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14. ABSTRACT
Previously published data from our lab suggested that alterations in the novel actin binding protein AIM1 could be associated with prostate cancer progression and more aggressive biological behavior. In this reporting period, we have established numerous cell lines in which we have robustly depleted AIM1 by shRNAs and we have generated domain and mutation specific AIM1 expression vectors, which will allow us to study structure function relationships. Furthermore, we have investigated the frequency of germline genomic alterations in 4 large prostate cancer cohorts and observed an enrichment in deleterious germline mutations in men who developed metastatic disease. This observation provides first evidence that germline changes in genes involved in cytoskeletal organization can be associated with aggressive variants of prostate cancer. To corroborate this finding, we developed a novel genetically modified mouse model by crossing hi-MYC mice to Aim1$^{+/-}$ mice. These crosses yielded viable offspring and preliminary analyses suggest that constitutive *Aim1* loss in this model results in accelerated tumor progression. We are currently expanding these cohorts to determine the impact of *Aim1* loss at different timepoints. Collectively, these studies will provide important insights in the role of AIM1 and the actin cytoskeleton in prostate cancer biology.

15. Absent in melanoma 1 (AIM1), Prostate cancer, actin, cytoskeleton, invasion, germline alteration, mouse model

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TABLE OF CONTENTS

1. INTRODUCTION

The ability of cancer cells to invade locally and spread systemically contributes to most cancer related deaths from prostate cancer (1,2). Cellular invasion and metastatic dissemination require changes in the cytoskeleton which is the dynamic scaffold that defines cellular shape. The most unabundant component of the cytoskeleton is actin (3-5). Structural changes in the actin cytoskeleton are almost universal in cancer, but the molecular alterations that cause changes in actin organization in cancer are not well understood (6-9). In prior work leading up to this proposal we have recently identified a novel protein, AIM1 (absent in melanoma 1, also known as CRYBG1) that is involved in regulating actin organization (9). We have shown that loss of AIM1 increases cytoskeletal remodeling, cell migration, invasion and micrometastatic dissemination. These findings suggest that AIM1 could function as a suppressor of invasion and metastasis formation by binding to actin and regulating actin cytoskeletal dynamics (9).
The purpose of this proposal is to investigate the biological role of AIM1 in prostate cancer progression and develop new approaches to target tumors with AIM1 alterations. This necessitates a better understand the molecular mechanism by which AIM1 regulates the cytoskeleton in health and disease. To understand the basic biology of AIM1 we will characterize the structural requirements necessary for the interaction between AIM1 and actin and determine the role of AIM1 in tumor progression *in vivo*. To gain insights into the translational relevance of AIM1 in prostate cancer we will investigate genomic and expression differences across different disease states. Finally, we will explore therapeutic targeting opportunities through synthetic lethality screens in cell line with AIM1 dysfunction. These studies will provide a comprehensive assessment of AIM1, its regulation of the cytoskeleton and its role in tumor progression and will provide new targeting approaches for advanced prostate cancer.

2. KEYWORDS

Absent in melanoma 1 (AIM1), Prostate cancer, actin, cytoskeleton, invasion, germline alteration, mouse model

3. ACCOMPLISHMENTS

To summarize the progress made on this project to date, all new data is listed below and itemized according to the specific aims of the original proposal.

SA 1: Define structural requirements and phenotypic consequences of altered AIM1-actin interaction

Task 1.1: Map site(s) required for AIM1-actin interaction and determine the mode by which AIM1 regulates the actin cytoskeleton ***IN PROGRESS****.*

To map the interaction sites required for AIM1-actin binding, we have begun to generate deletion mutant expression constructs by first cloning a human *AIM1* cDNA into the pcDNA3.2/capTEV-NT/V5-DEST vector using the Gateway cloning system. Using site directed mutagenesis we have generated 10 *AIM1* mutants disrupting putative actin binding sites. A biochemical characterization of this mutant constructs is currently ongoing.

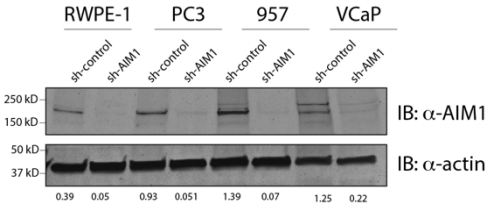


Figure 1. Efficient knock down of Aim1 in prostate cancer cell models.

