Enucleated Cells As Delivery Vehicles To Treat Cancer

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Abstract

Cell-based therapies hold incredible promise to treat a wide variety of diseases, but there are significant hurdles to widespread clinical success, including cost, controllability, consistency and source heterogeneity. Herein, we provide a novel platform for cell-based therapies in which any cell type can be extensively engineered and loaded with therapeutic cargo, and then rendered safe for patient administration through removal of the nucleus. Enucleated cells (cytoplasts) retain many desired biologic functions, such as retention of cell surface markers, secretion of bioactive molecules, viability up to 72 hours, in vitro and in vivo migration, and delivery of therapeutic cargos. This suggests that cytoplasts have therapeutic potential as cell-based delivery vehicles for disease such as cancer. Since the therapeutic functions of cytoplasts have not been previously examined, we designed a preclinical animal cancer model to investigate cytoplast engineering, cargo delivery, and effect on the tumor microenvironment. hTERT immortalized mesenchymal stem cells (hTERT MSCs) were engineered, enucleated, and the resulting cytoplasts were transfected with cytokine mRNAs. In vitro experiments suggest these engineered cytoplasts can secrete biologically functional cytokines for up to 72 hours, comparable to the parental engineered MSCs. Importantly, engineered cytoplasts were able to modulate tissue microenvironments in a preclinical animal model of cancer: human MSC-derived cytoplasts were transfected with IL-12 mRNA, and then intratumorally injected into syngeneic, immunocompetent C57Bl/6 mice bearing established subcutaneous E0771 murine breast cancers. Cytoplasts successfully delivered IL-12 to the tumor, activated IL-12 biomarkers, and induced infiltration of tumor-fighting immune cells that resulted in suppressed tumor growth and improved animal survival. Collectively, our findings indicate that cytoplasts may be a safe means to deliver a wide range of designed therapeutic compounds to diseased tissues, including malignant tumors.

Background

- MSCs are heavily investigated cell-based therapies for a wide variety of diseases based on their secretory, anti-inflammatory, tumor-trophic, regenerative, or wound healing properties [1]
- Current MSC therapies are limited by source heterogeneity, few engineering options, and safety issues such as tumor promotion [2]
- To address these limitations, we created a novel cell-therapy platform in which any cell can be extensively engineered, and then enucleated to be made safe for clinical use. Following ultracentrifugation in Ficoll density gradients [3], these enucleated cells (**cytoplasts**) retain functional organelles, cytoskeletal scaffolding, migrational abilities, survive up to 3 days, survive cryohibernation and cryopreservation, and can be engineered or loaded with many different combinations of therapeutic cargo.
- Cell-based cancer therapies aiming to stimulate the endogenous immune system frequently attempt delivery of cytokines such as IL-12 to induce T cell and NK cell proliferation and activation and IFNγ production. However, systemic delivery of IL-12 can cause adverse affects (fever, cytokine storms, rarely death) that has limited the therapeutic application [4].
- Intratumoral injections are a route to limit systemic toxicities while achieving local therapeutic effects.
- Checkpoint inhibitors like anti-PD-1 antibodies have been used alone or in conjunction with existing immunotherapies [5].
- This project investigates the use of engineered cytoplasts to secrete IL-12 at the site of syngeneic E0771 mouse breast cancer cells [6] in C57Bl/6 mice.

Figure 1. Cytoplasts are transfectable with and secrete sufficient quantities of exogenous, functional IL-12 mRNA

