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Stem cell-based gene therapy activated using magnetic hyperthermia to enhance the treatment of cancer

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ABSTRACT

Stem cell-based gene therapies, wherein stem cells are genetically engineered to express therapeutic molecules, have shown tremendous potential for cancer applications owing to their innate ability to home to tumors. However, traditional stem cell-based gene therapies are hampered by our current inability to control when the therapeutic genes are actually turned on, thereby resulting in detrimental side effects. Here, we report the novel application of magnetic core–shell nanoparticles for the dual purpose of delivering and activating a heat-inducible gene vector that encodes TNF-related apoptosis-inducing ligand (TRAIL) in adipose-derived mesenchymal stem cells (AD-MSCs). By combining the tumor tropism of the AD-MSCs with the spatiotemporal MCNP-based delivery and activation of TRAIL expression, this platform provides an attractive means with which to enhance our control over the activation of stem cell-based gene therapies. In particular, we found that these engineered AD-MSCs retained their innate ability to proliferate, differentiate, and, most importantly, home to tumors, making them ideal cellular carriers. Moreover, exposure of the engineered AD-MSCS to mild magnetic hyperthermia resulted in the selective expression of TRAIL from the engineered AD-MSCs and, as a result, induced significant ovarian cancer cell death *in vitro* and *in vivo*.

1. Introduction

Ovarian cancer currently ranks fifth in cancer mortalities among women and is the leading cause of death from gynecological malignancies [1]. The conventional mode of therapy for this cancer consists of cytoreductive surgery, followed by adjuvant platinum/taxane-based chemotherapy [2]. However, while most ovarian cancer patients exhibit initial sensitivity to chemotherapy, over 70% of these patients are diagnosed at an advanced stage, when the tumors have already metastasized throughout the peritoneal cavity [3]. As a consequence, the majority of ovarian cancer patients experience recurrence within 18–24 months of treatment and only 20% of them survive longer than 5 years after their initial diagnosis [4].

To enhance the treatment of advanced cancers, such as advanced ovarian cancer, mesenchymal stem cell (MSC)-based therapies have emerged as an attractive alternative that can overcome the limited tumor-targeting ability of conventional treatments [5]. MSCs have the intrinsic ability to self-renew and differentiate into multiple lineages including osteoblasts, chondrocytes, and adipocytes [6]. More importantly, several groups have demonstrated that these stem cells have the innate ability to migrate to tumors including ovarian tumors/metastases, even following systemic administration [7–10]. While the exact mechanism is still being elucidated, this tumor tropism has prompted the development of stem cell-based gene therapies, wherein MSCs are genetically engineered to express therapeutic molecules and therefore, act as targeted delivery vehicles to enhance our ability to treat metastatic cancers [11,12].

To this end, a number of therapeutic molecules have been investigated, including direct effectors of apoptosis, such as the cytokines, interferon-β (IFN-β) [13], and tumor necrosis factor-

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related apoptosis inducing ligand (TRAIL) [14], as well as more indirect immunomodulatory molecules like interleukin 12 (IL-12) [15]. Of these, TRAIL is a particularly attractive therapeutic candidate owing to its ability to selectively induce apoptosis in malignant cells, but not in most normal cells [16]. For example, Mueller et al. reported that multipotent MSCs that were genetically engineered to express TRAIL were able to induce apoptosis and inhibit the growth of colorectal carcinoma cells *in vivo* with no serious observable side effects [17]. Similarly, Loebinger and colleagues demonstrated that MSCs engineered to produce and deliver TRAIL could induce apoptosis in lung, breast, squamous, and cervical cancer cells [14]. Importantly, these engineered MSCs were able to significantly reduce tumor growth in a subcutaneous breast cancer xenograft and could home to and reduce lung metastasis. However, while TRAIL has largely been demonstrated to be biocompatible with normal cells, there have been a number of reports indicating potential hepatotoxicity upon treatment with TRAIL, thereby greatly dampening its clinical potential [18,19]. As such, to limit these potentially detrimental side effects and in order for stem cell therapies to reach their full potential, there remains a pressing need for approaches that can allow for the precise spatiotemporal control of therapeutic gene activation such that the engineered stem cells only express their therapeutic payload once they have reached the targeted tumor sites.

Herein, we report the novel application of magnetic core–shell nanoparticles (MCNPs), composed of a highly magnetic zinc-doped iron oxide ($ZnFe_2O_4$) core and a biocompatible mesoporous silica (mSi) shell, for the dual purpose of delivering and activating a heat-inducible gene vector that encodes a secretable form of TRAIL in MSCs (Fig. 1). For this purpose, we developed a plasmid with the heat shock protein 70B' (HSP70B′) promoter (Fig. 1B), which has previously been demonstrated to be more heat-specific than other heat shock promoters [20]. As such, the MSCs can first be engineered with MCNPs that are complexed with the heat-inducible TRAIL plasmid *in vitro*. Afterwards, following *in vivo* injection and migration of the engineered MSCs to the targeted tumor sites, TRAIL expression can be specifically activated via the induction of mild magnetic hyperthermia (~41 °C). In this report, we demonstrated the efficient and biocompatible uptake of MCNP-plasmid complexes into MSCs. In particular, we observed that the engineering process had no significant effects on MSC proliferation or differentiation. Moreover, the engineered MSCs retained their tumor tropism towards disseminated peritoneal ovarian cancer xenografts. Importantly, we demonstrated that mild magnetic hyperthermia, via exposure of the engineered MSCs to an alternating magnetic field (AMF), could be used to specifically raise the intracellular temperature to ~41 °C, which resulted in the selective expression of TRAIL in the engineered MSCs. As a result, significant ovarian cancer cell apoptosis and death was observed *in vitro* and *in vivo*. Overall, by combining the tumor tropism of MSCs with the spatiotemporal MCNP-based delivery and activation of TRAIL expression, this platform provides an attractive means with which to enhance our control over the activation of stem cell-based gene therapies.