Task 1.2: Test the phenotypic consequences of AIM1 perturbation in PC cell lines and organoid systems ***IN PROGRESS****.*

To study the consequences of AIM1 loss in *in vitro* systems we have generated stable derivatives of the prostate cancer cell lines PC3 and VCaP and of the benign prostate epithelial lines RWPE-1 and 957 expressing shRNA constructs targeting AIM1 with a knock down efficiency of >80% for all lines (**see Figure 1**). We were able to establish these lines as 3D organoid cultures in matrigel systems (**see Figure 2**). The phenotypic characterization of these cell lines is currently ongoing. In addition, we successfully established murine prostate organoids from C57BL/6 mice. These organoids will be further transduced with shRNA against *Aim1* and subjected to SiR-actin based live cell actin visualization studies, as described previously (10).

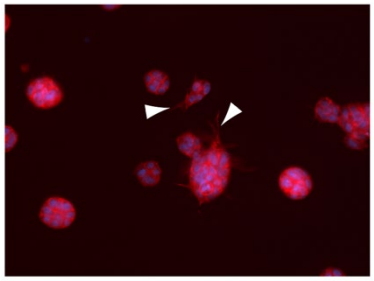


Figure 2. Prostate cancer organoid models stained for F-actin.

SA 2: Determine the consequences of AIM1 loss *in vivo*

Task 2.1: Investigate the spectrum of AIM1 alterations in advanced metastatic PC. ***IN PROGRESS***

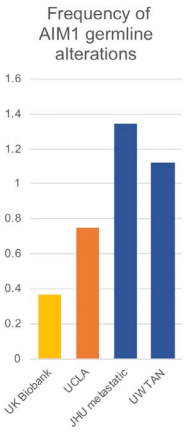


Figure 4. Frequency in percent of men with deleterious *AIM1* germline alterations in localized prostate cancer (yellow); locally advanced prostate cancer (orange) and metastatic prostate cancer (blue)

To begin to elucidate the pattern of genomic alterations of *AIM1* in prostate cancer we examined the association between deleterious (stop gain, frame shift) *AIM1* germline mutations and familial prostate cancer (defined as families with >3 members affected by PC). In collaboration with Dr. William Isaacs (The Johns Hopkins School of Medicine) we identified 4 families in which the majority of men diagnosed with prostate cancer showed germline changes in the *AIM1* gene locus (**Figure 3**). This supports our initial hypothesis that germline AIM1 alterations can contribute to prostate cancer progression.

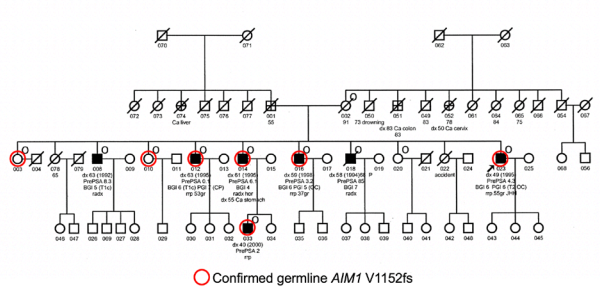
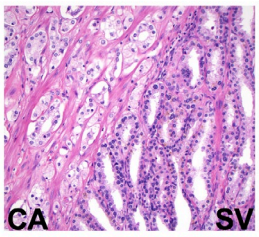


Figure 3. Pedigree of prostate cancer family with carriers of deleterious *AIM1* V1152fs mutation.

To further examine the association between germline *AIM1* alterations and disease progression, we analyzed the frequency of deleterious *AIM1* changes in germline samples from 4 cohorts. This included, 1086 men from the UK biobank cohort with low risk disease, 268 men from the Ontario Institute for Cancer Research/UCLA with locally advanced disease, 447 men with castration resistant prostate cancer from The Johns Hopkins School of Medicine and 89 samples with lethal metastatic prostate cancer from the University of Washington rapid autopsy cohort. Comparing these different datasets, we noted an enrichment of deleterious *AIM1* alterations in men with metastatic prostate cancer as compared to patients with localized disease (**Figure 4**). In addition, pathology review of cases with AIM1 mutations showed an association with higher tumor grade and stage. As shown in **Figure 5**, *AIM1* mutation carries exhibited a greatly increased rate of high-grade prostate cancer with 90% of tumors showing Grade Group 5 histology. In addition, a higher rate of locally aggressive disease, as evidenced by bilateral seminal vesicle involvement was observed (**Figure 6**).



