Biomaterials 91 (2016) 140–150

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

A prodrug-doped cellular Trojan Horse for the potential treatment of prostate cancer

Oren Levy [a, b, c, d, 1], W. Nathaniel Brennen [e, 1], Edward Han [a, b, c, d], David Marc Rosen [e], Juliet Musabeyezu [a, b, c, d], Helia Safaee [a, b, c, d], Sudhir Ranganath [a, b, c, d], Jessica Ngai [a, b, c, d], Martina Heinelt [a, b, c, d], Yuka Milton [a, b, c, d], Hao Wang [g], Sachin H. Bhagchandani [a, b, c, d], Nitin Joshi [a, b, c, d], Neil Bhowmick [f], Samuel R. Denmeade [e, **, 1], John T. Isaacs [e, ***, 1], Jeffrey M. Karp [a, b, c, d, *, 1]

[a] Division of Biomedical Engineering, Department of Medicine, Center for Regenerative Therapeutics, Brigham and Women's Hospital, United States
[b] Harvard Medical School, United States
[c] Harvard Stem Cell Institute, United States
[d] Harvard – MIT Division of Health Sciences and Technology, United States
[e] The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, United States
[f] The Samuel Oschin Comprehensive Cancer Institute at the Cedars-Sinai Medical Center, United States
[g] Department of Oncology, Division of Biostatistics at the Sidney Kimmel Comprehensive Cancer Center, United States

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form
21 February 2016
Accepted 15 March 2016
Available online 17 March 2016

Keywords:
Stem cells
Prostate cancer
Cell-based drug delivery

ABSTRACT

Despite considerable advances in prostate cancer research, there is a major need for a systemic delivery platform that efficiently targets anti-cancer drugs to sites of disseminated prostate cancer while minimizing host toxicity. In this proof-of-principle study, human mesenchymal stem cells (MSCs) were loaded with poly(lactic-co-glycolic acid) (PLGA) microparticles (MPs) that encapsulate the macromolecule G114, a thapsigargin-based prostate specific antigen (PSA)-activated prodrug. G114-particles (~950 nm in size) were internalized by MSCs, followed by the release of G114 as an intact prodrug from loaded cells. Moreover, G114 released from G114 MP-loaded MSCs selectively induced death of the PSA-secreting PCa cell line, LNCaP. Finally, G114 MP-loaded MSCs inhibited tumor growth when used in proof-of-concept co-inoculation studies with CWR22 PCa xenografts, suggesting that cell-based delivery of G114 did not compromise the potency of this pro-drug *in-vitro* or *in-vivo*. This study demonstrates a potentially promising approach to assemble a cell-based drug delivery platform, which inhibits cancer growth *in-vivo* without the need of genetic engineering. We envision that upon achieving efficient homing of systemically infused MSCs to cancer sites, this MSC-based platform may be developed into an effective, systemic 'Trojan Horse' therapy for targeted delivery of therapeutic agents to sites of metastatic PCa.

Prostate cancer (PCa) is the second most common cancer and the second leading cause of cancer-related deaths in American men. Currently affecting over 2.5 million Americans, 1 in 7 men in the U.S. will be diagnosed with PCa in their lifetime. PCa lethality is fueled by the development of disseminated metastases, which are commonly found in the bone, lymph nodes, liver and lungs [1–3]. Despite the impressive progress in PCa research and the availability of therapies such as surgery, radiation, hormonal therapy, immunotherapy and chemotherapy, there remains a significant need for more effective therapies for castration-resistant metastatic PCa [1,3–7]. Specifically, there is a major need to efficiently target systemically administered anti-cancer drugs to sites of PCa metastasis while minimizing host toxicity. Systemic administration of therapeutic agents typically encounters multiple challenges including

