AWARD NUMBER: W81XWH-18-1-0487

TITLE: The Impact of the Dura Mater on Prostate Spine Metastases

PRINCIPAL INVESTIGATOR: Nicholas Szerlip

CONTRACTING ORGANIZATION: University of Michigan

REPORT DATE: SEPTEMBER 2021

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE		Form Approved OMB No. 0704-0188
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1. REPORT DATE SEPTEMBER 2021	**TYPE** ANNUAL	**3. DATES COVERED** **1SEPT2020 - 31AUG2021**
4. TITLE AND SUBTITLE The Impact of the Dura Mater on Prostate Spine Metastases		**5a. CONTRACT NUMBER** **5b. GRANT NUMBER** W81XWH-18-1-0487 **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S) Nicholas Szerlip E-Mail: Nicholas Szerlip Szerlip@umich.edu		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Michigan		**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)** **11. SPONSOR/MONITOR'S REPORT NUMBER(S)**
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited		
13. SUPPLEMENTARY NOTES		
14. ABSTRACT Purpose: Understanding the influence of the dura and its secreted cytokines is the goal of the proposed project. Scope: We intend to examine the effects of dura and its released cytokines on the invasion and growth of prostate cancer cells in-vitro and in-vivo and explore a possible mechanism for these effects. Major findings: Over this period, we have found that when dura is exposed to tumor that it changes the dura and leads to increase in certain cytokines these do not significantly effect tumor growth, migration or invasion. It leads to increase in cytokine release of il-6 and other inflammatory cytokines		
15. SUBJECT TERMS Bone Metastases, Prostate Cancer, CXCR2, Dura		

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	8	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

TABLE OF CONTENTS

Introduction

Cancer can spread to nearly all tissues of the body, one of the most common sites of spread is to the bones. The majority of these metastases are found in the spine. About two-thirds of all symptomatic bone metastases are located in the spine with up to 74% of patients having spine metastases at autopsy and a majority of these in the posterior part of the vertebral body. A key feature of the spinal skeleton local environment is the proximity of the dura mater adjacent to the posterior aspect of the vertebral body, the covering of the central nervous system. The immediate objective is to examine the dura and its effects on prostate cancer cells as well as the effect of tumor cells on the dura. Tumor cells change the environment to help themselves grow we examine this feedback loop and ways of blocking it. Because the dura is next to the vertebral body we look to molecules that the dura releases. These molecules act as keys that unlock doors to pathways that increase tumor malignancy and growth. We will use different methods to quantify the effect and determine the clinical significance of these keys in animal models. Keyword

keywords

Bone Metastases, Prostate Cancer, CXCR2, Dura, Cytokines, CXCL1, CXCL8,

Accomplishments

What were the major goals of the project? Examine the in-vitro effects of dural secreted factors on prostate cancer growth, survival, and invasion Examine the in-vivo effects of dura on prostate cancer initial invasion using animal models of spine metastases.

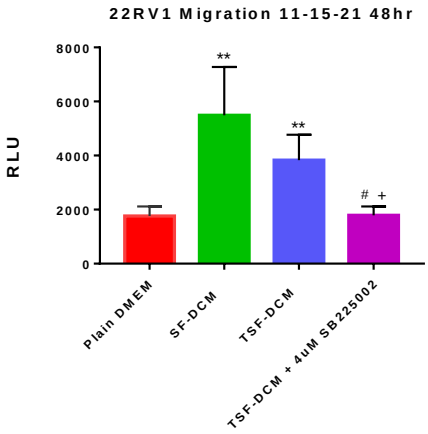
We continue to make progress albeit slowly in this covid arena. We are examining the role of CXCR2 receptor and activation of this receptor in prostate cancer by dural cytokines. We have done numerous experiments that show that the dural cytokines activate the CXCR2 receptor in prostate cancer and lead to increase growth and migration and that this effect is mitigated when a CXCR2 blocker is administered. We have finished all the in vivo studies with this and we are starting our animal experiments which will utilize SCID mice with an ossicle/vossicle model of metastases.