AIM1	Impact	GS	SV involvement
E830fs	Frameshift	5+5	BX only
V1152fs	Frameshift	4+5	positive
W1166X	Stop gain	4+5	positive bilat.
W1545X	Stop gain	4+3	BX only
V1152fs	Frameshift	4+5	positive bilat.
Y1324X	Stop gain	4+5	positive bilat.

Figure 5. Histopathological assessment of primary tumors of AIM1 germline mutation carriers shows enrichment for high grade disease with bilateral involvement of seminal vesicles (SV).

Task 2.2: Model the AIM1 alterations in prostate GEMMs. ***IN PROGRESS.***

To better understand the consequences of hemizygous *AIM1* loss *in vivo*, we generated a novel mouse model of prostate cancer. We crossed the Hi-Myc prostate cancer model, which is a well characterized prostate cancer GEM model to mice with constitutive hemizygous loss of *Aim1 (Aim1$^{+/-}$)* which were obtained from *the* knockout mouse project (KOMP) mouse consortium through the Hospital for Sick Children (Toronto). Notably, for all crosses, we used a Hi-Myc line that was backcrossed to the C57BL/6 background. This allowed us to follow a straightforward breeding scheme.

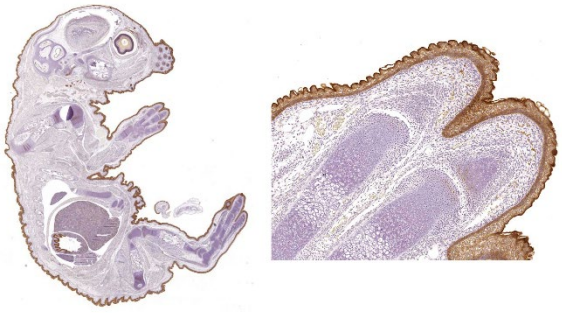


Figure 6. Assessment of Aim1 expression in murine embryos shows expression in epithelial tissues with highest level in epidermis and prostate.

We obtained all genotypes in the expected ratios, suggesting that *Aim1$^{+/-}$* is not embryonal lethal. We leveraged embryos from these early crosses to study Aim1 expression during development and observed that Aim1 is expressed in epithelial organs with highest expression in skin and prostate (**Figure 6**).

Although our mouse colonies are currenlty aging to obtained the desired number for all genotypes and timepoints (n=33 at 8mo, n=33 at 12mo, n=33 at 16mo; total n per group=99),

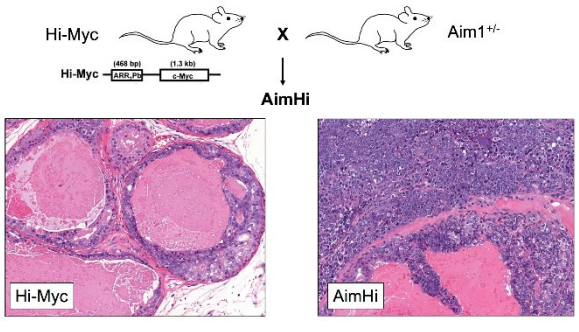


Figure 7. First evidence for accelerated disease progression in AIM1 heterozygous mice.

preliminary analysis of “canary” animals for each genotype at 8 months of age showed invasive carcinoma in 1/2 AimHi mice (Hi-Myc, *Aim1*$^{+/-}$) compared to high-grade PIN without any evidence for invasion in 2/2 Hi-Myc mice (**see Figure 7**). These very preliminary findings suggest a potential acceleration of the disease progression in the *Aim1*$^{+/-}$ *background.*

SA 3: Explore opportunities for synthetic lethal targeting in AIM1 altered cancers.