* Corresponding author. Division of Biomedical Engineering, Department of Medicine, Center for Regenerative Therapeutics, Brigham and Women's Hospital United States.
** Corresponding author. The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21287, United States.
*** Corresponding author. The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21287, United States.
E-mail addresses: denmesa@jhmi.edu (S.R. Denmeade), isaacjo@jhmi.edu (J.T. Isaacs), jeffkarp.bwh@gmail.com (J.M. Karp).
[1] Authors have contributed equally to this study.

http://dx.doi.org/10.1016/j.biomaterials.2016.03.023
0142-9612/

severe adverse effects due to systemic toxicity, uncontrolled drug levels, premature enzymatic/chemical inactivation and rapid drug clearance requiring repeated dosing [8]. While drug encapsulation in nano/micro delivery systems may reduce host toxicity and protect the drug from early degradation, effective targeting of tumors remains elusive [9]. Moreover, systemically infused micro/nanoparticles typically remain close to blood vessels and cannot efficiently distribute the drug throughout the tumor [10–13].

A potential approach to overcome such challenges is to use a cell-based platform for targeted delivery of therapeutics to sites of metastatic PCa. Known to display tropism towards cancer sites [14–16], mesenchymal stem cells (MSCs) are potential candidates for delivery of therapeutic agents to sites of PCa. One advantage of using particle-loaded cells is that they might migrate away from the vasculature and deeper into the tumor to effectively distribute their toxic payload throughout the tumor. Furthermore, allogeneic MSCs can be harvested from the bone marrow of healthy donors and expanded *ex-vivo* using well-established, FDA-approved protocols [17]. Displaying immune evasiveness, these allogeneic MSCs do not need to be host matched, providing yet another advantage towards their clinical translation [18]. Indeed, MSCs are being explored in over 500 clinical trials worldwide. Clinical studies have demonstrated that hundreds of millions of allogeneic MSCs can be safely administered intravenously (IV) without significant side effects [18].

To further reduce host toxicity and provide yet another layer of specificity to our delivery system, we chose to use the macromolecule G114, a thapsigargin-based Prostate Specific Antigen (PSA)-activated prodrug previously developed by our group and validated using preclinical in vitro and in vivo models of PCa [19–22]. PSA is a serine protease that is only secreted by prostate luminal epithelial cells [23–26]. Although PSA is detected in the blood of PCa patients, it is enzymatically inactive due to binding with ubiquitous serum protease inhibitors such as alpha-1-antichymotrypsin (α1-AC) and alpha-2-macroglobulin (α2M) [24]. Importantly, the enzymatically active form of PSA is only present in the extracellular fluid (ECF) within the prostate and sites of PCa including metastases [24]. We have previously engineered G114, a cell-impermeable PSA-activated prodrug (Fig. 1a) comprised of the potent cytotoxic molecule leucine-12-aminododecanoyl thapsigargin (Leu-12ADT), an amino acid-thapsigargin analog, conjugated to a unique, PSA-cleavable, five amino acid peptide substrate (i.e. HSSKLQ) [19–21,27]. Unproteolyzed G114 is inactive and cannot penetrate cells until it reaches PCa sites, where it is cleaved by PSA to liberate the active toxin, Leu-12ADT. The released lipophilic toxin rapidly enters adjacent cells and induces apoptosis in a proliferation-independent manner via inhibition of the sarcoplasmic/endoplasmic calcium ATPase (SERCA) pump; an essential pathway for maintaining calcium homeostasis in all cells [28–31]. Consequently, once liberated in the ECF, the active toxin effectively kills any adjacent cell independent of PSA expression resulting in a 'bystander effect' targeting the entire microenvironment to overcome tumor heterogeneity [22,28,32]. Therefore, G114 represents a PSA-targeted agent with previously validated selective anti-tumor efficacy in preclinical models of PCa [20]. Unfortunately, G114, like other peptide-based prodrugs, suffers from unfavorable pharmacokinetics with a plasma half-life of only a few hours due to renal clearance. Thus, G114 is a suitable pro-drug for initial evaluation of our particle-in-a-cell delivery platform.