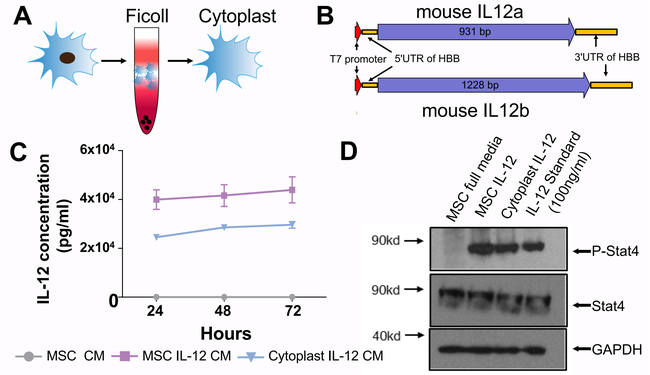

Fig. 1. A) Schematic of cytoplast generation from any cell type by ultracentrifugation in Cytochalasin B-treated Ficoll gradients to extrude nuclei. B) Schematic of IL-12 mRNA construct (a and b strands) including T7 promoter, human beta globin (HBB) mRNA at the 5' and 3' UTR and pseudouridine modification for mRNA stability. C) MSCs or MSC-derived cytoplasts were transfected with IL-12 mRNA in Lipofectamine suspension for 30 minutes, then plated at 2.5 x 10^4 cells/well of 24-well plate. Conditioned medium (CM) was collected at the indicated time points and the secreted IL-12 concentration determined by ELISA. MSC=non-transfected MSCs. MSC IL-12=MSC transfected with IL-12 mRNA. Cytoplast IL-12=cytoplasts transfected with IL-12 mRNA. D) Serum-starved mouse splenocytes were treated for 30 minutes with either full media, purified IL-12 protein standard or the indicated CM collected from MSCs or cargocytes transfected with IL-12. The phosphorylated/activated form of Stat4 (P-Stat4) was determined by western blot.

Figure 2. Cytoplasts secrete IL-12 in the tumor microenvironment with minimal amounts in blood

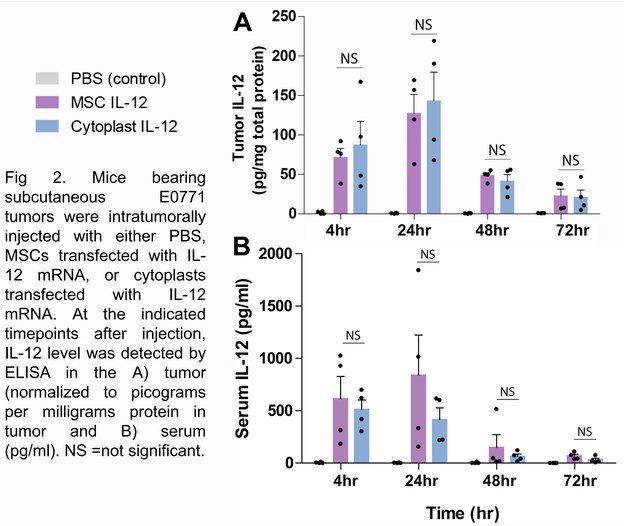

Fig 2. Mice bearing subcutaneous E0771 tumors were intratumorally injected with either PBS, MSCs transfected with IL-12 mRNA, or cytoplasts transfected with IL-12 mRNA. At the indicated timepoints after injection, IL-12 level was detected by ELISA in the A) tumor (normalized to picograms per milligrams protein in tumor and B) serum (pg/ml). NS =not significant.

Figure 3. Intratumoral injections of cytoplasts stimulate immunoregulatory cytokine cascades and increase anti-tumor immune cell recruitment/response

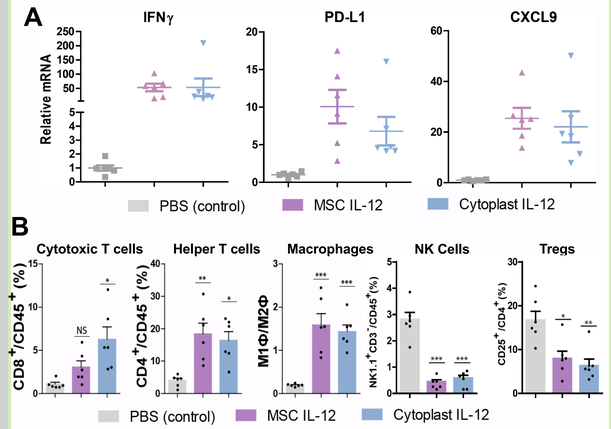

Fig 3. C57Bl/6 mice were injected subcutaneously with 1M E0771 syngeneic mouse breast cancer cells. After ~14 days, established tumors were injected with either 100µl PBS (control), 1M IL-12 transfected MSCs or 3M IL-12 transfected MSC-derived cytoplasts. For experiment A), tumors were harvested 48 after injection, lysed, and analyzed by real-time RT-PCR. Bar graph shows relative fold-change for expression of indicated mRNAs compared to housekeeping HPRT1 between controls, IL-12 transfected MSCs, or IL-12 transfected cytoplasts. For experiment B), mice were intratumorally injected every 2-3 days for a total of 3 injections. 48 hours after the last injection, tumors were analyzed by FACS for the presence of the indicated immune cells.

