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14. ABSTRACT We are evaluating polymorphisms in genes involved in the genesis of oxidative species, the detoxification of oxidative species, or the repair of oxidative DNA damage influence the risk of prostate cancer progression in men with clinically organ confined prostate cancer. We recently received a no-cost extension through 01/15/2007, and thus this progress report is an annual progress report rather than final. During the past funding year we finalized the definition, selection, and matching of 524 cases (progressors) and 524 controls (non-progressors) with input from pathologists and biostatisticians. We characterized these individuals with respect to baseline demographic and clinical data to confirm that they are otherwise comparable. The source of DNA for these men, unaffected paraffin-embedded lymph nodes removed routinely at prostatectomy, has been located and pulled thus far for 13% of the participants; pulling the blocks is ongoing. Candidate genes in these pathways have been selected and key single nucleotide polymorphisms in these genes are being selected based on the newly available HapMap.					
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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusions.....	7
References.....	7
Appendices.....	7

INTRODUCTION

We are evaluating whether polymorphisms in genes involved in the genesis of oxidative species, the detoxification of oxidative species, or the repair of oxidative DNA damage influence the risk of prostate cancer progression in men with clinically organ-confined prostate cancer who were treated with radical prostatectomy. We hypothesize that men with an inherently greater burden of oxidative stress or inability to repair DNA damage caused by oxidative stress is associated with a higher risk of men. We recently received a no-cost extension through 01/15/2007, and thus this progress report is an annual progress report rather than final progress report.

Dr. Platz, PI, also received a Career Development Award from the Hopkins Prostate Cancer SPORE to investigate genes involved in inflammation and prostate cancer progression in the same nested case-control set. Dr. Isaacs, a Co-investigator on this project, and Dr. Platz received an R01 on genes involved in metastasis and prostate cancer progression, which also will use the same nested case-control set. These additional funds allowed us to expand the number of cases and controls that we could include and also to examine three complementary pathways that likely act together to cause prostate cancer progression.

During the past funding year we finalized the definition, selection, and matching of 524 cases (progressors) and 524 controls (non-progressors) with input from pathologists and biostatisticians. We characterized these individuals with respect to baseline demographic and clinical data to confirm that they are otherwise comparable. The source of DNA for these men, unaffected paraffin-embedded lymph nodes removed routinely at prostatectomy, has been located and pulled thus far for 13% of the participants; pulling the blocks is ongoing. Candidate genes in these pathways have been selected and key single nucleotide polymorphisms in these genes are being selected based on the newly available HapMap.

BODY

The aims of this proposal were:

- 1) Using expression data from cDNA microarrays coupled with published information on the functionality of sequence changes, we plan to identify 5 single nucleotide polymorphisms (SNP) in each of 25 genes encoding enzymes involved in production of ROS, detoxification of ROS, and repair of oxidative DNA damage.
- 2) To test whether these SNPs are independently and in combination associated with risk of prostate cancer progression.

We had proposed that these aims be accomplished by the following tasks. After each task, progress is described.

Task 1. Select 25 polymorphic genes involved in production of ROS, detoxification of ROS, and repair of oxidative damage, Months 1-2

- a. Review cDNA expression data for prostate tumors generated in laboratory of Dr. Isaacs to identify genes involved in oxidation that are expressed above the 80th percentile or below the 20th percentile compared to normal tissue.

We began using this approach. We met with Drs. Isaacs and Luo about the utility of using this approach to selection of genes. We opted not to use this approach for two reasons: i) Expression studies in the literature have not produced consistent findings for genes that are over- or under-expressed in prostate cancer tissue from men who progressed who did not progress. Some of the variability in findings is likely due to issues sampling the tumor tissue, experimental conditions, as well as unaccounted for patient-to-patient variability in factors that influence gene expression. ii) The candidate gene approach has been replaced by the currently preferred focus on pathways; that is, several genes within the same pathway are investigated simultaneously. We have now selected this latter approach to gene selection and have generated the target gene list.

- b. Conduct searches of public and proprietary databases of the highly or lowly expressed genes to identify one or more single nucleotide polymorphisms with functional consequence.

We are now using a modification of this approach to the selection of SNPs. For each gene selected we are identifying both i) SNPs of known or suspected functional consequence and ii) SNPs that predict the most haplotypes in the U.S. white population. Evaluation of each SNP in a gene one at a time may or may not capture the variation in the production, stability, or activity of the gene product. Thus, haplotype analysis is now the gold standard approach to conducting studies of the association of genetic variation with disease risk.

We postponed the selection of SNPs until the HapMap was available. The HapMap provides information on which allelic combinations tend to be inherited in blocks. We are currently in the process of selecting the minimum number of SNPs per gene that best capture common haplotypes in the white population.

- c. Choose final set of genes using the criteria outlined in the proposal.

A comprehensive list of genes involved in the oxidation pathway has been selected.

