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TITLE: Exosomes from Mesenchymal Stem Cells for Treatment of Malignant Mesothelioma

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CONTRACTING ORGANIZATION: Massachusetts General Hospital, Boston, MA

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14. ABSTRACT
Over the past decade, trafficking of extracellular vesicles including exosomes has emerged as a mechanism of cell-cell communication and plays an important role in tumorigenesis and formation of tumor microenvironment. We hypothesize that different antitumor activities of CD90low and CD90high mesenchymal stem cells (MSCs) are applicable to treatment of malignant mesothelioma (MM) and that the effects of MSCs are related to their secreted exosomes. Exosome-based nanometric vehicles have a number of advantages: they are non-toxic, non-immunogenic, and can be engineered to have robust delivery capacity and targeting specificity. During this study period, we have successfully generated adipose tissue-derived mesenchymal stem cells (ADSCs) which were characterized as a typical MSC phenotypes (CD45-CD34-CD31-CD11b-CD29+CD44+CD73+CD90+CD105+CD106+SCA-1+, here briefly called CD90high). The cells were continuously cultured in MSC conditioned media with additional 1 ng/ml of LPS or 100 ng/ml of bacterial heat shock protein 70 (HSP70) and until passages 4 phenotypically differentiated into CD45-CD34-CD31-CD11b-CD29+CD44+CD73+CD90-CD105+CD106+SCA-1+, briefly called CD90low, while cells cultured without LPS or HSP70 maintained CD90high. Both CD90high and CD90low ADSCs were capable of further differentiating into osteoblasts and adipocytes in specific differentiation induction media. A week after luciferase-labeled mesothelioma cell line 40L (40L-luc) cells were intraperitoneally (i.p.) inoculated into C57BL/6j mice, tumor-bearing mice were treated i.p. with four doses of 1×10^6 of passage-4 CD90low or CD90high ADSCs at 7-day interval. Tumor growth was monitored by in vivo bioluminescent imaging weekly and tumors were collected a week after the last treatment and weighted. Treatment with CD90low ADSC significantly slowed tumor growth and reduced tumor mass compared to treatment with CD90high ADSCs or differentiated fibroblast cells as control.

15. SUBJECT TERMS
mesenchymal stem cells, adipose tissue-derived mesenchymal stem cells, CD90, exosomes, malignant mesothelioma, immunotherapy

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1. INTRODUCTION:

Malignant mesothelioma (MM) is an aggressive tumor that arises from the pleural and peritoneal mesothelium. It results from asbestos exposure and can present amongst military personnel and veterans. MM is refractory to conventional therapies and the median survival after symptom onset is less than 12 months. Standard surgery, radiotherapy and chemotherapy, as well as recent immunotherapy, have improved quality of life but have made little impact on survival with this tumor. There is a significant unmet need for new treatments. This research aims to generate a preclinical dataset that would support the development of mesenchymal stem cell (MSC)-derived exosome-based therapy in the context of regulation of inflammation and abrogation of immunosuppression as well as restoration of immune competence or homeostasis in the tumor microenvironment (TME) for treatment of MM.

2. KEYWORDS:

mesenchymal stem cells, adipose tissue-derived stem cells, exosomes, CD90, the tumor microenvironment, malignant mesothelioma, immunotherapy, immunomodulation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

***Specific Aim 1*:** To test the antitumor efficacy of $CD90^{low}$ and $CD90^{high}$ ADSCs.

***(1a)*:** Evaluation of the effects of $CD90^{low}$ and $CD90^{high}$ ADSCs on tumor growth and mouse survival in a mouse models of MM. Completed

***(1b)*:** Quantitation of phenotype and function of tumor infiltrating lymphocytes (TILs) in the murine models of MM. Completed

***Specific Aim 2*:** To test the antitumor efficacy of exosomes derived from $CD90^{low}$ and $CD90^{high}$ ADSCs.

***(2a)*:** Characterize and quantify exosomes derived from $CD90^{low}$ and $CD90^{high}$ ADSCs. To be completed 1/2022

***(2b)*:** Evaluation of the effects of exosomes derived from $CD90^{low}$ and $CD90^{high}$ ADSCs on tumor growth and mouse survival in the murine models of MM. 2/28/23

***(2c)*:** Quantitation of phenotype and function of TILs in the murine models of MM. 2/28/23

***Specific Aim 3*:** To evaluate of the antitumor efficacy of engineered exosomes and the translational potential of the delivery.