2. Materials and methods

2.1. Nanoparticle synthesis and characterization

The synthesis of $ZnFe_2O_4$ magnetic nanoparticles (MNPs) has previously been reported and modified by our group [21–25]. Briefly, 1.35 mmol, 0.3 mmol, and 0.7 mmol of $Fe(acac)_3$, $ZnCl_2$, and $FeCl_2$, respectively, were mixed into a round bottom flask with 20 mL of tri-n-octylamine, 6 mmol of both oleic acid and oleylamine, and 10 mmol of 1,2 hexadecanediol. The reaction mixture was then heated up to 200 °C for 2 h. From here, the mixture was heated to 305 °C for 2 h and the nanoparticles were purified by repeatedly washing with ethanol.

To coat the MNP cores with a mSi shell, a modified procedure from what was reported by Hyeon et al. was used [26]. 5 mg of the

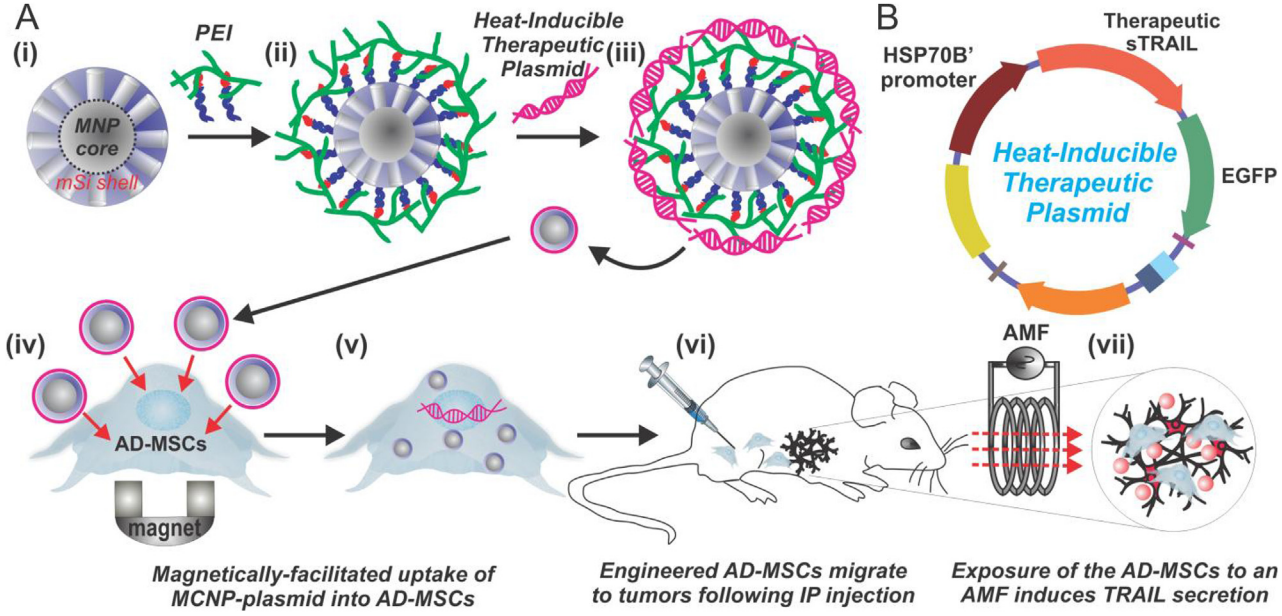

Fig. 1. Mild Magnetic Hyperthermia-Activated Stem Cell-Based Gene Therapy. A) MCNPs composed of a $ZnFe_2O_4$ magnetic nanoparticle (MNP) core and a mesoporous silica (mSi) shell (i) are functionalized with polyethyleneimine (PEI) to allow for complexing with a heat-inducible therapeutic plasmid (ii–iii). The MCNPs enhance delivery of the heat-inducible plasmid into the adipose-derived mesenchymal stem cells (AD-MSCs) via magnetically-facilitated uptake (iv-v). These engineered AD-MSCs can then be injected *in vivo* (vi), where they innately home to the tumors/metastases. Finally, mild magnetic hyperthermia, via exposure of the MCNPs to an alternating magnetic field (AMF), can be used to specifically activate the heat-inducible secretion of therapeutic TRAIL from the AD-MSCs (vii). B) The heat-inducible plasmid is composed of a HSP70B′ promoter and a secreted form of TRAIL (sTRAIL) that is fused to an EGFP reporter.