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14. ABSTRACT

Prostate cancer remains the most prevalent form of non-skin cancer in males and the second leading cause of cancer deaths in men within the U.S. MicroRNAs (MiRNAs) constitute an important class of non-coding RNAs that function as tumor suppressor genes, oncogenes, and metastatic factors in human prostate cancer and hold promise as novel therapeutic tools for prostate cancer. New evidence reveals miRNA activity is not confined to the cells in which they are produced, but can also signal intercellularly to other cells and tissues at distant sites via exosomal transport. We hypothesize that miRNAs specifically packaged in these 40-100 nm microvesicles and secreted from prostate cancer cells are important in the progression to aggressive disease. In this exploratory award, we are investigating the functional significance of exosomal miRNAs in prostate cancer. We are characterizing the miRNA composition within the cellular versus exosomal fractions of various syngeneic human prostate cancer cell lines that differ in their metastatic status in order to identify tumor suppressive exosomal miRNAs. We will subsequently test if exosomal delivery of tumor suppressor miRNAs modulates the behavior of aggressive human prostate cancer cell lines in vitro as well as in vivo using mouse xenograft models.

15. SUBJECT TERMS

microRNAs, exosomes, prostate cancer, intercellular signaling

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1. Introduction

Prostate cancer remains the most prevalent form of non-skin cancer in males and the second leading cause of cancer deaths in men within the U.S. MicroRNAs (MiRNAs) constitute an important class of non-coding RNAs that function as tumor suppressor genes, oncogenes, and metastatic factors in human prostate cancer and hold promise as novel therapeutic tools for prostate cancer. New evidence reveals miRNA activity is not confined to the cells in which they are produced, but can also signal intercellularly to other cells and tissues at distant sites via exosomal transport. We hypothesize that miRNAs specifically packaged in these 40-100 nm microvesicles and secreted from prostate cancer cells are important in the progression to aggressive disease. In this exploratory award, we are investigating the functional significance of exosomal miRNAs in prostate cancer. We are characterizing the miRNA composition within the cellular versus exosomal fractions of various syngeneic human prostate cancer cell lines that differ in their metastatic status in order to identify tumor suppressive exosomal miRNAs. During our "No-Cost-Extension" period, we will determine how exosomal delivery of these candidate tumor suppressor miRNAs modulates the behavior of aggressive, metastatic human prostate cancer cell lines in vitro as well as in vivo using mouse xenograft models. This proposal has high potential to lead to novel therapeutic methods for treatment of aggressive, lethal forms of prostate cancer.

2. Keywords

microRNAs, exosomes, prostate cancer, intercellular signaling

3. Accomplishments

What were the major goals of the project?

Goal 1. Characterize miRNA profiles in cellular vs exosomal fractions for syngeneic human prostate cell lines & identify tumor suppressor exosomal miRNAs enriched in non-aggressive prostate cells.

Goal 2. Determine if exosomal delivery of tumor suppressor miRNAs to aggressive human prostate cancer cells has a functional consequence in vitro on cancer-related pathways

Goal 3. Selectively load exosomes with prostate cancer-associated miRNAs and study their effects on tumor formation and metastasis using mouse xenograft models.

Goal 4. Data analysis, data interpretation, and manuscript preparation.

What was accomplished under these goals?

We have made significant progress since the activation of this grant on September 24, 2014. This DoD funding mechanism was for an exploratory proposal on a subject not currently

worked on in my laboratory. Therefore, in the last year we have learned much about exosome biology and isolation practices. Due to the successfully funding of this proposal by the DoD and added interest of our scientific colleagues on exosome research, my institution in collaboration with the Leroy T. Canoles Jr. Cancer Research Center at EVMS has shown support for this project by purchasing a NanoSight NS300 Instrument with a 488nm laser from Malvern ($85,000). This newly purchased piece of equipment housed down the hall from my lab in the cancer center has greatly aided our ability to move this project forward. The NanoSight allows us to accurately measure the purity and concentration of our exosome preparations, which is crucial for our *in vitro* assay analysis (Aim 2) and animal studies discussed (Aim 3).

