BIOBANKING OF DERIVATIVES FROM RADICAL RETROPUBIC AND ROBOT−ASSISTED LAPAROSCOPIC PROSTATECTOMY TISSUES AS PART OF THE PROSTATE CANCER BIOREPOSITORY NETWORK (PCBN)

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ABSTRACT

Introduction: The PCBN is a collaboration between Johns Hopkins University and New York University, and is funded by the Department of Defense (DOD). The goal of the PCBN is to develop a biorepository with high−quality specimens obtained using optimized and standardized protocols, and an infrastructure to facilitate the wide usage of the resource by the prostate cancer research community. We have developed a series of standard operating procedures (SOPs) for the extraction, quality assessment and biobanking of derivatives from frozen tissues harvested from radical retropubic prostatectomy (RRP) and robot−assisted laparoscopic prostatectomy (RALP) specimens. As part of developing protocols for derivative extraction from prostatectomy tissues, we aimed to scrutinize the potential use of RALP specimens for prostate cancer biomarker studies.
Methods: DNA, RNA and protein were extracted from RRP and RALP specimens. Quality assessment of DNA and RNA was conducted using spectrophotometric analysis and a series of quantitative real−time PCR assays for housekeeping genes such as 18S, GAPDH and β−globin. RNA samples from RRP versus RALP were analyzed for RNA integrity number (RIN) and by real−time PCR for biomarkers known to be differentially expressed in tumor and benign tissues such as racemase, hepsin, ERG, the TMPRSS−ERG gene fusion, and the microRNA miR−26a. We assessed protein quality by Western blot for the phosphoproteins pAKT and pS6.
Results: High quality derivatives were obtained from RRP and RALP tissues. No differences were observed between the quantity and quality of biospecimens derived from RRP versus RALP tissues. The average RIN for RRP samples was 7.6 +/− 1.9 and 7.8 +/− 1.6 for RALP samples. Likewise, expression levels of genes analyzed by real−time PCR and protein levels analyzed by Western blot did not differ between RRP and RALP−derived tissues.
Conclusions: We demonstrate that our SOPs for the extraction and biobanking of derivatives from frozen tissues harvested from RRP and RALP specimens yield samples of sufficient concentration and of high quality. Furthermore, our studies analyzing the differential expression of tumor−associated biomarkers in RRP versus RALP−derived specimens indicate that samples obtained from RALP specimens may be of suitable quality for prostate cancer biomarker studies.

INTRODUCTION

- One specific focus of the PCBN is to characterize critical parameters in the biospecimen "life cycle" that influence the molecular integrity of research tissues.
- **An emerging point of interest in the field of prostate cancer biobanking is an apparent shift in the proportion of surgical procedures performed for prostate cancer treatment from RRP to RALP.**
- Hesitation towards the use of RALP-derived biospecimens in prostate cancer research studies is driven by the fact that RALP specimens undergo a longer period of intra-operative warm ischemia time. This can be at least double to triple the period of warm ischemia time that RRP specimens undergo.
- We aimed to scrutinize the potential use of RALP specimens for prostate cancer biomarker studies.

SOP DEVELOPMENT

- The goal in the development of DNA, RNA and protein extraction SOPs was to choose the processing method that preserves the greatest number of analytes while maximizing quality/yield.

DNA	RNA	Protein
• DNeasy Blood and Tissue Kit (Qiagen)	• Trizol (Invitrogen)	• RIPA Buffer

- The following standard QC methods were established to assess the quality of derivatives:
  - Quantification of DNA/RNA using Nanodrop, RNA integrity number (RIN) obtained with Agilent Bioanalyzer.
  - Real time PCR protocols optimized to amplifying the housekeeping genes β globin and 18S for DNA and GAPDH and 18S for RNA.
  - Protein quantified via BCA assay and quality controlled via Western blot for phosphoproteins.

