Mesenchymal Stromal Cells as a Cellular Delivery Platform of Prostate Cancer Prodrugs

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Statement of Purpose: Prostate cancer, currently affecting 2.5 million Americans, is the second most common cancer and the second leading cause of cancer-related deaths in American men. Despite rapid and promising advances in prostate cancer research, there still remains an urgent need for development of more effective therapy for castrate resistant metastatic prostate cancer (CRPC). Specifically, there is a major need to efficiently target anti-cancer drugs to sites of prostate cancer metastasis, while sparing host toxicity. One potential approach is to use cell-based therapy for targeted delivery of therapeutics to sites of metastatic prostate cancer. Potential candidates for such an approach are human mesenchymal stromal cells, also known as mesenchymal stem cells (MSCs), known to display tropism towards cancer sites. Allogeneic MSCs can be harvested from bone marrow of healthy donors and expanded ex-vivo using FDA-approved protocols. Displaying immune evasiveness, these allogeneic MSCs do not need to be host matched and thus are used in over 350 clinical trials worldwide. Clinical studies have demonstrated that hundreds of millions of allogeneic human MSCs can be safely administered intravenously (IV) without significant side effects. In this study, MSCs were loaded with microparticles (MPs) that encapsulate a prostate specific antigen (PSA)-cleavable prodrug. With enzymatically active PSA present only in the extracellular fluid within sites of prostate cancers, and not in the blood or other normal tissues, this prodrug will be efficiently metabolized into a toxic agent only at sites of prostate cancer. This MSC-based platform for delivery of prostate cancer-specific prodrugs may be further developed into potential systemic therapy for CRPC.

Methods: A PSA-cleavable prodrug was encapsulated in poly(lactic-co-glycolic) acid (PLGA) microparticles (MPs) via a double emulsion protocol. Scanning electron microscopy (SEM) was used to image particle morphology. Mean MP size and size polydispersity were assessed via dynamic light scattering, and same solution was transferred into a zeta potential cuvette to determine MP surface charge. To determine drug loading and encapsulation efficiency in the PLGA MPs, drug MPs were lysed overnight in SDS-NaOH and then subjected to a microBCA assay to quantify drug amount inside the MPs. To determine the release kinetics of the drug from the PLGA MPs, MPs were incubated in PBS at 37^{o}C for up to 7 days. Supernatant was collected daily, following centrifugation (3500g, 15 min), and drug concentration in the supernatant was determined via HPLC. For MP internalization by MSCs, cells were incubated overnight with microparticles (0.1mg/mL, in MEM-α, supplemented with 1% FCS). DiO-loaded PLGA MPs were used to confirm MP internalization, assessed via flow cytometry and confocal microscopy. To evaluate the efficacy of drug MPs in inducing cancer cell death in-vitro, LnCAP (prostate cancer cell line known to secrete PSA) were incubated with MP supernatant or with supernatant of drug-MPs-loaded MSCs, followed by an XTT assay to assess cell viability.

Results: Spheroid shaped drug-PLGA MPs were fabricated and characterized for size, charge, release kinetics, drug loading and encapsulation efficiency. MPs were successfully internalized by MSCs, as demonstrated by flow cytometry and confocal microscopy (Figure 1A). Importantly, drug-MP internalization did not impact MSC viability. Drug released from MPs was shown to induce death of the prostate cancer cell line, LnCAP. Moreover, drug released from drug-MP-loaded MSCs was demonstrated to induce cancer cell death (Figure 1B).

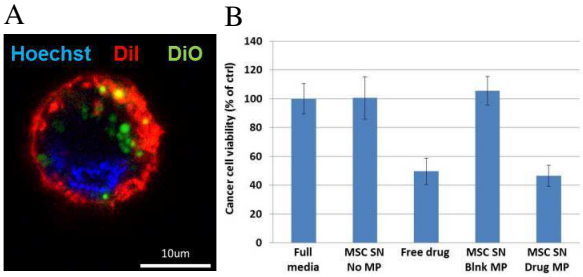

Figure 1: (A) PLGA microparticles internalized by MSCs. Cell nuclei (DAPI, blue), cell membrane (DiI, red) and PLGA microparticles (DiO, green) are shown in this confocal microscopy image (60X magnification). (B) Drug released from MP-loaded MSCs kills cancer cells (LnCAP cells incubated for 72h with supernatant from MP-loaded MSCs. SN=supernatant, MP=microparticles).

Conclusions: In this study we have developed a cell-based platform for potential delivery of prostate cancer-specific prodrugs. The drug-MP-loaded MSC platform was characterized in-vitro (to optimize MP size, charge, drug loading and efficacy in inducing cancer cell death) and may eventually be used as a Trojan horse systemic therapy for targeted delivery of therapeutic agents to sites of metastatic CRPC, while minimizing host toxicity.