In alignment with Aim 1, we have successfully isolated exosomes from syngenic human prostate cancer cell lines that differ in their metastatic status; specifically from PC3-N (non-aggressive, non-metastatic) & PC3-ML (highly aggressive & tumorigenic) cell lines derived from parental PC3 cells (from bone metastasis, androgen -independent); the LNCaP cell line (from lymph node metastasis, androgen-sensitive, low tumorigenicity) & more aggressive, androgen insensitive and metastatic LNCaP-derived cell lines C4-2; and the virally transformed normal prostate epithelial cell line RWPE-1 lacking tumorigenic potential & the highly aggressive RWPE-1-derived WPE1-NB26. Proper isolation has been confirmed for these preps by western blot analysis for the exosomal markers CD9, CD81, and CD63. (Figure 1.) Interestingly, we noted that more aggressive human prostate cancer cell lines secrete a greater number of exosomes then their non-aggressive syngeneic counterparts. (Figure 2)

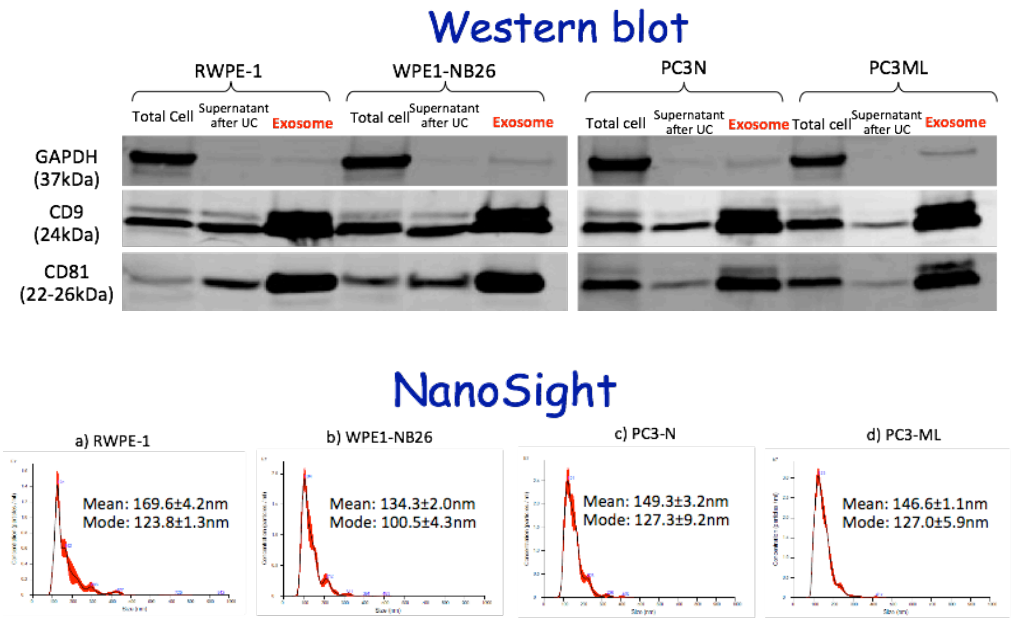

Figure 1. Exosomes were isolated by ultracentrifugation from paired syngeneic human prostate cancer cell lines: RWPE-1 (androgen sensitive, non-malignant) & aggressive WPE1-NB26; and PC3-N (hormone-refractory, non-aggressive) & metastatic PC3-ML. The exosomal markers CD9 and CD81 were highly enriched in all the exosome preparations by western blot. NanoSight analysis showed that our isolated exosomes were ~150 nm in size.

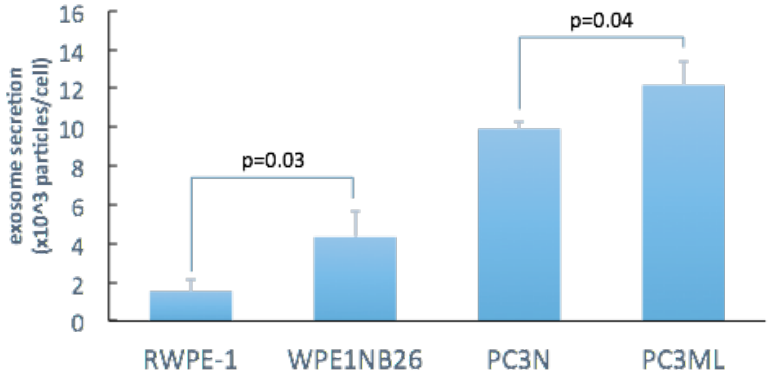

Figure 2. Exosomes were isolated from cell culture supernatant and the number of cells per flask was calculated. The exosome concentration was measured by NanoSight. We calculated the number of exosomes secreted per cell and found that cancer cells secrete more exosomes than non-malignant RWPE-1 cells, and aggressive PC3-ML cells secrete more exosomes than non-aggressive PC3-N prostate cells.

