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14. ABSTRACT

The Genitourinary Cancer Biorepository at the University of Washington joined the Prostate Cancer Pathology Resource Network (PCBN) September 30th 2014. The purpose of this interaction is to provide high quality, well annotated specimens that can be used by prostate cancer researchers through the PCBN. The University of Washington Biorepository has a focus on advanced stage disease. Specimens provided by the University of Washington site includes blood (serum, plasma, and buffy coat), prostatectomy tissues (frozen), biopsies and metastatic tissue from rapid autopsies (paraffin embedded material and tissue microarrays (TMAs)), prostate cancer patient derived xenografts (PDX) and derived specimens (DNA and RNA) from prostate cancer patients. These specimens are linked to clinical and outcome data and supported by an informatics infrastructure. In this 4th year of operation the University of Washington site has accrued new specimens from the clinic, surgery, and at autopsy, manufactured and provided TMAs, sera and derived RNA and DNA where required. Specimens were made available to prostate cancer researchers through the PCBN.

15. SUBJECT TERMS

Biorepository, prostatectomy, rapid autopsy, serum, patient derived xenografts

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Introduction

The Prostate Cancer Biorepository Network (PCBN) is a public bioresource that provides high quality, well annotated specimens that can be used by prostate cancer researchers through the PCBN http://prostatebiorepository.org. This biorepository is a collaborative effort between Johns Hopkins University (JHU), New York University (NYU), Memorial Sloan Kettering Cancer Center (MSKCC), University of Washington (UW), Washington University (WU), and the Department of Defense. The PCBN coordinating center is at JHU. UW is a network site.

The Genitourinary Cancer Biorepository at the University of Washington joined the Prostate Cancer Pathology Resource Network (PCBN) September 30th 2014. Access to clinical specimens from patients with advanced disease can be challenging so the Genitourinary Cancer Biorepository set up a rapid autopsy program to provide access to metastatic tissue and create patient derived xenograft (PDX) models of advanced disease. The biorepository also has an extensive collection of blood (serum, plasma, and buffy coat), prostatectomy tissues (frozen), and derived specimens (DNA and RNA) from prostate cancer patients; these specimens are linked to clinical and outcome data and supported by an informatics infrastructure.

Keywords

Biorepository, prostate cancer, patient derived xenografts, rapid autopsy, serum, radical prostatectomy, metastatic, biomarkers.

Accomplishments

The Major goals of the project were (1) patient accrual and biospecimen acquisition, (2) providing specimens to external investigators, and (3) improving biospecimen science.

Patient Accrual and Biospecimen Acquisition:
The adjacent table shows specimens prospectively accrued to the PCBN through the University of Washington during the 48 month period covered by this report. African American patients comprised only 3% of the patient specimens we accrued at UW. Increasing enrollment is an ongoing challenge for investigators at UW.

Biospecimen Acquisition October 2014 - September 2018	Total Specimens Collected
Serum	
Pre-RRP	414
Metastatic	264
Total	**678**
Tissue	
Prostatectomy	371
Metastatic Sites Sampled	620
Normal Sites Sampled	455
Metastatic Biopsy	24
Total	**1470**

Radical prostatectomies: During the year, we prepared frozen OCT embedded tissues from 371 prostatectomies. Ninety-five were from high risk patients (Gleason 8 and above), 231 were from medium risk (Gleason 7) and 40 were low risk (Gleason 6). Five had an undetermined Gleason due to prior hormone and radiation treatment.

Rapid autopsies: We performed forty-four rapid autopsies during the four years. The prostate cancer patients were approached by oncologists in the clinic and through the altruism and generosity of the patients and their families as soon as the patient passes we dispatch an ambulance to pick up the body and bring it back to the University of Washington where our autopsy team is prepared for a rapid autopsy and tissue acquisition. Based on our historic data over twenty years, we typically expect 8-9 autopsies a year, which has been exceeded in the last four years. All specimens have been processed and read by a pathologist.

Serum and Plasma Isolation: Sera were obtained from 414 prostatectomy patients and 245 metastatic patients. Plasma and buffy coats were obtained from 405 prostatectomy patients and 32 metastatic patients.

