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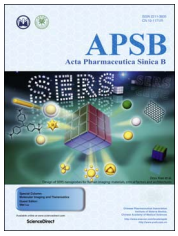

ORIGINAL ARTICLE

Gold nanorods together with HSP inhibitor-VER-155008 micelles for colon cancer mild-temperature photothermal therapy

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Abstract Enhancing the heat-sensitivity of tumor cells provides an alternative solution to maintaining the therapeutic outcome of photothermal therapy (PTT). In this study, we constructed a therapeutic system, which was composed of methoxy-polyethylene-glycol-coated-gold-nanorods (MPEG-AuNR) and VER-155008-micelles, to evaluate the effect of VER-155008 on the sensitivity of tumor cells to heat, and further investigate the therapeutic outcome of MPEG-AuNR mediated PTT combined with VER-155008- micelles. VER-155008- micelles down-regulate the expression of heat shock proteins and attenuate the heat-resistance of tumor cell. The survival of HCT116 cells treated with VER-155008- micelles under 45 °C is equal to that treated with high temperature hyperthermia (55 °C) *in vitro*. Furthermore, we proved either the MPEG-AuNR or VER-155008- micelles can be accumulate in the tumor site by photoacoustic imaging and fluorescent imaging. *In vivo* anti-cancer evaluation showed that tumor size remarkably decreased (smaller than 100 mm^3 or vanished) when treated with combing 45 °C mild PTT system, which contrasted to the tumor size when treated with individual 45 °C mild PTT (around 500 nm^3) or normal saline as control (larger than 2000 nm^3). These results proved that the VER-155008- micelles

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can attenuate the heat-resistance of tumor cells and enhance the therapeutic outcome of mild-temperature photothermal therapy.

1. Introduction

Although chemotherapy is one of the widely used clinical treatments for cancer[1] and hundreds of chemodrugs have been developed and applied in tumor therapy[2], the side-effects, auxiliary toxicity and the high risk excipients in traditional formulations limit the application of chemodrugs[3]. Finding an effective therapy with low side-effects deserves attention. The small-molecule inhibitors with low adverse reaction provide an alternative choice for cancer therapy, and the research and development of small-molecule inhibitors have been highlighted[4,5].

Small-molecule inhibitors inhibit tumors by inhibiting specific DNA, nucleic acid and proteins that relate to tumor growth and metastasis[6–8]. Unlike the non-targeting side-effects of chemodrugs, the small-molecule inhibitors specifically suppress the growth of the tumor cells with low adverse reaction[9]. Therefore, small-molecule inhibitors have attracted enormous attention in recent years[10–12]. VER-155008 is one of these potential small-molecule inhibitors, which can promote apoptosis to take place in the tumor cells by specifically reducing the expression of heat-shock proteins 70 and 90 (HSP70 and HSP90)[13–16]. As a member of the HSP family, HSP70 and HSP90 are involved in the folding and function of several proteins and essential for tumor cell survival by regulating the expression of oncogenic client proteins like RAS, p53 and AKT.[17] Besides, overexpression of HSP in the tumor site results in inefficiency of photothermal therapy (PTT) due to the tolerance of tumor cells to heat stress[18]. Therefore, reduction of HSP70 and HSP90 in tumor cells not only promotes the cells apoptosis, but also improves the heat-sensitivity of tumor cells[19–26]. Therefore, combining the PTT with VER might be a feasible way to enhance the anti-tumor effect[17]. However, the bioavailability of VER is also restricted by its hydrophobic character, similar to some other hydrophobic anti-tumor drugs[15,27].

Nano drug delivery systems have been applied to overcome the hydrophobicity of the cargo and enhance their delivery efficacy to the tumor site though the enhanced permeability and retention (EPR) effect[28–30]. More than 40 kinds of nano-formulations have been studied in clinical trials[31]. By being encapsulated into nanocarriers, the system improves the solubility of drugs, prevents renal clearance, promotes longer circulation in blood, responsiveness and enhances passive targeting to solid tumor sites[32–35]. Among numerous kinds of materials for nanocarrier construction, amphiphilic block copolymer methoxy polyethylene glycol-poly (D,L-lactic acid) (MPEG- PDLLA) has been approved by FDA in preparing the DTX micelle, which has been launched into the market in South Korea[36–41]. Bearing both hydrophobic and hydrophilic blocks within same polymer chain, amphiphilic copolymers can self-assemble and form different types of nanoparticular structures such as micelle[42]. Due to its superior drug loading ability and biocompatibility, we expected that MPEG-PDLLA could be used to load VER-155008 to form VER-155008 micelles (VER-M), therefore improving VER's water solubility and prolonging its blood circulation time. The introduction of VER-M may be more suitable for enhancing the therapeutic outcome of low temperature PTT (which is also called mild PTT)[43].

PTT is an efficient therapeutic process with low side-effects for cancer therapy[44,45]. Optically sensitive materials which exhibit efficient photothermal conversion can be used as PTT agents to generate heat under laser irradiation to ablate cancer[46,47]. Among different kinds of PTT agents, different types of gold nanoparticles (AuNPs) have long been a topic of intense research due to their size-related electronic, magnetic and optical properties[48], which was used for antibacterial, biosensing, imaging and cancer treatments[49]. Gold nanorod (AuNR) is one of the most effective agents in all these AuNPs for photothermal conversion[50–52]. By surface modification with MPEG-thiol (MPEG-SH, 5000 Da), more biocompatible AuNR (MPEG-AuNR) can be obtained[57] to overcome toxicity of AuNR[53]. Owing to the surface coating with MPEG and the EPR effect, MPEG-AuNR is more likely to accumulate in tumors[54]. Furthermore, AuNR can also be easily surface modified to functionalize[55,56]. MPEG-AuNR is a suitable agent for PTT.

According to the therapeutic temperature, PTT can be divided into hyperthermia ($>45\,^{\circ}C$) and mild PTT ($\leq 45\,^{\circ}C$)[58]. Although hyperthermia is usually more effective, adverse risks, including amatory disease and tumor metastasis, can happen. On the contrary, mild PTT has low side-effects but the ineffective therapeutic outcome as a result of high expression of HSPs remains a challenge[18]. Besides, improving the penetration depth of 700–950 nm NIR energy source to activate phototherapy is still desired, which lead to low temperature PTT[59]. Thus, it is imperative to attenuate heat-resistance in mild PTT by using an HSP inhibitor, and VER-M can be used as one of the potential high-performance HSP inhibitors[60].

Therefore, in this study, we plan to construct VER-M to evaluate the effect of HSP inhibition on the therapeutic outcome of MPEG-AuNR mediated low temperature PTT. MPEG coated AuNR (MPEG-AuNR) was used as the PTT agent for mild PTT. VER-M and MPEG-AuNR were characterized in detail. Both nano-sized VER-M and MPEG-AuNR could accumulate preferentially in tumor tissues through the EPR effect after intravenous injection as shown in Fig. 1, and then tumor cells were inhibited by a tumor inhibitor and enhanced mild PTT. The tumor inhibiting rate of these systems was further studied *in vitro* and *in vivo*. The inhibition of HSP70 and HSP90 expression was studied by western blot. To summarize, MPEG-AuNR@VER-M is a promising strategy for tumor inhibition.

2. Materials and methods

2.1. Materials

Sodium borohydride (NaBH4), ascorbic acid, tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$), *N*-cetyltrimethylammonium bromide (CTAB), DAPI, methyl thiazolyltetrazolium (MTT)

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