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TITLE: Proof of Concept for Systematic Collection of Optimal Molecular Quality Anatomically Oriented Normal Prostate from Diverse Age and Race Transplant Donors

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In a one-year Exploration-Resource Development project, we propose to collect additional normal prostate tissues from transplant donors, create tissue microarrays using these and previous samples, and use this experience to apply for funds beyond this pilot award for a large resource.

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Introduction

In a one-year Exploration-Resource Development project, we propose to collect additional normal prostate tissues from transplant donors, create tissue microarrays using these and previous samples, and use this experience to apply for funds beyond this pilot award for a large resource.

Body

This report applies to the Nov. 2004 to Dec 2005 time period, and is up to date to August 2, 2006.

I apologize for the delay in this report. My laboratory is devoted to improving very long term infrastructural issues in prostate cancer research, of which this pilot project is a part. I am finding it very hard to continue making progress on such long term issues in the current environment, when I am spending inordinate time keeping my lab funded, having to apply for funding much more often than in the past, with correspondingly less time to get actual research work done.

Nonetheless, significant progress has been made on this project:

1. In preparation for collecting additional tissues, and for creating tissue microarrays, we performed an exhaustive microscopic analysis of the tissues already available in the resource, including marking each block according to whether central zone (CZ), peripheral zone (PZ), and/or transitional zone (TZ) microanatomic. The results are shown in Table 1 below.

Table 1: Comprehensive Microscopic Analysis of Nl Prostate Resource by Subject, Zone, and Fixation Type

C Case#	NCI Study		Age	CZ-Fr	CZ-Et	CZ-F	PZ-Fr	PZ-Et	PZ-F	TZ-Fr	TZ-Et	TZ-F	# Fr	# Et	complete Et + Fr?
1		Cauc	35	1	0	0	0	1	0	1	1	0	2	2	y
2	Y	Cauc	30	1	1	0	1	1	0	1	0	0	3	2	y
3		Cauc	41	0	0	0	0	1	0	0	1	0	0	2	n
4		Cauc	32	1	0	1	0	1	1	0	1	0	1	2	y
5	Y	African American	19	1	1	0	1	1	0	0	0	0	2	2	n
6	Y	Cauc	41	1	0	0	1	1	0	0	1	0	2	2	y
7		African American	44	1	0	1	0	1	0	0	1	0	1	2	y
8		Cauc	4	0	0	0	0	0	0	0	0	0	0	0	n*
9	Y	Cauc	25	1	0	0	1	0	0	0	1	0	2	1	y
10	Y	African American	20	1	0	0	1	1	0	0	0	0	2	1	n
11		Cauc	31	0	1	0	0	1	0	0	0	0	0	1	n
13	Y	Cauc	35	1	0	0	1	1	0	1	1	0	3	2	y
12		Cauc	52	0	1	0	0	0	0	1	0	0	1	1	n
TOTAL				9	4	2	6	10	1	4	7	0	19	20	y=7

It is promising that we were able to identify CZ, PZ, and TZ on all three types of tissues collected (frozen, formalin fixed-paraffin embedded, and alcohol fixed-paraffin embedded), but still we were able to clearly identify all three: CZ, PZ, and TZ in both alcohol and formalin fixed in only a little over half the cases (7/13). The main reason we couldn't positively identify zones in all cases was that even though these prostates were carefully oriented and sectioned according to a 3D plan, because standard tissue cassettes were used, orientation of each piece relative to the periphery of the gland, and relative to other blocks, was difficult.

Based on our experience, saying with confidence that a portion of tissue is from the CZ, PZ, or TZ is based on both gross and microscopic anatomy, and our gross anatomy information was not clear enough. We thus determined that a thorough analysis of other collection methods must be performed prior to collecting additional tissues.

2. We were very impressed with the histological quality of the tissues of all three fixation types. The frozen tissue in particular is the finest we've seen, most likely due to the instant heat-sink method of freezing used, preventing ice crystal formation. Our studies to date have also shown that the availability of frozen and paraffin material appear to be the most important. With high quality frozen material, we can generate alcohol fixed tissue sections of high quality quite easily if they are desired. Thus, alcohol fixation-paraffin embedding should be dropped from future normal prostate tissue collection.

3. A detailed study of the current state of whole-mount prostate tissue processing was performed, and as a result of this research, I formed a collaboration between my laboratory and Dr. Michael Emmert-Buck's Pathogenetics Unit of the Pathology Branch of the National Cancer Institute. Dr. Emmert-Buck and his colleague Dr. John Gillespie have significant experience with whole mount tissue processing and in technology development, and together we are designing a new method for whole mount tissue processing to be employed for future tissue collections. I am meeting with them on August 11, 2006 to continue this planning. One issue is to design a method for performing flash freezing with whole mounts. This may require construction of a special device using their machine shop.

4. I have redesigned the tissue collection plan, and I will begin training the staff of the Transplant Resource Center of Maryland on performing the collections at a meeting on August 8th at their facility. We will begin additional collections shortly thereafter.

5. I have constructed a tissue microarray from the first 13 cases, but currently do not have adequate staff or web resources to provide these at cost to scientists from my laboratory directly. As an alternative, I've looked into providing them to researchers to commercial tissue microarray companies. I am currently in discussions with three of these, and would appreciate your guidance on this. I would recover my costs from such a supplier, but they would be free to charge whatever the market would bear. The advantage of doing this is that I think I could get this done sooner, or I could continue to put together the right web resources and staff to make them available. A third possibility that I'm pursuing is to allow the NCI tissue microarray resource provide them to others, but this would not allow my lab to recover costs, which reduces our ability to continue to collect tissues.

Key Research Accomplishments

-Examined existing collected tissues in detail morphologically, and found issues for identification of prostate zones using current collection method
-designed new collection method
-planned training of staff in new collection method
-created initial tissue microarray and currently exploring methods to make it available to others

Reportable Outcomes: Identified problems with current methods for identifying prostate zones. Created new method to eliminate these limitations.

Conclusions: A normal prostate tissue resource from individuals of diverse ages and races with optimally collected tissues will provide perhaps the most important resource possible to understand better how to prevent and detect prostate cancer. We will continue to develop the methods for such a resource, and using these new methods for additional collections to commence in the next several weeks.

References: None

Appendices: None