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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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14. ABSTRACT The lack of availability of high-quality whole normal human prostate tissue from subjects of various ages and races impedes prostate cancer research in several ways. It prevents examination of prostate molecular pathologic changes on a time continuum, thus preventing the establishment of a definition of what the range of "normal" cellular activity is in a human male prostate for any one age or race. Just as important, the lack of availability of well-curated whole normal prostate tissue across the age and race spectrum impedes the development of truly representative animal models of human disease. This project has completed or partially completed several key steps, each of which must be accomplished to a high degree of quality in order for this Resource Development prototype project to prove feasibility of a scaled-up version of this work, which will be necessary to provide sufficient sample numbers for many types of research. These steps are: IRB approval, Tissue Availability, Tissue Collection, Tissue Archiving and Preparation, Tissue Sharing Protocol, and Database Integration. Progress in each aspect is discussed.					
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Introduction

The bulk of valuable molecular research published in prostate cancer to date is based on radical prostatectomy specimens from men with prostate cancer, or on metastatic prostate cancer tissues collected at surgery or autopsy. The majority of “normal” control tissues for such studies come from the same radical prostatectomy specimens, and less commonly from TURP (transurethral resection of the prostate) samples from men with benign prostatic hyperplasia, cystoprostatectomy specimens in men with bladder cancer, or rarely from autopsy samples collected no sooner than 12-24 hours after death, although documentation is usually absent or scant²⁻⁵.

Using noncancerous areas of radical prostatectomy specimens or samples from men with other GU diseases as “normal” control tissue has critical drawbacks. Prostate cancer is often multifocal when it arises⁶⁻¹¹, and there is strong evidence that precancerous changes occur gradually over a period of years in men predisposed to the disease^{5,12-14}. Based on these observations, the noncancerous areas of prostates of men with prostate cancer are probably in a state more prone to the development of cancer than men of the same age and race without prostate cancer. Therefore, having a population-based sample of prostates from men of various ages and races is essential for us to understand whether this critical difference exists.

In a similar vein, when a potentially critical molecular finding is identified and is suspected to be specific to prostate cancer, the lack of definition of detailed molecular pathways used by “normal” human prostate cells *in situ* makes it much more difficult to identify the best way to apply chemical or other means to intervene in the aberration.

Moreover, the lack of availability of high-quality whole normal human prostate tissue from subjects of various ages and races impedes prostate cancer research in several ways.

First, it prevents examination of prostate molecular pathologic changes on a time continuum, thus preventing the establishment of a definition of what the range of “normal” cellular activity is in a human male prostate for any one age or race. Having a reference set of well-collected “normal” tissue (see proposal body a discussion of what is “normal” for this proposal) will increase the value of past prostate cancer research by establishing this reference set, and will markedly increase the value of future prostate cancer research by allowing researchers to obtain critical context at the time of their research studies.

Second, and just as important, the lack of availability of well-curated whole normal prostate tissue across the age and race spectrum impedes the development of truly representative animal models of human disease. We need to know how aging, heredity, and exposure gradually erode natural defense mechanisms against cancer in some men more than others, and we need to know how similar these various defense mechanisms are in specific animal models under study. The relevance of animal model-based prostate cancer experiments is tenuous unless comparisons of normal tissues are made between model animals and human. They are currently not possible because of the lack of resources proposed to be developed.

Body

Please note that this is an Exploration-Resource Development project, with specific goals but no specific statement of work requested or provided.

The overall goal of this project, in a nutshell, is to develop a good way to eliminate many of the problems listed in the Introduction by collecting a series of normal prostates from men of various ages and races, and then make these tissues available to other researchers in a way that optimally supports good prostate cancer research, and good collaboration. This might sound easy to the uninitiated or to those who do not understand the quality

requirements for such work, but several aspects of the project have never before been accomplished even in rudimentary form, let alone with the high quality required to support prostate cancer research progress.

There are several key steps, each of which must be accomplished to a high degree of quality in order for this Resource Development prototype project to prove feasibility of a scaled-up version of this work, which will be necessary to provide sufficient sample numbers for many types of research. These steps are: IRB approval, Tissue Availability, Tissue Collection, Tissue Archiving and Preparation, Tissue Sharing Protocol, and Database Integration. Each of these is discussed in turn.

IRB approval took longer than expected but was obtained after one year of recurrent administrative time expenditure.

