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TITLE: The Roles of the Bone Marrow Microenvironment in Controlling Tumor Dormancy

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14. ABSTRACT

The purpose of this study is to identify the mechanisms whereby the bone marrow microenvironment is involved in regulation of tumor dormancy. Aim1 will identify and explore how DTCs stay dormant for long periods of time. We postulate that DTCs drive the bone marrow niche into dormancy through the GAS6 pathway. Aim2 will determine how DTCs escape dormancy, consequently rendering them more susceptible to the chemotherapy.

As a major accomplishment of this study during this period is that the PI, Dr. Yusuke Shiozawa, accepted a position as an Assistant Professor at Wake Forest School of Medicine as of 03/01/15. The PI obtained the necessary institutional approvals (IACUC, IRB, IBC) and submitted the grant transfer request (06/11/2015) to gain approval from the Department of Defense for a transfer of the award from the University of Michigan to Wake Forest School of Medicine. As a result, the progress of this award was suspended at Wake Forest School of Medicine during this period of time. Once the PI obtains the grant transfer approval from the Department of Defense, he will initiate the proposed research as soon as possible.

15. SUBJECT TERMS

Prostate Cancer; Bone metastasis; Disseminated tumor cells; Bone marrow microenvironment; Tumor dormancy; GAS6; BMP2

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1. INTRODUCTION:

Despite improvements in treatments for primary prostate cancer (**PCa**), bone metastasis remains a major cause of death in PCa patients. Several studies have shown that disseminated tumor cells (**DTCs**) shed from a primary tumor may lie dormant in distant tissues for long periods of time, retaining the potential for activation resulting in metastatic growth. Understanding the underlying mechanisms of metastasis is therefore crucial for effective treatment of this disease. Bone marrow has been well established as a regulatory site for hematopoietic function. In the marrow, hematopoietic stem cells (**HSCs**) are believed to localize to a specific microenvironment, the "niche", where they reside in a dormant state. Likewise, growing evidence has suggested that disseminated PCa also resides within the marrow niche. In fact, disseminated PCa uses similar mechanisms as HSCs in order to gain access to the marrow microenvironment, and DTCs target and displace HSCs, establishing metastatic foci within the hematopoietic niche. As a result, these cells parasitize the niche to become dormant, utilizing the mechanisms that keep HSCs in a dormant state. Although bone marrow is known as a fertile microenvironment ("soil") for metastatic tumor cells ("seed"), little is known about how dormancy is established or what leads to re-activation of the dormant cells. Therefore, we hypothesize that **once DTCs become dormant within the bone marrow niche, they stay dormant by stimulating the niche to remain dormant, and eventually escape from dormancy when the niche matures.**

To address our hypothesis the following aims are proposed:
Aim1: Determine the mechanisms whereby DTCs control the dormancy of the niche cells.
Sub hypothesis: DTCs drive the niche into dormancy via GAS6 signaling.
Aim2: Determine if the differentiation of the niche cells triggers the regrowth of DTCs.
Sub hypothesis: Dormant DTCs exit from dormancy when the niche is differentiated via BMP2 signaling.

The proposed studies will provide significant insight into the mechanisms whereby the bone marrow microenvironment is involved in regulation of tumor dormancy. Aim 1 allows us to identify and explore how DTCs stay dormant for long periods of time. We postulate that DTCs drive the bone marrow niche into dormancy through the GAS6 pathway. Aim2 will determine how DTCs escape dormancy, consequently rendering them more susceptible to the chemotherapy. Results from this work will lead to a greater understanding of niche aging effects on metastatic growth, and could result in valuable new treatment approaches.

2. KEYWORDS:

Prostate Cancer; Bone metastasis; Disseminated tumor cells; Bone marrow microenvironment;
Tumor dormancy; GAS6; BMP2

3. ACCOMPLISHMENTS:

What were the major goals and objectives of the project?

The goal of this project is to understand the mechanisms of tumor dormancy and metastatic outgrowth of disseminated prostate cancer within the bone marrow microenvironment.

Task 1: Complete the grant transfer from University of Michigan to Wake Forest School of Medicine.

Months 1-3.