Tissue Microarrays: We constructed a tissue microarray (TMA) of specimens from forty-five patients from the rapid autopsy program (two bone and two visceral metastasis per patient across four blocks; UWTMA79). However, the TMA has been almost completely exhausted, so we manufactured a new tissue microarray (TMA) with metastatic castration-resistant tissue from forty-five different patients (2010-2017) with three cores from each site, with three sites per patient, across four blocks (UWTMA92). We also constructed a twenty patient TMA (UWTMA81; 2005-2014) with three cores from each site, with two sites per patient. Later we updated this TMA with a further twenty patients (UWTMA93; 2014-2017) with three cores from each site, with two sites per patient. Additionally, a TMA of thirty LuCaP PDX models was constructed (UWTMA78). This was replaced by a TMA consisting of 42 PDX models three xenografts per model, three cores per xenograft (UWTMA89). Associated de-identified clinical data was also abstracted.

Patient Derived Xenografts: Five new lines were established over the four year period. We are currently maintaining 42 lines and continue to passage tumors in animals to develop new lines.

DNA/RNA Isolation: We have a bank of RNA/DNA from primary prostate, xenografts and metastases, but as we collect more tissue, we isolate additional RNA and DNA. During the last four years RNA was isolated from a 138 metastases, 112 xenograft tissues, 16 benign prostates, 20 primary prostate cancers, and 10 normal control tissues and DNA from 26 metastases for the biorepository.

Providing Specimens to External Investigators:
We have provided specimens and de-identified clinical data for forty-six different requests detailed below.

1. Serum samples from 108 non-recurrence and 104 recurrent prostate cancer patients.
2. Ten 4+3, ten 3+3, ten normal, and ten metastatic RNA tumor specimens.
3. Fifty normal and 50 cancer serum specimens.
4. RNA from fifteen normal, primary, and metastatic prostate cancer specimens.
5. Xenograft RNA.
6. Serum from twenty CRPC and twenty benign patients.
7. Serum from 70 patients with benign prostate, 70 Gleason 3+3, 70 Gleason 3+4, 70 Gleason 4+3, and 50 Gleason 8/9.
8. Three metastases TMAs.
9. Four metastases TMAs.
10. One metastasis TMA.
11. Two metastases TMAs.
12. Twenty RNA specimens from CRPC metastases.
13. One metastases TMA.
14. Two metastases TMAs.
15. Four xenograft TMAs.
16. Two metastases TMAs.
17. Matched RNA and DNA from five xenograft lines and ten metastases.
18. One metastasis TMA.
19. One metastasis and xenograft TMA.
20. Sixteen sections of six neuroendocrine metastases, three xenograft lines, and normal prostate.
21. Sections of bone and soft tissue metastases from three rapid autopsy patients.
22. A metastasis TMA.
23. Two Metastases and two LuCaP xenograft TMAs.
24. Two LuCaP TMAs.

25. Nine LuCaP TMAs.
26. One LuCaP TMA.
27. Serum from 46 patients with CRPC.
28. Paraffin sections from 53 CRPC patients (three sections/patient) and six patients with primary disease.
29. We provided frozen tissue from 48 LuCaP xenografts and four primary prostates.
30. Two xenograft TMAs.
31. Three sections of a metastasis TMA.
32. Paraffin embedded sections and RNA from seven xenograft models.
33. Five sections of a patient derived xenograft TMA.
34. Three sections of a xenograft TMA.
35. RNA from five normal prostate and seven metastases.
36. Five frozen xenograft tumors.
37. Three sections of a xenograft TMA and a metastasis TMA.
38. RNA and FFPE sections from five neuroendocrine xenografts.
39. Serum and FFPE tissue sections from twenty patients with CRPC.
40. One section of a xenograft TMA.
41. Frozen tissue from three different xenografts.
42. One xenograft TMA.
43. Two xenograft TMAs and frozen tissue from three xenograft models.
44. Three metastases and three xenograft TMAs.
45. Ten frozen metastases and ten DNA specimens from same metastases.
46. Matched Serum, DNA, and RNA from thirty patients with CRPC.

Improving Biospecimen Science:

Quality Assurance Study (1): In discussions with Dr. De Marzo agreement was reached to create 2 LuCaP xenograft series TMAs to assess the effect of sectioning and the storage of tumor specimens on antigenicity. The LuCaP xenograft tissues were perfect models to test our hypothesis in. Drs. Corey, Morrissey and De Marzo discussed and designed a template of the first TMAs to assess the stability of protein and RNA over 5 years. The availability of the LuCaP PDX paraffin blocks over prolong period of time is a great resource to perform this stability study. We selected four different tumors with variable AR, TMPRSS2/ERG, PTEN and MYC expression and used three different tumors and 5-8 cores for each within the 5 year period. The TMA blocks were constructed and sent to Dr. De Marzo for analysis.