Tissue Availability was initially through the Maryland Transplant Resource Center, with study team members participating in the collection, but this was found to be highly cumbersome and reduced available prostates because the research team staff could not always be available to fly or drive to transplant donor organ harvesting procedures. This led to more detailed analysis of the problem in meetings with the MTRC in August and September 2006, where the MTRC agreed to do the collection directly without outside help if provided a simplified, easy to follow protocol and all the materials needed. An additional source of tissue was identified in January 2007 through meetings with the Maryland Medical Examiner's office. Activation of this collection plan thus awaits only the completion of the simplified protocol and associated devices described below.

Tissue Collection was completed for an initial 13 cases, and results from this pilot work has been analyzed in detail to provide a foundation for scale-up. Methods used to test and verify the quality of the pilot prostate collection method was described in a previous annual report. Analysis of various aspects of the quality of these initial samples has continued since the last report. Critical issues for tissues to form a solid basis for long term prostate cancer research include:

- quality of fixation
- histologic quality
- immunostaining quality
- ability to define prostate zones
- ability to fully reconstruct prostate from individual blocks at gross and histologic levels
- quality of material for molecular analysis
- metadata collection and validation

Analysis of results from the first 13 cases revealed several positive features, and several important limitations of the pilot collection technique that would hinder ability to support high quality research if scaled up to collection of hundreds of prostates. Positive features of the pilot collection technique include high quality frozen section histology, high quality ethanol fixed tissue morphology (but difficult to interpret because its artifacts are not well understood in relation to artifacts commonly seen in standard formalin fixed tissue), high quality immunostain results, and high quality RNA.

Limitations discovered through this ongoing analysis are (in order of importance):

- ✓ relative difficulty defining prostate zones using standard size tissue blocks (many otherwise excellent blocks contain tissues that could not be accurately defined as part of Peripheral Zone (PZ), Central Zone (CZ), or Transition Zone (TZ))
- ✓ limited availability of tissue for a given project because tissues were divided into three groups, fixed either by formalin, ethanol, or freezing. So, for example, for a given normal prostate, we might have no or only very limited tissue from the TZ available for study.

- ✓ variations in thickness of the blocks led to variation in thickness of tissue microarray (TMA) cores inserted into tissue microarray blocks, adding complexity to the TMA construction process, and causing tissue to be unnecessarily wasted.
- ✓ Inability to use blocks to register the prostate blocks in 3D space. Thus, the blocks could not be used to do analyses plotting molecular data to specific cellular locations in the prostate. This is an important limitation because little is known about the importance of anatomic location within the prostate in terms of the origin or progression of cancer, and without such “volume registration” such studies cannot be performed.
- ✓ excessive time needed for initial sectioning/labeling/processing of prostate after surgical removal from transplant donor because instant-freezing machine (Gentle Jane Snap Freezer, Instrumedics) can freeze only a single section at a time
- ✓ Metadata collection for each block of tissue (relative location in the prostate, fixative used) even with checklists and well designed forms, was too prone to error and confusion for use by technicians in the field, working without direct professional supervision.

In order to retain the positive features described above, while eliminating all or at least some of the problems listed, we have identified the following solutions. The status of each portion of this solution is listed.

Problem	Solution/Status
Difficulty defining prostate zones	Do whole mount processing of prostates only, and limit fixation methods to two (formalin fixation, and frozen blocks). Fix end pieces with formalin, and alternate sections in between between formalin and frozen. Use formalin sections to identify zones primarily, then identify zones in frozen tissue through comparison to formalin fixed sections in combination with direct histologic analysis. More on whole mount processing below.
Limited availability of tissue from all zones for a given project	Solved by limiting fixation methods to two, and ensuring as above that all portions of each prostate will be able to be identified by zone
Variations in thickness of the tissue blocks	This requires that all tissue blocks be cut to the same thickness as the prostate is handled in the fresh state. We examined an experimental prostate sectioning machine produced in England for this purpose (see PubMed ID 15858122), but found that this produces tissue blocks in the 5 mm thickness range, which is too thick for good fixative penetration and good division of the prostate into alternating formalin and frozen components (too few slices per prostate). Based on our experience, a prostate slicing device which is safe and easy to use, and produces sections that are consistently 3.25 mm thick is needed, and is unavailable. We have created a preliminary design for such a device, and worked with a mechanical engineer to develop a prototype, but this engineer was not able to come up with solutions that would allow initiation and uniform cutting of whole prostates. We had to abandon work with this engineer and instead identified a potential off the shelf solution described in Jhavar et al{7907}, and since that time have been negotiating a Material Transfer Agreement to obtain a copy of the reported device sometime in September or October 2007. If this device works well in testing, this problem will have been solved.
excessive time needed for initial sectioning/labeling/processing of prostate after surgical removal from transplant donor	This requires a method of rapid freezing that allows multiple sections to be conveniently and rapidly labeled and frozen simultaneously. We performed detailed searches and were unable to find such a device. We have a preliminary design for such a device, and have identified an alternate mechanical engineering resource to help build a prototype, with efforts to develop a prototype to be started in September 2007. This is another critical step before allowing collection to begin again.