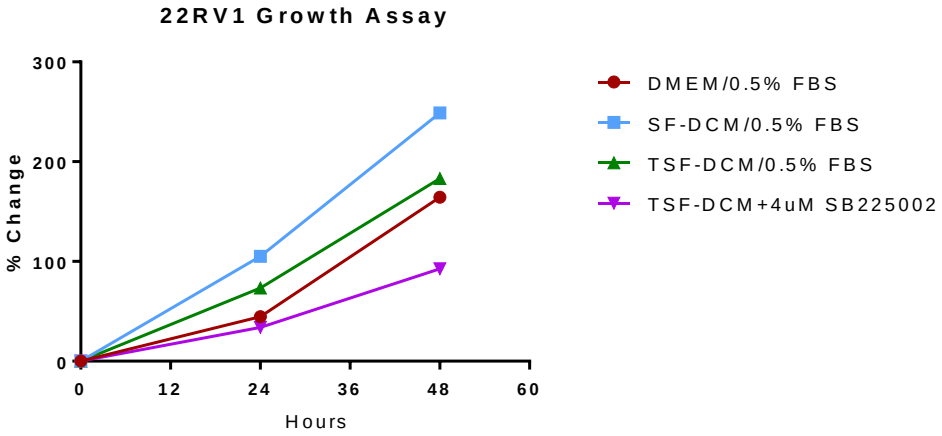
In this model we proposed putting fragments of vertebral bodies in a subcutaneous compartment of a mouse and examining the effects of dura and no dura on tumor spread after a cardiac injection of PC3 cells. We have done numerous preliminary experiments with this model and found it to be wanting. Although this model had been utilized in the past and published on by our colleagues it is not very robust. We got only one mouse out of every 20 that developed tumor in the subcutaneous bone fragments. This is not enough to show anything statistically or to power a study.

We applied for and made alterations to our animal protocol and model and will now inject tumor cells into the bone fragment before putting it subcutaneous. We will than use IVIS luciferase expression to follow the growth of these tumors in this model in a group of animals with CXCR2 inhibitor and one group without. This will show whether in vertebral bodies this inhibitor will modify the tumor growth and this could lead to human clinical trials using such an inhibitor. For the SOW this is **major task 2 subtask 1** this is the major experiment remaining to be completed.

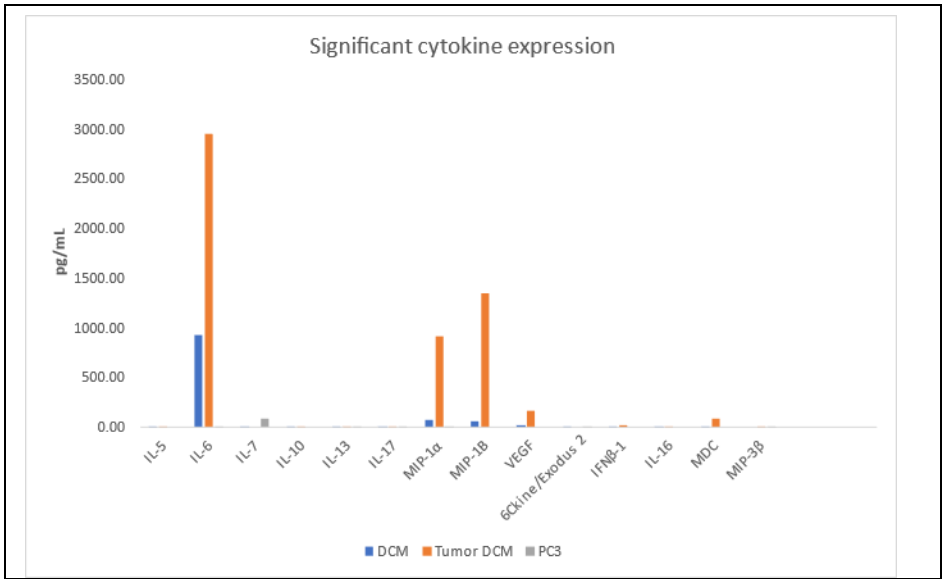
Our preliminary data suggested that CXCR ligand RNA expression is high in dura and increases when dura is exposed to tumor cytokines. This raised the idea of a reciprocal loop of increased expression of CXCR-ligand and increase tumor effects over time.