- Upon arrival at Wake Forest School of Medicine, the PI will seek to obtain the necessary approvals (IACUC, IRB, IBC) to complete the grant transfer, and then will initiate the proposed research as soon as possible (**Months 1-3**).

Task 2: Determine the mechanisms whereby DTCs control the dormancy of the niche cells.

Months 4-18.

- To determine the effects of GAS6 on the dormancy of niche cells *in vitro*, co-culture of bone marrow stromal cells (**BMSCs**) (pre-stained with DiD fluorescent dye) with either GAS6-downregulated PCa cells (**PCa^{shGAS6}**) or control PCa (**PCa^{Control}**) will be performed. At the termination of experiments, BMSCs will be harvested, and the retention of DiD dye will be measured with FACS (**Months 4-7**).

  To further characterize the difference, gene and protein expression of proliferation markers and cell cycle status will be analyzed using those isolated BMSCs (**Months 7-9**).

- To determine the effects of GAS6 on the dormancy of niche cells *in vivo*, we will perform a vertebral body implant (vossicle) experiment. We will implant BrdU-incorporated vossicles directly injected with PCa^{shGAS6} or PCa^{Control} into immunocompromized mice, and then will determine the effects of GAS6 on the dormancy of the microenvironment by immunohistochemistry for BrdU (**Months 9-14**).

  Additionally, using immunohistochemistry we will also visualize co-localization of PCa cells with the dormant microenvironment cells using these vossicles (**Months 14-19**).

Task 3: Determine if the differentiation of the niche cells triggers the regrowth of DTCs.

Months 19-36.

- To determine if the differentiation of the niche following exogenous BMP2 treatment stimulates the regrowth of DTCs *in vitro*, co-culture of BMSCs with G1-Red and SG2M-Cyan co-infected PCa cells will be performed. The differentiation of the niche, and the dormancy, proliferation, and cell

cycle status of PCa cells after treatment with recombinant mouse (**rm**) BMP2 will be analyzed (**Months 19-22**).

- To determine if the differentiation of the niche following the exogenous BMP2 treatment stimulates the regrowth of DTCs *in vivo*, we will implant vossicles directly injected with G1-Red and SG2M-Cyan co-infected PCa cells into immunocompromized mice. The differentiation of the niche, and the dormancy, proliferation, and cell cycle status of PCa cells after treatment with rm BMP2 will be analyzed (**Months 22-26**).

- To determine whether BMP2 expressed by DTCs is crucial for metastatic progression *in vitro*, co-culture of BMSCs with BMP2-downregulated PCa (**PCa$^{\mathbf{shBMP2}}$**), upregulated PCa (**PCa$^{\mathbf{BMP2OE}}$**), or control PCa (**PCa$^{\mathbf{Control}}$**) will be performed. Thereafter, the differentiation of the niche, and the dormancy, proliferation, and cell cycle status of PCa cells will be analyzed (**Months 27-30**)

- To determine whether BMP2 expressed by DTCs is crucial for metastatic progression *in vivo*, we will implant vossicles directly injected with PCa$^{\text{shBMP2}}$, PCa$^{\text{BMP2OE}}$, or PCa$^{\text{Control}}$. Thereafter, the differentiation of the niche, and the dormancy, proliferation, and cell cycle status of PCa cells will be analyzed (**Months 31-36**).

What was accomplished under these goals?

As of 03/01/15, thanks to receiving this Idea Development Award for Young Investigators, the PI, Dr. Yusuke Shiozawa started an independent faculty job as an Assistant Professor at Wake Forest School of Medicine. Upon his arrival at Wake Forest School of Medicine, the PI obtained the necessary institutional approvals (IACUC, IRB, IBC) and submitted the grant transfer request (06/11/2015) to gain approval from the Department of Defense for a transfer of the award from the University of Michigan to Wake Forest School of Medicine. As a result, the scientific progress of this award has been suspended at Wake Forest School of Medicine during this period of time. The PI is prepared to begin this work immediately when transfer details are completed.

What opportunities for training and professional development did the project provide?

Thanks to receiving an Idea Development Award for Young Investigators, the PI obtained independent status at Wake Forest School of Medicine with lab space, office space, and start-up costs provided.

How were the results disseminated to communities of interest?

There is nothing to report at this time, as progress has been postponed due to the grant transfer process from University of Michigan to Wake Forest School of Medicine.

