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TITLE: Discover Novel Therapeutic Strategies for Peritoneal Metastases from Gastric Adenocarcinoma

PRINCIPAL INVESTIGATOR: Samir M. Hanash

CONTRACTING ORGANIZATION: The University of Texas M.D. Anderson Cancer Center
Houston, TX 77030

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Hanash, Samir, M, Ph.D., M.D.

E-Mail:SHanash@mdanderson.org

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The University of Texas MD
Anderson Cancer Center
1515 Holcombe Blvd Unit 207
Houston TX 77030

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The major goals of the project during the reporting period were to (1) optimize purification of tumor cells and exosomes from gastric adenocarcinoma ascites, and (2) to initiate proteomic profiling of these disseminated GAC cells and exosomes. We completed untargeted MS proteomic profiling of a total of 10 (52.5% of SOW) gastric cancer ascites derived cell specimens. We commenced proteomic profiling of exosomes derived from gastric cancer cell lines.

15. SUBJECT TERMS
Ascites; Cancer stem cell; Carcinomatosis; Exosome; Gastric Adenocarcinoma; Molecular profiling; Metastasis; Novel therapeutics; Peritoneal; Proteomics; RNA; Therapeutic target

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Gastric adenocarcinoma (GAC) is prevalent with ~1 million new cases diagnosed per year globally. The majority of GAC patients are diagnosed at an advanced stage with a median survival of ~9 months. Peritoneal carcinomatosis (PC) originating from GAC is frequently observed either at diagnosis (~15% of patients) or later in the course of the disease (~40%), with dismal prognosis. The purpose of this research is to better understand the molecular drivers of peritoneal metastases that result from primary GAC, with an ultimate aim to identify new and exploitable therapeutic targets for PC. Research in this specific area is limited and comprehensive profiling of metastatic GAC is incomplete. Our central hypothesis is that cancer stem cells (CSCs) seed the peritoneal cavity and that detailed assessments including integrated analyses of high-quality multiplex data will identify new and exploitable therapeutic targets. To this end, the scope of our work is to perform comprehensive proteomic profiling on metastatic PC cells and exosomes from malignant ascites of GAC patients as part of a multi-omic effort to identify specific molecular features and novel therapeutic targets in human PC cells. These data will be integrated with miRNA/lncRNA, and genomic analyses to provide a wide-ranging molecular understanding of peritoneal metastases that disseminate from primary gastric adenocarcinomas.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Ascites; Cancer stem cell; Carcinomatosis; Exosome; Gastric Adenocarcinoma; Molecular profiling; Metastasis; Novel therapeutics; Peritoneal; Proteomics; RNA; Therapeutic target

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The major goals of the project during the reporting period were to (1) optimize purification of tumor cells and exosomes from gastric adenocarcinoma ascites, and (2) to initiate proteomic profiling of these disseminated GAC cells and exosomes.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We optimized tumor cell purification from ascites fluid and confirmed enrichment of tumor cells using EpCAM+ and CD45- immunofluorescence. We also optimized a method for exosome isolation from ascites based on extension of or our established exosome isolation methods and conformed exosome recovery by nanoparticle counting, electron microscopy and western blotting for exosome and tumor surface markers.

We completed untargeted MS proteomic profiling of a total of 10 (52.5% of SOW) gastric cancer ascites derived cell specimens.

Analysis of the initial 10 GAC-derived cell specimens as well as 6 gastric cancer cell lines identified 12,945 gene products from total cell lysate and the cell surface compartments. Intersection of these datasets resulted in a panel of 49 surface enriched proteins. This list included membrane-localized proteins previously identified in independent analyses of tumor tissue but not normal tissue, and was concordant with high TCGA mRNA expression that indicated significantly poor prognosis in gastric cancer.

We commenced proteomic profiling of exosomes derived from gastric cancer cell lines.

During the next period, we will complete MS proteomics profiling on an additional 19 newly collected ascites tumor cell samples as well as ascites-derived exosomes. We will also perform in-vitro validation experiments on identified surface target candidate proteins.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The research activities supported by the project have provided training and professional development opportunities for post-doctoral fellows with both basic science and clinical background and expertise.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Interim results have been disseminated to communities of interest primarily at monthly team meeting, local and inter-instructional public seminars as well as scientific meetings. Final results will be published in peer-reviewed journals and presented at international scientific meetings.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next reporting period we will commence profiling of PC malignant ascites derived exosomes and perform proteomic profiling on additional PC cells derived from malignant ascites.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The proteomic data resulting from the project activities is a fundamental component in establishing a complete molecular signature of drivers of metastatic disease in the context of GAC. Initial analyses of total cell and surface compartments has yielded novel surface protein candidates that are currently being further validated.

Importantly, the integrated molecular profiling efforts of this collaborative project will facilitate development of new and exploitable therapeutic targets for peritoneal carcinomatosis. During the next reporting period, we will integrate the proteomic data with the corresponding miRNA/lncRNA, and genomic analyses to provide a wide-ranging molecular understanding of peritoneal metastases that disseminate from primary gastric adenocarcinomas.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The metastatic signature identified in this study could be applied to other tumor types.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

The results of the project are likely to be translatable into clinical practice to improve outcomes for patients with gastric adenocarcinoma or other cancers

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Optimized techniques for exosome isolation from ascites.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*

- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Dr. Samir Hanash*
Project Role:	*PI*
Nearest person month worked:	*1*
Contribution to Project:	Oversight of the project and coordinated activities with co-investigators
Name:	*Jody Vykoukal*
Project Role:	*Sr. Research Scientist*
Nearest person month worked:	*1*
Contribution to Project:	Develop and apply novel, optimized workflows for the isolation of extracellular vesicles from ascites, serum, plasma, cell line conditioned media, and other biofluids, as well as companion procedures that enable molecular profiling of circulating vesicle-associated protein and nucleic acid markers in the context of gastric cancer.
Name:	*Makoto Kobayashi*
Project Role:	*Post Doc*
Nearest person month worked:	*1 person month*
Contribution to Project:	*Protein extraction from gastric cancer ascites fluid-derived tumor cells and sample prep for LCMS proteomics platform. Pickup novel therapeutic target for gastric cancer from LCMS data and perform candidate validation using proteomics, pathology, and molecular biology methods.*
Funding Support: CPRIT	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

See attached

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.