Quality Assurance Study (2): In the past we have questioned whether the quality of RNA from rapid autopsy tissue was due to warm ischemia time or due to the pathology of the tumor (in some cases a significant amount of the tumor is necrotic). We are analyzing RNA specimens from the rapid autopsy program in an attempt to assess the impact of time on RNA quality. We have identified 90 specimens from 49 patients. We are cross referencing the tissue acquisition time, % necrosis in the tissue, tissue type and RIN to determine if there is a linear relation between time and RNA quality. Further, we have observed an increased number of patient specimens from the rapid autopsy program that appear to have lost androgen receptor expression. We are now starting to assess the specimens for androgen receptor, chromogranin A and synaptophysin expression to 'phenotype' the metastases. We have isolated RNA and DNA from the metastases from rapid autopsy patients for RNAseq and exome-seq. The exome-seq analyses are ongoing, however the RNAseq analyses in combination with immunohistochemical analyses have provided critical characterization data on castration-resistant specimens from the rapid autopsy program that appear to have lost androgen receptor expression or display neuroendocrine features. 'Phenotyping' the specimens gives the investigator a greater understanding of the specimens they are analyzing enhancing their research.

This work from quality assurance (1) has resulted in a publication on Biospecimen Science with the Johns Hopkins site. The work from quality assurance (2) on the immunohistochemical analysis of the CRPC metastases above has also contributed to an article we are working on currently.

Impact

New prostate cancer PDX models were developed. Biospecimens from the clinic, operating room and at autopsy were collected for distribution and future use by the prostate cancer research community. Clinical specimens and associated data were provided to researchers. An article on biospecimen scicence was published, with an additional article in preparation.

Changes/Problems

In the initial application, one of the focuses was to obtain metastatic biopsies for distribution. This proceeded in year one, however, after the initial collection, we were unable to obtain additional metastatic biopsies for the biorepository or distribution. Repeated attempts were made to access this material, however, given the limited amount of material collected, we were unable to 'link-in' with ongoing studies to obtain specimens for research for the biorepository.

Products

The reportable outcomes for the project include tissue acquisition, PDX development, and TMA construction, and specimen distribution are already discussed under accomplishments.

Publications

Rapid Loss of RNA Detection by In Situ Hybridization in Stored Tissue Blocks and Preservation by Cold Storage of Unstained Slides. Baena-Del Valle JA, Zheng Q, Hicks JL, Fedor H, Trock BJ, Morrissey C, Corey E, Cornish TC, Sfanos KS, De Marzo AM. Am J Clin Pathol. 2017 Nov 2;148(5):398-415. PMID:29106457.

Presentations

Rapid Loss of RNA Detection by In Situ Hybridization in Stored Tissue Blocks and Preservation by Cold Storage of Unstained Slides. Baena-Del Valle JA, Zheng Q, Hicks JL, Fedor H, Trock BJ, Morrissey C, Corey E, Cornish TC, Sfanos KS, De Marzo AM. Am J Clin Pathol. 2017 Nov 2;148(5):398-415.

Rapid Loss of RNA Detection by In Situ Hybridization in Stored Tissue Blocks and Preservation by Cold Storage of Unstained Slides (2113) Javier A Baena Del Valle, Qizhi Zheng, Jessica Hicks, Helen Fedor, Bruce J Trock, Colm Morrissey, Eva Corey, Toby C Cornish, Karen S Sfanos, Angelo M De Marzo. [Abstract of a poster presentation at USCAP, San Antonio, TX. March 2017].

Website

http://prostatebiorepository.org/

Participants & other Collaborating Organizations

This program involves interactions with the coordinating site at Johns Hopkins for the distribution of specimens.

What individuals have worked on the project?