We created dural conditioned media by growing mouse dura in culture and than grew it in non contact co-cultures with tumor cells (PC3). We completed experiments looking at this conditioned media compared to the dura conditioned media. We examined growth, invasion, migration as we did previously(Major task 1 of aim 2.1). We saw that the dura conditioned media again increases growth and migration compared to plain media (with serum) but that the addition of the tumor conditioned dura media has little effect on growth or migration. Again the effect seen is mitigated by the CXCR2 inhibitor. This is despite the fact that the RNA levels of the CXCR2 ligand increased on the RNA sequencing we originally observed.

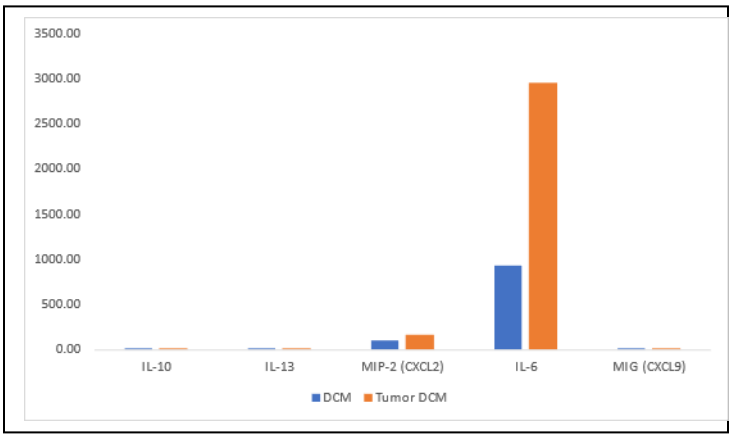




To further investigate this we performed ELISA measurements on the conditioned media and the tumor media to examine the cytokine levels in the media. **(subtask 2 Aim 2.1b)** described above. When this was done we found that the actual values of the ligand does not increase in the media even though we previously have seen RNA increases. This could be a ceiling that is reached. We have done experiments in most of our cell lines we have 2 lines left to examine the effects of tumor dural conditioned media. We attempted to obtain human dura and see if we could grow it and obtained from adults dura does not grow in sufficient quantities for a sufficient length of time to obtain conditioned media. It takes us weeks to grow the dura we have from mice and to than co-culture with tumor cells.



We have the mouse experiments to go and are all set for those over the next couple of months as well as finishing up the last of the in vivo cell characteristic experiments. At this time we are creating large enough quantities of dural tumor conditioned media to do the experiments in one go.



Impact:

We think that the results we have seen so far should be enough to push forward with a clinicl trial in the

future with CXCR2 inhibitors

Changes/Problems

There have been changes (see above) we had to abandon the intracardiac injection model after a few preliminary attempts had such a low rate of metastases that it would be prohibitive to use that many animals

to see a difference. We switched to a model where we inject the bone fragments with tumor and implant them. We can follow growth with IVIS as the cells have luciferase. We will follow local growth in the bone fragment as well as the rate of metastases from the fragments to other areas of the mouse.
We also attempted to grow human dura with no good results. We can only procure adult human dura and it looks like it does not grow as well as immature dura. This will not allow us sufficient samples to perform experiments with human dura, but since the cytokines are very similar we have strong faith that the results would be similar.

10. Products

11. Participants & Other Collaborating Organizations: University of Michigan

What individuals have worked on the project?

Name: Sabrina Rocco Project Role: Laboratory Technician Researcher Identifier (e.g. ORCID ID): NA Nearest person month worked: 6 Contribution to Project: Ms. Rocco has performed the bench experiments Funding Support: NA Name: Nicholas Szerlip Project Role: PI Researcher Identifier (e.g. ORCID ID): 0000-0003-1116-3422 Nearest person month worked: 8 Contribution to Project: Dr. Szerlip has organized and set up experiments and oversees work on project Funding Support: NA Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? No

12. Special Reporting Requirements NONE

13. Appendices