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

Medha Darshan1, Helen L. Fedor1, Qizhi Zheng1, Laxmi G. Pellakuru1, Patricia Kolmer2, Ruth Pe Benito3, Monica Gorman3, George Netto1, Jonathan Melamed3, Peng Lee3,4, Angelo M. De Marzo1, Bruce J. Trock2, Karen S. Sfanos1

Departments of Pathology and Urology2, Johns Hopkins University School of Medicine, Baltimore, MD
Departments of Pathology1 and Urology4, New York University School of Medicine, New York, NY

ABSTRACT

INTRODUCTION

• One specific focus of the PCBN is to characterize critical parameters in the biobank "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.

METHODS

• DNeasy Blood and Tissue Kit (Qagen) • Tris-oHCl (Qiagen) • 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 amplify the housekeeping genes β-actin and tubulin for 18S and β-globin.
  - Protein quantified via BCA assay and quality controlled via Western blot for phosphoproteins.

SOP DEVELOPMENT

• The goal is the development of DNA, RNA and protein extraction SOPs to allow the processing method that preserves the greatest number of analytes while maximizing quality/yield.

RESULTS

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

CONCLUSIONS

• Studies analyzing tumor-associated biomarkers in RRP versus RALP-derived specimens including pCPR for hepatocellular carcinoma 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.

REPORTABLE DATA ON PCBN SAMPLES

DNA
• 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 were reviewed for % tumor/normal by a pathologist.
• Quantification (Nanodrop)
• RNA quality (real-time PCR for 18S, GAPDH)
• RNA quality (real-time PCR for 18S, GAPDH)
• Additional biomarker QC (real-time PCR for hepsin/caspinase, ERG, TMPRSS-ERG, mir26a)

METHODS

Nucleic Acid and Protein Extraction. All DNA, RNA and protein samples were extracted from matched radical prostatectomy (RRP) and robot-assisted laparoscopic prostatectomy (RALP) specimens. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). RNA was extracted using Trizol (Invitrogen). Protein was extracted using standard RIPA buffer. Further details of SOPs and extraction protocols can be found at http://www.prostatebiorepository.org/protocols.

Quantitative PCR (qPCR), RNA for hepsin, caspinase, and ERG 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 4°C with α-pAKT antibody, β-actin, or tubulin. Membranes were probed with LI-COR Odyssey secondary antibody (1:1000, LI-COR Biosciences) and visualized with a LI-COR Odyssey Imaging System (LI-COR Biosciences).

ACKNOWLEDGEMENTS

This work was supported by Department of Defense (DOD) grant number W81XWH-10-2-0016. We would like to acknowledge Maria Gresczak and Kriessler Lecksel for prostate harvesting efforts.