Metadata collection for each block of tissue	In our past annual report, we detailed efforts (see screenshots in previous report) underway to create an integrated database of clinical phenotype and molecular data together will comprehensive management of tissue blocks, slides, and images. Based on our direct experience, if such data are not managed with appropriate data-validation methods using a modern database, serious errors and omissions in downstream research are inevitable. Progress in this area has continued since the last report and is now sufficient to support release of initial slides directly once we complete a “collaboratory” portion of the web application as described below.
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Tissue Archiving and Preparation. Our existing system for managing standard-size tissue blocks is highly functional, and supported creation of initial tissue microarrays to be released to investigators. It will need to be modified to handle large format blocks. This will require new block storage boxes, and new slide files, both of which are available on the market. Small supplies of large format cassettes, and large format glass slides have been obtained to test collection of large format samples. A new tissue microarray block designed for collaboration has been created, and 50 tissue microarray slides have been cut and made ready for use in initial sharing. Funds obtained through initial sharing will be used to fund cutting and processing of additional slides if initial collaborations are successful.

Tissue Sharing Protocol. The original proposal included release of slides to investigators. Simple release of slides will assist in research (and is supported as below), but based on discussions with various researchers, the ability to search a database for other data obtained from the same slide by other researchers would greatly magnify the value of the slides and speed research, and researchers in an informal poll indicated a willingness to do this. On a test basis, a series of tissue microarray slides containing the normal prostate tissue samples were released to Drs. David Berman, Dr. Vasanth Yegnasubramanian, Dr. Alan Meeker, and Dr. Ben Casero, for immunohistochemistry studies of important new potential molecular markers, with the proviso that images of each spot of the stained slides would be uploaded to the PELICAN database and used for integrated research (after primary publication by each investigator). These researchers not only agreed to this, but followed through and provided the slides for automated imaging and upload to the integrated PELICAN database. In order to formalize this process on the web, we are working with Joe Gusmano J.D. of the Office of Technology Transfer of Johns Hopkins University to create a simple process for researchers to obtain and use the slides as above, maintaining research subject protections and allowing each party specified rights and responsibilities. A major overhaul of our web database has yielded a highly efficient means for researchers to view both slides available and tissue microarray spot images from each slide.

Database Integration. Is sufficient for collection and processing of the samples. We have made progress in creating a collaboration store, where researchers will be able to conveniently order the slides, and if they choose to participate in the collaboration portion of the site, to have images from their slide uploaded to the database, and for the researchers to search staining results from other researchers for the same slides. As stated in the original Resource Development application, we will charge researchers for the slides, to help defray the costs of continued support and development of this major new prostate cancer research resource. We have also created a convenient web-based tissue microarray slide search and viewer module as shown (Figures 1 and 1). This system is not yet ready to go live, but is anticipated to be ready by the end of 2007.

Figure 1. Tissue Microarray Search Module to be used by collaborators

JUMP TO BLOCK:

ADD A SLIDE SERIES
ADD A SLIDE

▼ **Array Block 160**
 ▸ Slide #1 (ID# 256147)
 ▼ **Series 1 (Slides 1 - 7)**
 Slide #1 (1 of 7) (ID# 256210)
 Slide #2 (2 of 7) (ID# 256211)
 Slide #3 (3 of 7) (ID# 256212)
 Slide #4 (4 of 7) (ID# 256213)
 Slide #5 (5 of 7) (ID# 256214)
 Slide #6 (6 of 7) (ID# 256215)
 Slide #7 (7 of 7) (ID# 256216)
 ▼ **Series 2 (Slides 8 - 17)**
 Slide #8 (1 of 10) (ID# 257116)
 Slide #9 (2 of 10) (ID# 257117)
 ▸ Slide #10 (3 of 10) (ID# 257118)
 ▸ Slide #11 (4 of 10) (ID# 257119)
 ▸ Slide #12 (5 of 10) (ID# 257120)
 ▸ Slide #13 (6 of 10) (ID# 257121)
 ▸ Slide #14 (7 of 10) (ID# 257122)
 ▸ Slide #15 (8 of 10) (ID# 257123)
 ▸ Slide #16 (9 of 10) (ID# 257124)
 ▸ Slide #17 (10 of 10) (ID# 257125)
 ▼ **Series 3 (Slides 18 - 27)**
 ▸ Slide #18 (1 of 10) (ID# 257126)
 ▸ Slide #19 (2 of 10) (ID# 257127)
 ▸ Slide #20 (3 of 10) (ID# 257128)

Tissue Microarray Primary Info. Update Page

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(* denotes a required field.)