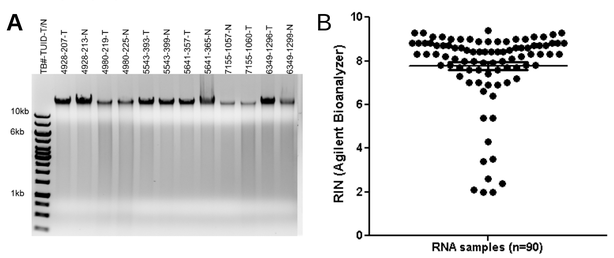

Figure 1. Assessment of nucleic acid quality as part of SOP development. **(A)** Agarose gel of DNA samples extracted from frozen prostate tissue specimens **(B)** RIN of RNA samples extracted from frozen prostate tissues (Agilent Bioanalyzer). Average RIN = 7.8, 91% RIN > 5, 84% RIN ≥ 7.

RESULTS

Comparative Biomarker Expression between RRP and RALP by Real-time PCR

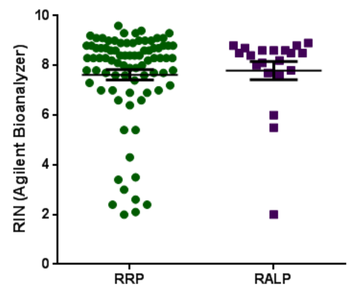

Figure 2. Distribution of RIN numbers obtained for RNA extracted from RRP (n=83) versus RALP (n=20) specimens. The average RIN for RRP samples was 7.6 +/− 1.9 and 7.8 +/− 1.6 for RALP samples.

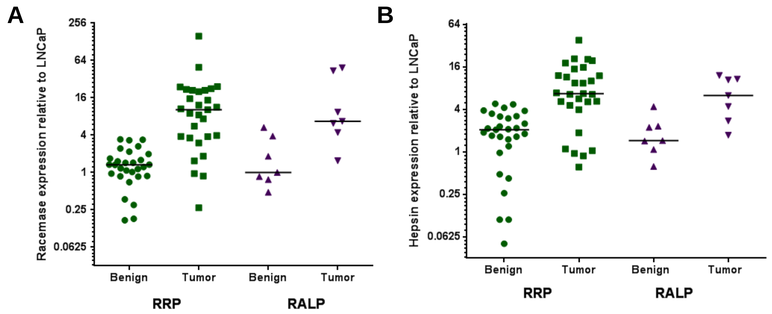

Figure 3. Distribution of the relative expression (Log 2) of **(A)** racemase and **(B)** hepsin as compared to LNCaP among tumor/benign RNA pairs from open radical prostatectomy and robot-assisted laparoscopic prostatectomy.

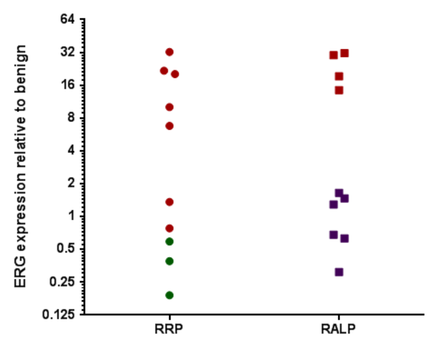

Figure 4. Distribution of the relative expression (Log 2) of ERG in tumor samples as compared to benign among RNA pairs from open radical prostatectomy and robot-assisted laparoscopic prostatectomy. *Samples shown in **red** were positive for the TMPRSS-ERG fusion via qPCR.

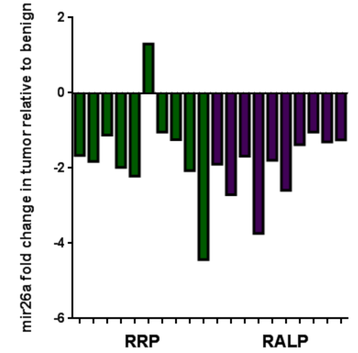

Figure 5. Fold change in mir26a expression in tumor samples relative to benign as assessed by TaqMan® Small RNA Assay. RRP sample, RALP sample. Note consistent downregulation of mir26a in tumor samples relative to benign.

