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Foot-and-mouth disease virus-like particles as integrin-based drug delivery system achieve targeting anti-tumor efficacy

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Abstract

The surface of foot-and-mouth disease virus (FMDV)-like particles (VLPs) contains a conserved arginine–glycine–aspartic acid (RGD) motif. Natural FMDV specifically attaches to overexpressed integrin receptors in several cancer cells. The FMDV VLPs produced in *Escherichia coli* were used for the first time as a delivery system of anti-tumor drug doxorubicin (DOX). The DOX-loaded VLPs exhibited a distinct release profile in different physiological conditions. The effects of FMDV-VLPs-DOX on cellular internalization and viability were evaluated *in vitro* by cell imaging, MTT assay and apoptosis, respectively. The anti-tumor efficacy *in vivo* was also determined in a nude mouse xenograft model based on tumor volume/weight and histological changes. The FMDV-VLPs-DOX complex significantly inhibited the proliferation of tumor and improved the pathological damage of DOX to non-targeting tissues. All results supported the potential of FMDV VLPs as a platform for specific targeted delivery of drugs or chemical reagents.

Key words: FMDV VLPs; Integrin receptor; Drug delivery; Dox; Tumor therapy

Traditional chemotherapy is the major choice for tumor treatment, but its effectiveness is restricted by the toxic side effects of chemical drugs. Properly designed delivery systems are essential to reduce non-specific distribution and toxicity of chemotherapeutics in healthy organs and increase drug accumulation in tumor tissues.[1,2] Virus-like particles (VLPs) exhibit well-defined structures and can be chemically or biologically engineered in several ways. Therefore, these particles are uniquely attractive platforms for drug delivery applications. Thus far, several plant viruses have been proven safe and non-toxic based on the results of *in vivo* studies[3,4]; such viruses include the cowpea mosaic virus,[5,6] red clover necrotic mosaic virus,[7] hibiscus chlorotic ring spot virus,[8] and cucumber mosaic virus.[9,10] However, VLPs of plant virus are noninfectious toward mammals and must be modified with a specific ligand recognized by the receptor on the tumor cell surface to achieve the targeted delivery. Several researchers have explored animal virus-based VLPs as delivery systems to avoid additional modifications. Animal VLPs that naturally display large numbers of cell- or tissue-targeting ligands have considerable potential as targeted delivery systems. Animal VLPs are also useful vectors for conjugation of imaging moieties and/or therapeutic agents in a well-defined manner through various chemical means.[11–13] The particles will improve the targeting treatment and reduce the side effects of the drug because of their targeted binding to tumor tissues/cells by mimicking the native

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virus that infects the host cell; the binding is mediated by cell receptors and ligand interactions. For example, canine parvovirus VLPs containing transferrin were used in a targeted delivery system based on the recognition of transferrin receptors that are overexpressed in tumor cells.[14–16] However, the use of foot-and-mouth disease virus (FMDV) VLPs as nano-carriers for chemotherapy agents has not been reported yet. In the present study, the native features of FMDV VLPs were exploited to develop a targeted delivery system for the anti-tumor drug doxorubicin (DOX).

FMDV is the type species of the *Aphthovirus* genus of the *Picornaviridae*, which are non-enveloped virus particles that consist of 60 copies (each) of four virus-encoded structural proteins, namely, VP1 to VP4. These proteins form an icosahedral capsid, which encloses a single-stranded positive-sense RNA genome. A major structural feature of FMDV capsid is a surface-exposed, flexible conformational GH loop of VP1, which includes a highly conserved RGD tripeptide motif. Several studies indicated that melanoma cells express high levels of integrin receptors, especially αvβ3 and αvβ5; several integrins recognize the RGD sequence in their matrix proteins.[17,18] The integrin receptors are mostly undetectable in normal adult kidney, lung, and skin but detected at high expression levels in several types of carcinoma; thus, these receptors play a central role in tumor cell invasion and metastasis.[18,19] Therefore, the integrin receptors are regarded as promising targets for cancer treatment.[20–22] Consequently, some anticancer drugs are loaded in some carriers with RGD-targeting ligands to enhance drug delivery and achieve the growth inhibition of cancer cells; in this technique, integrin trafficking can be effectively targeted for clinical applications, which has long been one of the major issues in cancer therapeutics.[23–25] Therefore, FMDV VLPs with the RGD motif in their matrix proteins have the potential to be developed into a targeting drug delivery system. Fortunately, FMDV is an animal pathogen, whereas virus–host interactions occur in mammalian cells but cannot cause serious diseases in humans. Therefore, FMDV VLPs are safe for humans and may have broad biological uses in medicine.