Name:	*Colm Morrissey*
Project Role:	*PI*
Nearest person month worked:	*1.2 calendar months per year*
Contribution to Project:	*Coordinating with Dr. Trock, managing the biorepository, obtaining permissions and distribution of specimens.*
Funding Support:	*NA*

Name:	*Eva Corey*
Project Role:	*Investigator*
Nearest person month worked:	*1.0 calendar months per year*
Contribution to Project:	*Coordinating with Dr. Morrissey, involved with the development, maintenance, study and distribution of our LuCaP series of prostate cancer xenografts.*
Funding Support:	*NA*

Name:	*Lawrence True*
Project Role:	*Investigator*
Nearest person month worked:	*1.0 calendar months per year*
Contribution to Project:	*Coordinating with Dr. Morrissey, Dr. True is a genitourinary research pathologist who has been a leader of the Biorepository for some time now. He primarily participated in the rapid autopsy program, and assessing tissue from radical prostatectomies, including providing histological analysis of all tissue acquired.*
Funding Support:	*NA*

Name:	*Oliva Sommers*
Project Role:	*Research Scientist*
Nearest person month worked:	*2.4*
Contribution to Project:	*Maintained patient derived xenograft models and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Alex Van-Den-Ende*
Project Role:	*Research Scientist*
Nearest person month worked:	*3*
Contribution to Project:	*Tissue acquisition from radical prostatectomies and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Olena Tseona*
Project Role:	*Research Scientist*
Nearest person month worked:	*2.4*
Contribution to Project:	*Tissue acquisition from radical prostatectomies and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Halima Essien*
Project Role:	*Research Scientist*
Nearest person month worked:	*3.6*
Contribution to Project:	*Tissue acquisition from radical prostatectomies and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Rebecca Ann De Frates*
Project Role:	*Research Scientist*
Nearest person month worked:	*1.8*
Contribution to Project:	*Tissue acquisition from radical prostatectomies, blood processing and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Fung Man Poon*
Project Role:	*Research Scientist*
Nearest person month worked:	*2.4*
Contribution to Project:	*Tissue acquisition from radical prostatectomies, blood processing and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Belinda Nghiem*
Project Role:	*Research Scientist*
Nearest person month worked:	*2.4*
Contribution to Project:	*Tissue processing, blood processing, phlebotomy, consenting patients and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Jennilee Kho*
Project Role:	*Research Scientist*
Nearest person month worked:	*1.8*
Contribution to Project:	*Consenting patients, phlebotomy and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Khanh Thy Kin Doan*
Project Role:	*Research Scientist*
Nearest person month worked:	*1.2*
Contribution to Project:	*Tissue acquisition from radical prostatectomies, blood processing, tissue microarray construction and a role in rapid autopsy program.*

Funding Support:	*NA*

Name:	*Lisha Brown*
Project Role:	*Research Scientist*
Nearest person month worked:	*1.2*
Contribution to Project:	*Immunohistochemistry, tissue processing and a role in rapid autopsy program.*
Funding Support:	*NA*

Name:	*Jennifer Noteboom*
Project Role:	*Research Scientist*
Nearest person month worked:	*1.0*
Contribution to Project:	*Managing the Biorepository and the biorepository database, and clinical data abstraction.*
Funding Support:	*NA*

Name:	*Lori Kollath*
Project Role:	*Research Scientist*
Nearest person month worked:	*2.4*
Contribution to Project:	*Managing the Biorepository and the biorepository database, and clinical data abstraction, and a role in the rapid autopsy program.*
Funding Support:	*NA*

Name:	*Bryce Lakely*
Project Role:	*Research Scientist*
Nearest person month worked:	*1.8*
Contribution to Project:	*DNA and RNA isolations, server maintenance, tissue processing and a role in the rapid autopsy program.*
Funding Support:	*NA*

CHANGES IN SUPPORT

Title: W81XWH-13-PCRP-PCPRNA (Morrissey)
Time Commitment: 1.2 calendar
Supporting Agency: DOD Prostate Cancer Research Program (PCRP)
Grants Officer: Kathy Robinson, Grants Officer, help@cdmrp.org, (301)682-5507
Performance Period: 9/30/14 – 9/29/18 (No cost extension)
Level of Funding: $805,476
Goals/Aims: The Prostate Cancer Biorepository Network (PCBN) is a public bioresource that provides tissue and other biospecimens to all prostate cancer investigators. The goal of the PCBN is to develop a biorepository with high quality, well-annotated specimens obtained in a systematic, reproducible fashion using optimized and standardized protocols. The PCBN derives its specimen resources from extensive, well-characterized patient populations with a long history of supporting clinical and biomarker research.
Role: PI
Overlap: None