Array ID	160
Array Name *	Metastatic Prostate Car
Array Job ID	107
Array Job Name	40 core array05212003
Array Core Diameter (mm) *	0.60
Center to Center Spacing (mm) *	1.20
Array Total Columns	10
Array Total Rows	4
Array Maker's Lab Name *	PELICAN Laboratory
Array Maker's Name *	Yi, Xiande
Focus Dx *	METASTATIC CARCINOMA
Array Start Date	2003-05-21 00:00:00.0
Array Completion Date	2003-05-21 00:00:00.0
Embedding Medium *	Embedding Medium-Paraffin, Type 6, Stephens Scientific
Currently Processed By *	Bova, G. Steven

Figure 2. Tissue Microarray Viewing Module to be used by collaborators

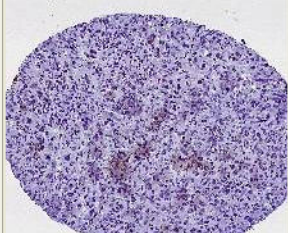
Tissue Microarray Slide Viewer

Array ID	Array Name	Slide ID
160	Metastatic Prostate Cancer/Normal Prostate/Normal Tissue 40 Core Optimization Formalin Array	257118

Slide Label:
TA 160 11/23/2003
CD31

	1	2	3	4	5	6	7	8	9	10
1	27724 A32 L Kidney 1 NL (A32)	1786 A1 Subd Met (A1)	2953 A2 Liver Met 8 (A2)	2116 19 Met (A4 Para LN Met) (A4)	2307 Hilar LN Met (A9 Hilar LN Met) (A9)	23918 Pericardial Bulk Mets 41 (A16) (A16)	2035 3LP CA (A17) (A17)	28395 Liver 1 NL (A33) (A33)	31505 LNCaP Formalin (CLORG1)	31507 PC3 Formalin (CLORG3)
2	31509 DU145 Formalin (CLORG4)	31422 22Rv1 2 (CLORG2)	31426 PC-3 3 (CLORG3)	24317 PR 6-1-1 (C1) (C1)	24496 PR 7-1-1 (C4) (C4)	31217 Testicle B (A25) (A25)	24430 NT-3 (C3 Transverse Colon) (C3)	16526 L psoas (C7) (C7)	28986 Iliac Nerve 2 (C13) (C13)	16559 Bladder (C7) (C7)
3	2409 12 NL (A12 Pancreas) (A12)	1589 L lung B NL (A5) (A5)	1598 L Kidney NL (A5) (A5)	1604 R Adrenal NL (A5) (A5)	2397 2 NL (A12 Left Ventricle) (A12)	1611 Abd Skin NL (A5) (A5)	752 30 NL (A22 Frontal Cortex) (A22)	2761 Stomach and Pancreas tail NL (A21) (A21)	31644 Broncheal cartilage-2 (C22) (C22)	3965 L Cervical Spinal Cord A3 NL (A28) (A28)

Image



Meta Information

Image ID	34472
array column number	3
array image slide code	257118
array image x coord	286
array image y coord	122
array row number	2
block image type code	8

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Key research accomplishments

- ✓ Further defined the first robust system for researchers to obtain, share, and potentially collaborate using a set of extremely high quality normal prostate samples
- ✓ Tested and implemented new methods at each stage of the complex process needed for this resource to function at a high level
- ✓ Identified and designed new technologies to fill out the stepwise process to allow the resource to function at a high level. Now seeking funds to build these tools.
- ✓ Designed, partially implemented and now testing web interface for researchers to obtain pilot samples in tissue microarray slide format, planned to go live by the end of 2007.

Reportable outcomes:

- ✓ None so far. When the resource website is available we will publicize it through journal articles and announcements to research societies.

Conclusions:

- ✓ We have made substantial progress in the project, despite being unprecedented and highly complex at every level.
- ✓ With extremely high quality well-curated tissue, and a far more convenient and advanced way of obtaining and sharing qualified collaboration information than currently available methods, we expect that with continued development of this pilot resource will greatly facilitate prostate cancer and related research.

References: None so far

Appendices : None