***(3a)*:** Engineering or modification of exosomes to enhance their antitumor immune modulation.

***(3b)*:** Evaluation of engineered exosomes of their antitumor efficacy in the murine models of MM.

***(3c)*:** Evaluation of exosomes of their antitumor efficacy in *in vitro* 3D simulation models of human MM.

What was accomplished under these goals?

Hand over of datasets and know-how on assay execution under Aims 1 and 2 from Dr. Chen to Dr. Poznansky.
Once transfer of PI from Dr. Chen to Dr. Poznansky was approved by the DoD, all technical know-how, records, lab notebooks and datasets from the first year of research work were reviewed together between Dr. Chen, Dr. Poznansky and Ms. Mukherjee during a series of in person and virtual meetings during September 2022. All questions arising from the original datasets were reviewed between the outgoing and incoming PI's around the execution of cell culture, flow cytometry, in vitro and in vivo assays of exosome characterization and function in vitro and vivo. The first order of lab research transfer involved determining the robustness by which CD90hi cells were derived from ADSCs given that these cells were the origin of the exosomes to be used in the subsequent Aim 2.

Re-establishment of collaborative subprojects to support the completion of Aim 2 and 3.
In addition, we have established a strong collaborative component involving Prof. Xandra Breakefield here at Massachusetts General Hospital, Harvard Medical School regarding intellectual and material support for the completion of Aims 2 and 3. Prof. Breakefield is an internationally recognized expert in exosome biology and use as therapeutics.

Replication of ADSC differentiation to obtain CD90 high & low cell populations
Due to inconsistent yield of exosomes under the supervision of Dr. Chen, growth conditions of CD90 high cells derived from ADSCs, were optimized to be performed in 5% O_2 5% CO_2, hypoxic conditions, as recommended by the manufacturer of specialized growth medium required by these cells (STEMCELL Technologies). Emulating physiologic conditions in-vivo significantly enhances MSC cell culture and proliferation. (Boregowda, S.V., Krishnappa, V., Chambers, J.W., Lograsso, P.V., Lai, W.T., Ortiz, L.A. and Phinney, D.G., 2012. Atmospheric oxygen inhibits growth and differentiation of marrow-derived mouse mesenchymal stem cells via a p53-dependent mechanism: implications for long-term culture expansion. Stem cells, 30(5), pp.975-987) Boregowda and colleagues have also demonstrated that hypoxia significantly enhances MSC cell proliferation kinetics and alters metabolism. We confirmed this finding in this study.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

We will commence work on Aim 2 in collaboration with Dr. Xandra Breakefield at MGH who has expertise in exosome biology and characterization now that we have consistently derived CD90hi cells from ADSCs. Ms. Mukherjee will complete this work under the direction of Dr. Poznansky at the Vaccine and Immunotherapy Center at MGH. All assays are up and running the PI's and collaborator's laboratories to complete this research work.

Aim 2 To test the antitumor efficacy of exosomes derived from $CD90^{low}$ and $CD90^{high}$ ADSCs cells.

***(2a)*:** Characterize and quantify exosomes derived from $CD90^{low}$ and $CD90^{high}$ ADSCs.

***(2b)*:** Evaluation of the effects of exosomes derived from $CD90^{low}$ and $CD90^{high}$ ADSCs on tumor growth and mouse survival in the murine models of MM.

***(2c)*:** Quantitation of phenotype and function of TILs in the murine models of MM.

***Specific Aim 3*:** To evaluate of the antitumor efficacy of engineered exosomes and the translational potential of the delivery.