In this proof-of-concept study, we aimed to engineer MSCs as a G114 delivery platform for PCa therapy independent of genetic manipulation (Scheme 1). First, we sought to encapsulate G114 in poly(lactic-co-glycolic acid) microparticles (PLGA MPs). PLGA is already present in FDA-approved products; it is a biodegradable, biocompatible polymer that enables tunable drug release [33–35] and we have previously used PLGA MPs to encapsulate small molecules to control cell phenotype [36–38]. Following an intricate iteration of a double emulsion protocol [39,40], we succeeded in encapsulating G114, a peptide-containing prodrug (M.W. >1600 g/mole), in PLGA particles (~950 nm in diameter), achieving high drug loading (>13%) and encapsulation efficiency (>88%). G114 MPs were then successfully internalized by MSCs without compromising their viability. G114 was released as a functional, intact prodrug in significant levels from G114-MP-loaded MSCs for up to 7 days and selectively induced cell death of PSA-secreting PCa cells *in-vitro*. Finally, co-inoculation *in-vivo* studies demonstrated the therapeutic efficacy of G114-MP-loaded MSCs, suggesting that cell-based delivery of G114 did not compromise the potency of this pro-drug *in-vitro* or *in-vivo*. Overall, this study highlights the potential of G114-MP-loaded MSCs as cell-based delivery vehicles for PCa therapy. Furthermore, achieving efficient targeting of systemically infused MSCs to sites of PCa metastasis in future studies will facilitate the development of such drug-loaded cells into a potent systemic therapy for metastatic PCa.

1. Results

1.1. Encapsulation of G114, a PSA-cleavable macromolecule pro-drug, in PLGA microparticles

G114 is composed of a thapsigargin-based toxin and a previously optimized PSA-cleavable peptide sequence (i.e. HSSKLQ) [20,27], which confers its specificity (Fig. 1a). Hence, we modified a double emulsion protocol [38–40] to encapsulate G114 in PLGA MPs. Drug amounts, polymer molecular weight, MP size, solubility parameters and homogenization velocity were rigorously iterated to accomplish high drug loading and encapsulation efficiency of the intact, peptide-containing prodrug (M.W. = 1684 g/mole) in PLGA MPs. To determine drug loading in the G114-MPs, MPs were lysed overnight using NaOH-SDS, and G114 levels in MP lysates were measured using a microBCA assay against a standard curve of free G114. G114 was successfully encapsulated in spherical PLGA MPs (Fig. 1b), with drug loading (mass of drug over total MP mass) of approximately 13% and encapsulation efficiency of over 88% (fraction of drug mass encapsulated in MPs of the initial mass of drug used in MP fabrication), which are indicative of an efficient encapsulation process (Fig. 1c). The average size of a G114-MP was ~950 nm (Fig. 1c), within the range which was previously shown by our group to be suitable for successful internalization of PLGA MPs by MSCs followed by sustained release of drugs from MP-loaded cells [36–38]. Importantly, LCMS analysis demonstrated that drug-loaded MPs (without cellular internalization) displayed sustained release of significant amounts of the intact prodrug over time (Fig. 1d), with 2.5 mg of G114-MPs releasing more than 70 μg of drug within 10 days, which is equivalent to 20.56% of the total encapsulated drug.

1.2. Intact G114 pro-drug is released from G114-MP-loaded cells

Next, we sought to assess the impact of the G114-loaded PLGA MPs on MSCs, which are the intended cellular carriers for the MPs. Following incubation of the G114-MPs with MSCs at different concentrations (0.025–0.5 mg/mL for 15 h), we assessed MSC viability via XTT. As shown in Fig. 2a, G114-MPs did not induce significant toxicity in MSCs at concentrations up to 0.5 mg/mL. Based on this data and our previous work [38], 0.1 mg/mL was chosen as the working concentration for subsequent experiments. Of note, MSC immunophenotyping analysis to assess the expression levels of surface markers in response to incubation with blank MPs and G114-MPs demonstrated that MSC expression of key surface markers was not altered in response to MP incubation (Supp. Fig. 1).