Our results confirmed the potential of FMDV VLPs as a platform for specific targeted delivery systems. DOX was first covalently conjugated to FMDV VLPs. The resulting FMDV-VLPs-DOX particles were then characterized based on size and stability. Furthermore, the biotechnological potential of VLPs–DOX was assessed by targeting HeLa cells *in vitro* and *in vivo*. This study provides a basis for the scientific community to manipulate FMDV VLPs for diverse purposes.

Methods

Materials

Feline kidney (F81) cells and human cervical cancer (HeLa) cells (American Type Culture Collection, ATCC, USA) were cultured in DMEM containing 10% (v/v) fetal bovine serum and 1% (w/v) penicillin–streptomycin at 37 °C with 5% CO_2. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxy sulfosuccinimide (NHS), and doxorubicin hydrochloride (DOX) were purchased from Sigma-Aldrich (USA). 4,6-Diamidino-2-phenylindole (DAPI) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenltetrazolium bromide (MTT) were obtained from Promega (USA). Anti-caspase-3 and anti-total poly(ADP-ribose) polymerase (PARP) rabbit monoclonal antibodies were acquired from Cell Signaling Technology (USA). All other chemicals used were of analytical grade.

Expression and assembly of FMDV VLPs

The expression and assembly of FMDV VLPs by prokaryotic system were established in our laboratory according to previously described methods.[26] Proteins were detected by SDS-PAGE and Western blot analyses. The FMDV VLPs were confirmed by dynamic light scattering (DLS; Malvern Zetasizer-Nano ZS90, Britain) or transmission electron microscopy (TEM; FEI Tecnai G2 F20 S-Twin transmission electron microscope, USA).

Preparation of DOX-loaded FMDV VLPs

The concentration of the FMDV-VLPs was measured by the Coomassie plus Protein Assay (Thermo Fisher Scientific Inc. Madison, WI, USA) and adjusted to 2 mg/mL. The FMDV-VLPs-DOX conjugation was obtained by the following method. Briefly, 8 mg of EDC (~40 mM) and 12 mg of NHS (~100 mM) were added to 1 mL of FMDV-VLPs (2 mg/mL) solution and the pH value was adjusted to 6.0. After the 0.5 h reaction at room temperature. 2 mg of DOX (dissolved in PBS buffer) was added to the activated FMDV-VLPs and the pH value was adjusted to 8.0. The final FMDV-VLPs-DOX complex was dialyzed against a 10 mM sodium phosphate buffer pH of 8.0 for 24 h at 4 °C after the reaction with gentle stirring for 2 h at room temperature, using 10 kDa molecular weight cutoff membranes (Sangon Biotech, China) to remove the excess unbound DOX. The percentage of DOX in the complex was measured with a UV–Vis spectrophotometer by using standard curve method (data not shown).

Characterization of FMDV-VLPs-DOX complex

The size distribution of the FMDV-VLPs-DOX complex was measured by DLS on a Malvern Zetasizer-Nano ZS90. The fluorescent absorbance was analyzed with the UV–Visible Spectrophotometer BioMate 3S (ThermoFisher Scientific, Inc., Madison, WI, USA). The UV absorbance spectra of DOX, FMDV VLPs, and FMDV-VLPs-DOX complex were recorded from 250 nm to 700 nm. The morphology of FMDV-VLPs-DOX complex was observed by TEM.

Detection of DOX release in vitro

The release of DOX from the FMDV-VLPs-DOX complex *in vitro* was detected by monitoring the quantity of DOX at different pH values (5.0, 6.0, 7.0, and 8.0) or different temperatures at pH 7.4, in which 37 °C was the bloodstream environment temperature and 4 °C was the control temperature. The detailed protocol was as follows: The FMDV-VLPs-DOX complexes were placed in dialysis cassettes (MWCO 10 kDa) and dialyzed at 4 °C. The remaining DOX in each dialysis cassette was quantified by UV–Vis spectrophotometer (495 nm) at different time points, including 4,