Title: W81XWH-15-1-0430 (Nelson)
Time Commitment: 0.6 calendar
Supporting Agency: Fred Hutchinson Cancer Research Center
Grants Officer: Mackenzie Krouse, Research Program Coordinator, / mkrouse@fredhutch.org, (206)667-1315; Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N., Seattle, WA 98109
Performance Period: 9/1/15-8/31/18
Level of Funding: $573,149
Goals/Aims: This project centers on the treatment of castration resistant prostate cancer (CRPC) metastases. The specific aim is to determine the molecular identity and genomic diversity across the spectrum of metastasis found within individual men with CRPC via the tissue acquisition necropsy program where multiple metastases are acquired from each patient.
Role: PI of Subcontract
Overlap: None

Title: W81XWH-16-1-0584 (Petros)
Time Commitment: 0.12 calendar
Supporting Agency: US Department of Defense
Sponsor Contact: Emory University
Performance Period: 07/01/2016 – 06/30/2019
Level of Funding: $18,466
Goals/Aims: The purpose of this application is to determine the molecular consequences of the 10398 mtDNA mutation in three distinct models of prostate cancer bone metastases: (1) the human, (2) human tumor nude mouse xenografts and (3) genetically modified mouse models of prostate cancer. Thereby testing the broad hypothesis that the 10398 bone metastasis-specific mutation is crucial to the development of prostate cancer bone metastasis.
Role: Co-Investigator
Overlap with proposed research: None

Title: Targeting AR-NULL CRPC, W81XWH-17-1-0414 (Morrissey)
Time Commitment: 3.0 calendar
Supporting Agency: US Department of Defense
Sponsor Contact: Janet Kuhns, janet.p.kuhns.civ@mail.mil, 301-619-2827.
Performance Period: 07/01/2017 – 06/30/2020
Level of Funding: $805,613

Goals/Aims: Targeting the Mechanisms Driving Double-Negative Basal-Like Prostate Cancer. The goal of this application is to identify targets for the treatment of androgen receptor null castration-resistant prostate cancer in *in vitro* and pre-clinical models of disease.
Role: PI
Overlap with proposed research: None

Title: Targeting chemokine signaling and MAPK/ERK pathway in advance prostate cancer 17CHAL22 (Yu)
Time Commitment: 0.6 calendar
Supporting Agency: Prostate Cancer Foundation 2017 Movember-PCF Challenge Award
Sponsor Contact: Mark Buzza, mark@movember.com
Performance Period: 12/31/2017-12/31/2018
Level of Funding: $112,163 **(UW subcontract)**
Goals/Aims: To assess the activation of the MAPK/ERK pathway in CRPC.
Role: Co-Investigator
Overlap with proposed research: None

Title: Therapeutic Targeting of Neuroendocrine Prostate Cancer,
Time Commitment: 1.5 calendar
Supporting Agency: CDMRP
Performance Period: 9/30/18-9/29/21
Level of Funding: $193,540 (**UW subcontract**)
Brief description of the project's goals: Neuroendocrine prostate cancer incidence has increased with wide clinical use of abiraterone and enzalutamide. This project focuses on investigation of neuroendocrine prostate cancer and specifically role of BRN4.
Role: Co-PI
Overlap with proposed research: None

Title: Pacific Northwest SPORE
PI: Nelson
Supporting Agency: Fred Hutchinson Cancer Research Center (NIH/NCI)
Performance Period: 09/01/18 – 08/31/23

Project 5: Basic Science Leader
Time Commitment: 1.2 calendar
Level of Funding: $75,000 yearly
Goals/Aims: The major goals of P2 are to determine the anti-tumor activity of the BET bromodomain inhibitor ZEN-3694 in patients with AR-independent CRPC. Identify and target critical Master Regulator transcription factors and kinases that promote AR-independent cell survival. Identify molecular markers of transition from AR-active to AR-independent CRPC subsets and identify and target critical pathways that promote survival of specific AR-independent subsets.

Core B: PI Morrissey
Time Commitment: 1.2 calendar
Level of Funding: $198,000 yearly
Goals/Aims: The major goal of the Biospecimen Core is to provide a well-organized and standardized system of specimen collection, storage, distribution and related clinical/research information dissemination that is based on over two decades of experience. The Core will ensure consistency and quality assurance in the pathological analysis of tissue specimens. It will maintain a large series of prostate cancer xenograft lines developed by Core investigators, which will be used for proposed studies by the SPORE investigators.
Role: Core Co-Director

Special Reporting Requirements
N/A

Appendices

N/A