Figure 4. Cytoplast and checkpoint inhibitor combination therapy design

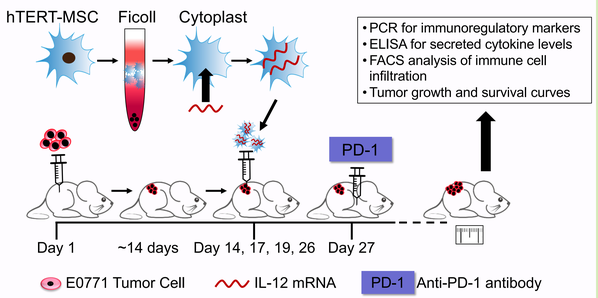

Fig 4. Schematic of cytoplast experimental design. C57Bl/6 mice were injected subcutaneously with 1M E0771 syngeneic mouse breast cancer cells. After ~14 days, established tumors were injected with either 100µl PBS (control), 1M IL-12 transfected MSCs or 3M IL-12 transfected MSC-derived cytoplasts. Anti-PD-1 antibody or anti-IgG was administered intraperitoneally 24 hours after the third injection. One week later, a fourth injection followed by PD-1/IgG was administered. Animals were monitored at least weekly and tissues collected when tumors reached 2cm diameter in any direction or became ulcerated.

Figure 5. Intratumoral injections of cytoplasts with PD-1 inhibitors leads to tumor growth suppression and increased animal survival

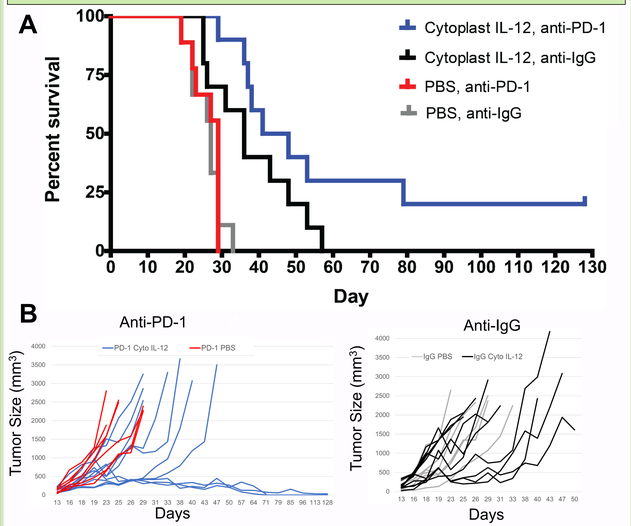

Fig 5. A) Survival curves for animals. Day 0=tumor initiation. Day 13= intratumoral cytoplast or PBS injections. Day 19=anti-PD-1 or IgG injection. B) Tumor growth curves for PBS and IL-12 cytoplasts with anti-PD-1 or IgG injections.

Conclusions

- *Cytoplasts are a viable cell-like entity for delivery of therapeutic cargo to tumors in vivo.*
  - *Secrete IL-12 in the tumor microenivoronment*
  - *Minimal IL-12 secretion in blood*
  - *Induce immunoregulatory cytokines/markers*
  - *Recruit anti-tumor immune cells into the tumor*
- *IL-12 secretion by cytoplasts in conjunction with anti-PD-1 therapy suppressed tumor growth and prolonged animal survival.*

Future directions:

- **Validate survival effects of cytoplast and combination therapies**
- **Investigate long-term tumor immunity in mice with regressed tumors by re-challenge**
- **Analyze effects of injection of cytoplasts derived from MSCs engineered to express 3 cytokines and multiple anti-tumor antibodies**
- **Determine cytoplast homing into subcutaneous and metastatic tumors following intravenous injection**

References

1. Galipeau J, Sensebe J. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. Cell Stem Cell (2018) 22:824-33.
2. Chen YS, Chen YA, Tsai PH, Chen CP , Shaw SW, Hsuan Y. Mesenchymal Stem Cell: Considerations for Manufacturing and Clinical Trials on Cell Therapy Product. Int J Stem Cell Res Ther (2016) 3:29.
3. Wigler MH, Weinstein IB. A preparative method for obtaining enucleated mammalian cells. BiochemBiophResCo (1975) 63.3:669-74.
4. Colombo MP, Trinchieri G. Interleukin-12 in anti-tumor immunity and immunotherapy. Cytokine Growth Factor (2002) 13: 155–168.
5. Waldmann TA. Cytokines in Cancer Immunotherapy. Cold Spring Harb Perspect Biol (2017) doi: 10.1101/cshperspect.a028472.
6. Johnstone CN, Smith YE, Cao Y, Burrows AD, Cross RS, Ling X, Redvers RP, Doherty JP, Eckhardt BL, Natoli AL, Restall CM, Lucas E, Pearson HB, Deb S, Britt KL, Rizzitelli A, Li J, Harmey JH, Pouliot N, Anderson RL. Functional and molecular characterisation of EO771.LMB tumours, a new C57BL/6-mouse-derived model of spontaneously metastatic mammary cancer. Dis Model Mech. (2015) 8(3):237-51.

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