*Task 2. Select 200 cases (progressors) and 200 matched controls (nonprogressors)
Months 3-5*

- a. Link the Hopkins Pathology Tissue Core database to electronic hospital records to identify prostate cancer patients treated with radical prostatectomy and who experienced biochemical failure.
- b. From the total set of eligible patients, select 200 men who had biochemical failure and 200 men who still had undetectable PSA at the date of the case's failure, same follow-up time, and who are similar on demographic and tumor characteristics.

A total of 4,860 men were treated for prostate cancer by radical prostatectomy at the Brady Urological Institute in 1993 or later. Of these men, we excluded 365 because they received hormonal or radiation therapy prior to prostatectomy (46.9%), positive surgical margins or we could not confirm organ confined disease (26.8%), or follow-up was incomplete (17.0%). Of the 4,495 eligible men, 524 subsequently experienced

biochemical failure (73.8%), local recurrence (7.8%), local and/or distant metastasis (15.7%), or death from prostate cancer (2.6%).

Because we anticipate that the prostate cancer case-control set that we prepared will be used by other prostate SPORC investigators, Dr. De Marzo (director of the tissue core) convened a meeting of potential users, including Drs. Epstein and Piantadosi. We also consulted with two other statistical epidemiologists to confirm the approach to sampling and to ensure that planned analytical approach could handle the data structure that would be imposed by the method chosen.

We decided to use the approach to the selection of matched controls from among men who underwent radical prostatectomy called incidence density sampling. In this method, a man's person-time at risk is sampled and thus, a man may be sampled more than once represent different person-years at risk and a man who goes on to recur may be sampled as a control prior to failure and then also be counted as a case. Because stage and grade are such strong predictors of recurrence and because our goal is to study genetic variants that influence recurrence independent of stage and grade at diagnosis, we matched the cases and controls very closely on stage and grade. We also matched the men on age at diagnosis and race. For each case then, we sampled another man who had not yet recurred, who was still alive and under follow-up, and who had the same stage, grade, and age at diagnosis and was the same race as the case in question. We used a SAS macro (Tassoni et al. "One-to-one matching of case/controls using SAS software") to optimize the closeness of the matching. The final set contains 524 cases and 524 controls (see Table), of which the controls are 326 unique men (204 men selected 1 time, 74 men selected 2 times, etc.). Of the 326 unique controls, 108 later became cases. Thus, the total number of unique men is 742. 95% of the matches are exact on stage, grade, and race and are within 10 years of age.

Characteristics of 524 cases and 524 controls matched on age, race, pathologic stage, and pathologic Gleason sum among men who underwent radical prostatectomy, Johns Hopkins Hospital 1993–2004

	Cases	Controls
Mean age (yr)	58.9 ± 6.3	59.1 ± 5.9
Race (%)		
White	85.9%	88.6%
Black	9.2%	7.6%
Hispanic	1.3%	0.6%
Asian	0.4%	0%
Other	3.2%	3.4%
Mean PSA at prostatectomy (ng/mL)*	12.3 ± 10.2	11.0 ± 8.3
Mean pathologic Gleason sum	7.2 ± 0.8	7.2 ± 0.8
Pathologic stage (%)		
T2	13.6%	13.7%
T3a	51.7%	51.7%
T3b or N1	34.7%	34.5%
Mean time since prostatectomy to progression or last follow-up (yr)*	2.5 ± 1.8	5.9 ± 2.4

* Not a matching factor

Task 3. Genotyping, Months 6-12

- a. Pull samples for the 400 patients from Hopkins Pathology Tissue Core archive and review for normal regions.
- b. Extract genomic DNA in laboratory of Dr. Isaacs.
- c. Ship samples to laboratory of Dr. Xu and perform high throughput genotyping.

DNA for these cases and controls will be extracted from frozen tissue, where available, or archived blocks in the laboratory of Dr. Isaacs. In discussions with Dr. De Marzo, we have decided to use paraffin-embedded unaffected lymph nodes removed at the time of radical prostatectomy because lymph nodes contain large numbers of lymphocytes and thus are a good source of germline DNA for genotyping and because archived lymph node blocks have not been accessed for these men and thus we expect that blocks will be available and locatable. Thus far, samples have been pulled from the repository for 13% of the selected cases and controls. High throughput genotyping will be done in the laboratory of our collaborator at Wake Forest.

Task 4. Data management and interim analysis, Months 13-18
Not done.

Task 5. Final analyses and report/manuscript preparation, Months 19-24
Not done.

KEY RESEARCH ACCOMPLISHMENTS

- None to date specifically from this project.
- Accomplishments of related to this New Investigator Award
 - Dr. Platz, principal investigator, was promoted July 2005 to Associate Professor.
 - Dr. Platz has received subsequently two grant on prostate cancer:
 - She is the PI of a DOD Idea Development award also on prostate cancer: Telomere Length as a Predictor of Prostate Cancer Aggressiveness.
 - She is the leader of Project 4, Genotypic and Phenotypic Indicators of Inflammation in the PCPT, of the NCI-funded P01 entitled "The Biology of the Prostate Cancer Prevention Trial".

REPORTABLE OUTCOMES

- None to date

CONCLUSIONS

- None to date

REFERENCES

- None to date

APPENDICES

- None