces [6]. VLNPs have a large surface-to-volume ratio compared to traditional delivery vehicles which offer a greater capacity for drugs and/or imaging reagents, and the ability to decorate VLNPs with specific ligands means these diagnostic and therapeutic payloads can be delivered to tumor cells [8].

EXPERIMENTAL SECTION

RESULT AND DISCUSSION

Truncated Hepatitis B core antigen (tHBcAg) as a nanocarrier

Truncated Hepatitis B core antigen (tHBcAg), an HBcAg mutant, produced in Escherichia coli self-assembles into VLNPs in discrete size and with a high degree of geometric symmetry and polyvalency [9,10]. tHBcAg VLNPs are very robust and preserve their integrity at 70 °C for more than 1 h. Due to their topology, tHBcAg NPs can be considered as a large dendrimer with the multiple copies of the asymmetric unit which provide consistently spaced attachment units [11]. As a result, different moieties can be attached and presented on the exterior surfaces of tHBcAg NPs which make them a useful nanoscaffold for chemistry, biomaterials, and functional display of ligands by bioconjugation [11]. Besides, tHBcAg VLNP has a hollow inner core of about 26 nm in diameter [12], equivalent to about an empty room of 8600 nm^3 [13] which can be used to package anticancer drugs. These characteristic along with ease of production and high yield products suggest that tHBcAg NPs can be exploited as carriers for drug delivery.

Conjugation of anticancer drug and targeting moieties to tHBcAg VLNPs

The specific amino acids present naturally in the VLNPs [14] or the genetically modified VLNPs [14,15] can be used as conjugation sites. These conjugation sites facilitate attaching of different cell targeting moieties and/or therapeutic drugs such as Doxorubicin (DOX) [16,17]. tHBcAg VLNPs possesses a series of amino acid residues including Asp, Glu, Lys and Cys, that contains several functional groups. These functional groups are protruding on the capsid surface or/and in the interior cavity which are normally charged and hydrophilic [9]. In our previous study, we showed that these functional groups can be used for chemical modifications throughout bioconjugation in order to develop targeted drug delivery system for cancer treatment [11]. About 1600 DOX molecules were directly coupled covalently on the external surface of each tHBcAg VLNP via carboxylate groups using the EDC/Sulfo-NHS method. The VLNPs were then conjugated covalently with folic acid (FA) to target them to cancer cells over-expressing folic acid receptor (FR). Transmission electron microscopy proved that the tHBcAg VLNPs preserved their integrity during the conjugation of DOX and FA to these particles. The cytotoxicity and cellular uptake of conjugated tHBcAg VLNPs were evaluated by MTT assay, live cell imaging, and quantified using spectrophotometer. The result indicated that the conjugated VLNPs improved the uptake of DOX in the human cervical cancer cell line and increased the cytotoxicity of DOX in these cells. However, these nanoparticles were less toxic to the 3T3 cells. The results revealed that of conjugated tHBcAg VLNPs nanoparticles enhance cellular uptake and therapeutic efficacy of DOX in cancer cells while minimizing the side effects of DOX to normal cells [11].

Loading anticancer drug into tHBcAg VLNPs

It has been shown that tHBcAg VLNP can be simply subjected to dissociation and re-association process [18]. These attractive biophysical properties make the tHBcAg VLNP a potential nano-container to package gene and/or therapeutic molecules freely in its interior room. Cargo accommodated by this method can be captured easily and released from the nanoparticles at a targeted destination. Fluorescein labelled oligonucleotides and green fluorescent protein (GFP) were packaged into the tHBcAg VLNPs by this method [18,19]. These biophysical properties have made the tHBcAg VLNP a potential nanocarrier to package drug molecules. By taking advantage of these prop-

erties, we developed a pH-responsive drug delivery system based on tHBcAg VLNP [20]. DOX was mixed with polyacrylic acid (PAA) and loaded into the tHBcAg VLNPs during the re-association of the nanoparticles. About 946 DOX molecules were packaged in each tHBcAg VLNP. FA was then conjugated to the loaded tHBcAg VLNPs to increase the specificity of the VLNPs. The loaded tHBcAg demonstrated a sustained anticancer drug release profile in vitro under tumour tissue conditions. The release of drug was in a controlled manner. The loaded tHBcAg VLNPs significantly increased the accumulation of DOX in the colorectal cancer cells and improved the uptake of DOX, leading to enhanced antitumor effects. The result indicated that the anticancer drug can be loaded inside the tHBcAg VLNPs without any modification and the pharmacological activity of the loaded DOX is preserved [20].

CONCLUSIONS

The tHBcAg VLNPs have been widely exploited as nanocarriers to display foreign immunological epitopes in multicomponent vaccines and diagnostic reagents; however the potential of the HBcAg VLNPs as a nanocarrier for the delivery of drugs and genes has not been fully studied yet. The findings from our studies indicated that the anticancer drug can be conjugated to or encapsulated in VLNPs for the destruction of cancer cells, suggesting that these smart nanocarriers could serve as promising candidates in targeted therapy against cancer cell lines, and may open an avenue for cancer therapy.

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