As described in Aim 1, we are now profiling miRNA expression in the cell lysate and exosomal components from the cell lines described above to determine if miRNA populations, particularly those already characterized to act as tumor suppressor genes and oncogenes in the prostate, are differentially packaged into exosomes for cell lines differing in their metastatic status. We are also initiating our in vitro studies to test if exosomes isolated from non-aggressive human prostate cancer cell lines can suppress proliferation, migration, and invasion activities of aggressive. metastatic prostate cells.

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

Thanks to this DoD funding, I successfully recruited an outstanding Postdoctoral Fellow, Tsuyoshi Hasegawa, MD, PhD from Osaka City University School of Medicine, Department of Surgical Oncology, Osaka, Japan. Before relocating to EVMS from Japan to begin his postdoctoral training in my lab this February, he was previously employed as a general surgeon specializing in gastric, colon, pancreas, and breast cancers at Aomatsu Memorial Hospital in Osaka. Dr. Hasegawa was very interested in extending his formal research training in cancer progression pathways into the involvement of small non-coding RNAs and applied to my laboratory last Fall 2014 for his postdoctoral fellowship. I am VERY fortunate to welcome him to my research team due to his extensive expertise in cancer biology, surgical & clinical training, and familiarity using mouse models to study tumor formation and metastasis. This experience has aided the project immensely. Tsuyoshi Hasegawa receives salary support (30% effort) and is an important asset to this project to perform the experiments described in my funded proposal.

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 have been granted a one-year extension without funds to the expiration date of September 23, 2016. The extension will not involve a change in the approved objectives or scope of the project. We are beginning our in vitro studies to test if exosomes isolated from non-aggressive human prostate cancer cell lines can suppress proliferation, migration, and invasion activities of aggressive. metastatic prostate cells (Aim 2). Once this is completed and we identify tumor suppressor exosomal-miRNAs that can inhibit prostate cancer related activities, we will quickly move our studies into the mouse xenograft models (Aim 3).

4. Impact

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

This project is still in progress and we have not yet shared our results at professional meetings or in peer-reviewed journals – so there is nothing to report. However, successful completion of this grant would reveal a novel therapeutic strategy to treat aggressive, metastatic prostate cancer, which is currently incurable and lethal.

What was the impact on other disciplines?

Although this project is still in progress, successful completion of this grant could impact other fields. Similar use of exosomes as delivery vehicles for tumor suppressive miRNAs could be applied to other types of cancers.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report, however successful application of this approach to prostate cancer could make a significant impact to society since prostate cancer is the second leading cause of cancer-related males deaths, behind lung cancer.

5. Changes/Problems

Nothing to report – for all sections

6. Products

Nothing to report - for all sections

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name	Aurora Kerscher, PhD
Project Role	Principle Investigator
Researcher Identifier	
Nearest person month worked	2.4
Contribution to project	Dr. Kerscher supervises, coordinates and trains personnel on this project. She has assisted in all aspects of miRNA isolation from exosomes and functional analysis of exosomal small RNAs.
Funding Support	1 R21 CA175894-01A1 (PI: Kerscher)

Name	Tsuyoshi Hasegawa, MD, PhD
Project Role	Postdoctoral fellow
Researcher Identifier	
Nearest person month worked	3.6
Contribution to project	Dr. Hasegawa has been trained by Dr. Kerscher and has assisted in all aspects of miRNA isolation from exosomes and functional analysis of exosomal small RNAs.
Funding Support	1 R21 CA175894-01A1 (PI: Kerscher)

Name	Amy Tang, PhD
Project Role	Collaborator
Researcher Identifier	
Nearest person month worked	1
Contribution to project	Dr. Tang has assisted Dr. Kerscher's lab personnel in preparation for the *in vivo* mouse xenograft experiments for this project. Personnel have been trained by Dr. Tang to perform the animal techniques.
Funding Support	1 R01 CA140550-01 (PI: Tang); Breeden-Adams Foundation Grant; 1 R21 CA175894-01A1 (PI: Kerscher)

Name	Hind Beydoun, PhD
Project Role	Biostatistician
Researcher Identifier	
Nearest person month worked	1
Contribution to project	Assisted in all aspects of biostatistical analysis for this project that included aiding in the experimental design and animal power umber calculations.
Funding Support	1 R21 CA175894-01A1 (PI: Kerscher); Doris Duke Clinical Scientist Development Award (PI: Troy);

Name	Raymond Lance, MD

Project Role	Collaborator
Researcher Identifier	
Nearest person month worked	1
Contribution to project	Dr. Lance has provided advisory support on this project based on his expertise in clinical study design, prostate cancer oncology, and experience using prostate cancer mouse models.
Funding Support	none

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

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. Special Reporting Requirements

Not Applicable

9. Appendices.

Not Applicable