Comparative Biomarker Expression between RRP and RALP by Western Blot

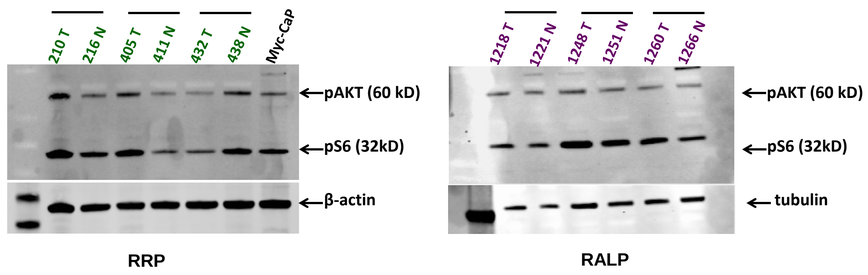

Figure 6. Western blot detection of pAKT and pS6 in protein extracts from matched pairs of tumor and benign frozen tissues from RRP (left) and RALP (right) specimens. Myc-CaP used as positive control and β-actin or tubulin used as loading control.

The **Prostate Cancer Biorepository Network** (PCBN) is a public biorepository open to all prostate cancer investigators. Please visit http://prostatebiorepository.org if you are interested in obtaining biospecimens or other services.

REPORTABLE DATA ON PCBN SAMPLES

DNA	RNA
➢ H&E stained frozen section taken immediately before and immediately after frozen sections collected for sample preparation (scanned slides)	➢ H&E stained frozen section taken immediately before and immediately after frozen sections collected for sample preparation (scanned slides)
➢ For tumor/normal pairs, H&E sections reviewed for % tumor/normal by a pathologist	➢ For tumor/normal pairs, H&E sections reviewed for % tumor/normal by a pathologist
➢ Quantification (Nanodrop)	➢ Quantification (Nanodrop)
➢ Quantification (real-time PCR for β-globin)	➢ RNA quality (RIN number, Agilent Bioanalyzer)
➢ DNA quality (real-time PCR for 18S, β-globin)	➢ RNA quality (real-time PCR for 18S, GAPDH)
	➢ Additional biomarker QC (real-time PCR for hepsin,racemase, ERG, TMPRSS-ERG, mir26a)

CONCLUSIONS

- SOPs for the extraction and biobanking of derivatives from frozen tissues harvested from RRP and RALP specimens yield samples of equally high quality.
- Studies analyzing tumor-associated biomarkers in RRP versus RALP-derived specimens including qPCR for hepsin, racemase, ERG, and mir26a as well as western blot for the phosphoproteins pS6 and pAKT indicate that samples obtained from RALP specimens may be of suitable quality for prostate cancer biomarker studies.

METHODS

Nucleic Acid and Protein Extraction. All DNA, RNA and protein samples were extracted from frozen prostate tissues harvested from radical prostatectomy specimens. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). RNA was extracted using Trizol (Invitrogen). Protein was extracted using a standard RIPA buffer. Further details of SOPs and extraction protocols can be found at **http://www.prostatebiorepository.org/protocols**.
Quantitative PCR (qPCR). RNA for hespin, racemase, and ERG qPCR was reverse transcribed using the SuperScript First Strand Synthesis System for RT-PCR (Invitrogen, Cat # 11904-018) . qPCR was carried out using gene-specific primers and SYBR Green PCR Master Mix (Invitrogen Catalog # 4309155). RNA for mir26a qPCR was reverse transcribed using the TaqMan® MicroRNA Reverse Transcription Kit (Life Technologies) and miRNA-specific primers. qPCR was performed using the TaqMan® Small RNA Assay (Life Technologies).
Western blot. Protein lysates (10 µg) were run on a 4-12% polyacrylamide gel and transferred to nitrocellulose membranes (Invitrogen). After blocking in 5% BSA, membranes were probed overnight at 4ºC with α-pAKT antibody (Cell Signaling, 1:1000), α-pS6 antibody (Cell Signaling, 1:2000) and α-tubulin (CP06, Calbiochem, 1:2000) or β-actin (Sigma, 1:5000). After washing, membranes were probed with LI-COR Odyssey secondary antibody (1:10,000, LI-COR Biosciences) and visualized with a LI-COR Odyssey Imaging System (LI-COR Biosciences).

ACKNOWLEDGEMENTS

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