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

Once the PI obtains the grant transfer approval from the Department of Defense, he will initiate the proposed research as soon as possible.

4. IMPACT:

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

There is nothing to report at this time, as progress has been postponed due to the grant transfer process from University of Michigan to Wake Forest School of Medicine.

What was the impact on other disciplines?

There is nothing to report at this time, as progress has been postponed due to the grant transfer process from University of Michigan to Wake Forest School of Medicine.

What was the impact on technology transfer?

There is nothing to report at this time, as progress has been postponed due to the grant transfer process from University of Michigan to Wake Forest School of Medicine.

What was the impact on society beyond science and technology?

There is nothing to report at this time, as progress has been postponed due to the grant transfer process from University of Michigan to Wake Forest School of Medicine.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

Since the PI moved to a new institution (Wake Forest School of Medicine), the progress of this award has been delayed at the Wake Forest School of Medicine due to the grant transfer process (The grant transfer request was submitted to the Department of Defense on 06/11/15). Once the PI obtains the grant transfer approval from the Department of Defense, he will initiate the proposed research as soon as possible (see also **ACCOMPLISHMENTS**).

Changes that have a significant impact on expenditures

Nothing to report.

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

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal Publication

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?

Name: Yusuke Shiozawa
Project Role: PI
Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0001-9814-9230
Nearest person month worked: 2.4
Contribution to Project: Dr. Shiozawa provides oversight of the entire program and development and implementation of all policies, procedures, and processes. In this role, Dr. Shiozawa is responsible for the implementation of the specific aims, and for ensuring that systems are in place to guarantee institutional compliance with US laws, including biosafety and animal research, data and facilities. Dr. Shiozawa supervises other personnel on the project to ensure timely and effective studies.
Funding Support: Department of Defense; and National Cancer Institution

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

Nothing to report.

What other organizations have been involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS:

The Quad Chart is attached as an appendix.

9. APPENDICES:

Nothing to report.

The Role of the Bone Marrow Microenvironment in Controlling Tumor Dormancy

PC130359
W81XWH-14-0403

PI: Yusuke Shiozawa **Org:** Wake Forest School of Medicine **Award Amount:** $225,000

Study/Product Aim(s)

- **Aim1**: Determine the mechanisms whereby disseminated tumor cells (DTCs) control the dormancy of the niche cells.
- **Aim2**: Determine if the differentiation of the niche cells triggers the regrowth of DTCs.

Approach

The goals of these experiments are to explore the mechanism(s) behind the transition of DTCs to dormancy in their interaction with the marrow niche, and to determine why the cells leave dormancy to grow into full-blown metastases.

Thanks to receiving this Idea Development Award for Young Investigators, the PI, Dr. Yusuke Shiozawa accepted an appointment as Assistant Professor at Wake Forest School of Medicine as of 03/01/15.

The PI obtained the necessary institutional approvals (IACUC, IRB, IBC) and submitted the grant transfer request (06/11/2015) to gain approval from the Department of Defense for a transfer of the award from the University of Michigan to Wake Forest School of Medicine.

Timeline and Cost

Activities / CY	11	12	13	14
Aim 1 (in vitro study)				
Aim 1 (in vivo study)				
Aim 2 (in vitro/in vivo study)				
Aim2 (in vitro/in vivo study)				
Estimated Budget ($K)	**$50**	**$60**	**$55**	**$60**

Updated: 09/30/15

Goals/Milestones

CY11 Goal – in vitro study

☐Determine how disseminated prostate cancer (PCa) controls the dormancy of the niche

CY12 Goals – in vivo study

☐Determine how disseminated PCa controls the dormancy of the niche

CY13 Goal – in vitro/in vivo study

☐Determine how differentiation of the niche affects the cell-cycle of the disseminated PCa

CY14 Goal – in vitro/in vivo study

☐ Determine how differentiation of the niche affects the progression of the disseminated PCa

Comments/Challenges/Issues/Concerns

- Since the PI moved to Wake Forest School of Medicine, the progress of this award has been delayed due to the grant transfer process, which was approved by the Department of Defense.

Budget Expenditure to Date

Projected Expenditure: $0
Actual Expenditure: $0