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TITLE: Telomere Length as a Predictor of Aggressive Prostate Cancer

PRINCIPAL INVESTIGATOR: Elizabeth A. Platz, ScD, MPH

CONTRACTING ORGANIZATION: Johns Hopkins University School of Medicine Baltimore MD 21205

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We are evaluating whether men with shorter telomere length in prostate cancer tissue and in normal appearing prostate tissue adjacent to adenocarcinoma have a higher risk of aggressive prostate cancer than men with longer telomere length. Since the last progress report (Nov 2005) the tissue microarrays were assembled by our Harvard collaborators. At Hopkins, we completed staining of the tissue microarrays for telomere length and are in the processing of imaging the spots and estimating telomere length.
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**INTRODUCTION**

We are investigating whether telomere length in prostate cancer cells (prognostic factor) and in normal prostate epithelial cells (risk factor) predicts risk of aggressive prostate cancer, that is, prostate cancer with a greater potential to progress. We hypothesize that men with shorter telomeres will be at higher risk of high grade prostate cancer, biochemical failure, metastasis, and death from prostate cancer. Because telomere shortening occurs with each round of replication and with oxidative damage, we also hypothesize that men with dietary and lifestyle profiles predicted to enhance cellular proliferation or oxidative stress are more likely to have shorter telomeres in normal prostate epithelial cells.

**BODY**

The aims of this proposal were to:

1. To test whether median telomere length in prostate cancer cells differs between men with aggressive prostate cancer and nonaggressive prostate cancer.

2. To test whether median telomere length in normal-appearing prostate epithelial cells differs between men with aggressive prostate cancer and nonaggressive prostate cancer.

3. To test whether median telomere length in normal-appearing prostate epithelial cells differs across levels of factors predicted to influence:
   a) Cellular proliferation – total energy intake, obesity, physical inactivity
   b) Oxidative stress – antioxidants (lycopene, selenium, vitamin E) and oxidants (cigarette smoking)

4. Secondary objective: To support the use of telomere length in peripheral blood lymphocytes as a surrogate for telomere length in prostate epithelium, we will determine the correlation between telomere length in peripheral blood lymphocytes and in normal-appearing prostate epithelial cells.

We had proposed that these aims be accomplished by the following tasks:

*Task 1: Select study subjects from among participants in the Health Professionals Follow-up Study who have been diagnosed with prostate cancer, Months 1-4*

Task 1 was completed by the end of the prior progress report period. We stated the following in the last progress report: “Depending on the decision about tissue microarrays described below in Task 2, we will select either a) 200 aggressive cases (all progressed plus Gleason sum 8-10) and 200 nonaggressive cases (no progression and Gleason sum <5) or we will use all 966 prostate cancer cases for whom blocks are available. We are holding off on the selection until we make a decision as outline below.” We made the decision to use tissue microarrays because of their greater efficiency.
**Task 2: Obtain biological samples for study subjects, Months 2-6**

Our collaborators at the Health Professionals Follow-up Study completed making the tissue microarrays from all of the blocks collected from the prostate cancer cases (under separate funding). We have opted to use these tissue microarrays. They cut the blocks and sent them to us in September 2006.

**Task 3: Determination of telomere length in prostate cancer cells (Aim 1) and in normal adjacent epithelial cells (Aim 2), Months 7-22**

Since receiving the tissue microarrays in September 2006, we have stained them for telomeres, centromeres, high molecular weight cytokeratins (which are indicative of the basal layer of the epithelium), and for DAPI (fraction of the area that is nuclear). The quality of the staining is good and is consistent across the TMAs. We will be quantifying telomere length separately in areas of cancer and normal epithelium.

Examples of representative images are shown for the HPFS prostate samples. The images are annotated to show regions of normal epithelium and adenocarcinoma.

Fig 1. a) This image and the following one are of the same case, the first is shown at 200x magnification and b) the second at 400x magnification. As expected, telomere staining (red) intensity is greater in normal epithelium than in adenocarcinoma.
Prostate cancer mixed with normal glands.

Red = telomeres
Blue = DNA
Green = basal cell - specific cytokeratin
(orig. mag. = 400X)

b) the second at 400x magnification

Fig 2. This image is of a different case at 200x magnification.
Fig 3. This image shows prostate adenocarcinoma at 400x magnification. In this image, centromeric regions are shown in green.

Fig 4. Below is an example image depicting high-grade prostatic intraepithelial neoplasia. As expected, telomere staining intensity appears strong in the basal cells, but weaker in the PIN cells.
Fig 5. This image shows telomere staining (green) in a positive control.

Fig 6. This image shows centromere staining (green) in a positive control.
Task 4: Determination of telomere length in lymphocytes (Secondary aim), Months 23-30

In the last progress report we indicated that “In preparation for this work, we have optimized a protocol for staining peripheral blood lymphocytes from stored buffy coat, the type of sample that we have available in the Health Professionals Follow-up Study. This protocol yields adequate staining for imaging of in situ telomere staining for individual cells applied to microarray slides.” We have not yet applied this method to the blood specimens.

Task 5: Data management and interim analysis, Months 25-30

No work has been done on this aim.

Task 6: Final analysis and report manuscipt preparation, Months 31-36

No work has been done on this aim.

KEY RESEARCH ACCOMPLISHMENTS

- None to date specifically from this project.
- Other accomplishments of the research team related to this funded project
  - The research team continues to collaborate effectively on the etiology of prostate cancer and other prostate diseases. Shown below are publications since the date of the last progress report.


REPORTABLE OUTCOMES

- None to date

CONCLUSIONS

- None to date

REFERENCES

- None

APPENDICES

- None