Task 3.1: Perform a genome-wide CRISPR-Cas9 knockout screen in isogenic AIM1-deficient and -proficient PC cell line models. ***IN PROGRESS.***

To prepare for the proposed genome wide screens, we have taken 2 steps. First, we prepared prostate cancer cell lines with robust AIM1 knock down (see **Figure 1**). Second, we obtained a genome wide CRISPR library which we expanded in bacteria and prepared plasmids for lentiviral packaging (11). We are planning to begin with the first cell line screens in the coming months.

KEY RESEARCH ACCOMPLISHMENTS:

- Demonstrated an enrichment of *AIM1* germline alterations in familial prostate cancer.
- Demonstrated that *AIM1* germline mutations are associated with lethal metastatic prostate cancer.
- Developed novel cell line models with *AIM1* alterations.
- Generated a new prostate cancer GEM model that mimics the hemizygous germline loss of *Aim1* observed in prostate cancer patients.

OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT:

This reporting period included my first year as an independent investigator in the Prostate Cancer Program in the Human Biology Division at the Fred Hutchinson Cancer Research Center. My laboratory now includes 1 post-doctoral fellow, a technician, a computational biologist and a staff scientist that I directly oversee and mentor. I have been expanding my professional development though involvement in the digital pathology committee at the University of Washington as well as my participation on the Prostate Cancer Program Faculty Search Committee and in the Pacific Northwest Prostate Cancer SPORE. Since January 2021, I am also a member of the editorial board for the journal The Prostate.
In the past year, I have received a Clinician Scientist award from the Doris Duke Foundation and a Young Physician-Scientist Award from the American Society for Clinical Investigation.
As part of my junior faculty mentoring committee, I meet with my faculty mentors Drs. Pete Nelson, Valeri Vasioukhin, and Julian Simon on a regular basis.

DISSEMINATION OF RESULTS:

The preliminary findings of this project were presented at the Fred Hutchinson Cancer Research Center Human Biology Faculty this spring. As our data matures, we hope to be able to assemble a first manuscript in the next 16-20 months.

PLAN FOR THE NEXT REPORTING PERIOD:

In the next reporting period, we will continue expand our *in vivo* modeling and perform more detailed characterization of the novel Hi-Myc, *Aim1*$^{+/-}$. We will expand our human tissue-based assessment of AIM1 alterations and perform further *in vitro* experiments to elucidate the role of AIM1 loss on actin dynamics. Lastly, we will initiate first genome wide *in vitro* screens to determine new targeting opportunities in cancers with AIM1 loss of function.

4. IMPACT

In this reporting period we provide first evidence that germline alterations in AIM1 are associated with more aggressive disease biology in prostate cancer. This established a novel link between genomic changes in genes involved in regulating the cytoskeleton and cancer progression. If validated in other cohorts, assessment of *AIM1* germline mutation status could be used to assess germline risk for aggressive prostate cancer. Although our project is focused on prostate cancer biology, it is possible that AIM1 genomic alterations are also relevant in other solid tumors. Therefore, the concepts established here in prostate cancer models, could inform future studies in other cancers.

5. CHANGES/PROBLEMS

The project was impacted by the COVID-19 pandemic which decreased the access to crucial samples, limited mouse breeding and diminished the workforce in our laboratory. Although the pandemic restrictions resulted in a slightly reduced productivity for this funding period, I am confident that the proposed aims can be completed in time without any major adjustments to the propose tiemline.

6. PRODUCTS

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	**Michael C. Haffner, MD, PhD**
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4.0
Contribution to Project:	Dr. Haffner performed data analysis and supervised the project
Funding Support:	N/A
Name:	**Jin-Yih Low**
Project Role:	Staff Scientist
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2
Contribution to Project:	Dr. Low performed wet lab-based experiments and assisted with data analysis.
Funding Support:	N/A
Name:	**Radhika Patel**
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3
Contribution to Project:	Ms. Patel performed wet lab-based experiments.
Funding Support:	N/A

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William B Isaacs (The Johns Hopkins Schools of Medicine), Paul Boutros (UCLA) and Peter S. Nelson (Fred Hutchinson Cancer Research Center) provided data for the germline genomics studies and assisted with the analysis.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

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10. APPENDIX

None