***(3a)*:** Engineering or modification of exosomes to enhance their antitumor immune modulation.

***(3b)*:** Evaluation of engineered exosomes of their antitumor efficacy in the murine models of MM.

***(3c)*:** Evaluation of exosomes of their antitumor efficacy in *in vitro* 3D simulation models of human MM.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The ultimate bench-to-bedside delivery from this study is expected as ADSC-derived exosome-based therapeutic approach to mobilize immune responses against MM. This approach is designed to generate favorable effects on the tumor microenvironment that will overcome the limitation of other current therapies in failure to target tumor mutagenicity and immune evasion. Therefore, the findings will be potentially applicable to a myriad of solid tumors and the impact could be broad.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

The findings of murine CD90low and CD90high ADSCs and their derived exosomes may translate into humans in terms of antitumor activity which is evidenced by the findings in human that decreased positivity for CD90 on human MSCs was associated with a loss of immunosuppressive activity by MSCs. The relevance of this work to the clinical setting is maximized by the fact that both active elements of this therapy can be accessed and approved for use in humans with cancer. If this study is successful, ADSC-derived exosomes could be pushed to clinical study in a fresh cancer-targeting angle. This might be beneficial to treatment of not only MM but also certain other types of cancers.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

No significant changes in objectives or scope.

Actual or anticipated problems or delays and actions or plans to resolve them

Dr. Huabiao Chen, Principal Investigator (PI) of the award left his employment with the Massachusetts General Hospital as of March 31, 2022, to begin an appointment in industry. On March 3, 2022, MGH requested a change of PI from Dr. Chen to Dr. Mark C. Poznansky, to be effective April 1, 2022, with no change in project objectives or scope. This change of PI was approved by the agency on August 8, 2022. Dr. Poznansky, as Director of the MGH Vaccine and Immunotherapy Center where Dr. Chen's laboratory is located, was already familiar with the project, has experience with the relevant models and techniques, and is well-positioned to ensure successful completion of the remaining project objectives.

There was some delay in project progress during the 4 months while awaiting the approval of the PI change. In addition, the graduate student working on the project under Dr. Chen completed his degree and moved to a postdoctoral position elsewhere. Work on the project is once again underway under the direction of Dr. Poznansky, with the expert assistance of Sonia Mukherjee, MS, a senior research technologist at the MGH Vaccine and Immunotherapy Center.

Due to the delay while awaiting approval of the PI change, this report covers through September 14, 2022 (14 months rather than 12). In addition, given these delays in research transfer and the need to establish collaborative components under the new PI leadership it is highly likely that we will have to consider applying for a 4 month no cost extension from 2/28/23 to 6/30/23 in order to complete the proposed project work to time and cost.

Changes that had a significant impact on expenditures

Project expenditures were reduced during while the change of PI approval was in progress. The rate of spending has since increased as laboratory work has resumed.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Not applicable—nothing to report.

Significant changes in use or care of vertebrate animals

No protocol changes to report, beyond transfer of protocol PI and addition of new study stuff, both approved by the MGH IACUC on March 14, 2022, and by ACURO on April 21, 2022.

Significant changes in use of biohazards and/or select agents

No changes to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

From July 15, 2021-March 31, 2022:
Name: Huabiao Chen, MD, PhD
Project Role: PI
Nearest person-month worked: 5 (56.50% effort)
Contribution to Project: Dr. Chen oversaw project design and execution, reviewed data analysis, led animal studies, and performed flow cytometric analyses as needed.

Name: Sen Han, PhD
Project Role: Graduate student/Postdoctoral fellow
Nearest person month worked: 4 (50% effort)
Contribution to Project: Dr. Han carried out cell culture and flow cytometric experiments, assisted in animal studies and performed initial exosome studies.

From April 1, 2022-September 14, 2022
Name: Mark C. Poznansky, MD, PhD
Project Role: PI (new)
Nearest person-month worked: 0.4 (10% effort)
Contribution to Project: Dr. Poznansky oversees experimental design and overall project execution and strategy, and reviews data analysis and interpretation.

Name: Sonia Mukherjee, MS
Project Role: Senior Research Technologist
Nearest person-month worked: 2 (50% effort)
Contribution to Project: Ms. Mukherjee has reviewed the project data and records received from Drs. Chen and Han, identified next steps, and performed cell culture experiments to determine the reproducibility of prior results and to optimize ADSC differentiation conditions.

From July 15, 2021-September 14, 2022
Name: Ann E. Sluder, PhD
Project Role: Project Manager
Nearest person month worked: 0.3
Contribution to Project: Dr. Sluder ensures compliance with agency and institutional requirements, tracks project finances, and has provided day-to-day guidance as needed to Ms. Mukherjee as she became familiar with the project.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Up-to-date Other Support information for Dr. Poznansky was submitted with the change of PI request.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Not applicable.

QUAD CHARTS:

Not applicable.

9. APPENDICES: