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PRINCIPAL INVESTIGATOR: Hans-Olov Adami, M.D.

CONTRACTING ORGANIZATION: Harvard College
Harvard School of Public Health
Boston, MA 02115

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Hans-Olov Adami, MD, PhD and Lorelei A. Mucci, ScD

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Harvard School of Public Health
677 Huntington Avenue –Rm 506
Boston, MA 02115

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**14. ABSTRACT**

Accumulating evidence points to a role of chronic inflammation in the pathogenesis and progression of cancers, including prostate. Infections are important agents in the genesis of inflammation. For prostate cancer, several lines of evidence point to a role of infections as important agents, although no specific infection has consistently been identified. In this project, we are examining two specific infectious agents with respect to prostate cancer: T vaginalis, the most common non-viral sexually transmitted infection, and the recently identified retrovirus XMRV. The aims of this study are 1-) To assess the role of the newly identified XMRV virus in prostate carcinogenesis and progression; 2-) To characterize the role of the infectious protozoa *T. vaginalis* in prostate carcinogenesis and progression. The current study is nested within the Swedish Watchful Waiting Cohort, a population-based cohort of 1,256 Swedish men diagnosed with localized prostate cancer. During 28 years of follow-up, 320 men have died of cancer, and thus this is a powerful population in which to examine determinants of prostate cancer progression. A tumor repository from archival tissue specimens have been collected from all men in the cohort and will be used to assay for presence of the infections.

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## INTRODUCTION

Prostate cancer has considerable biologic heterogeneity, such that some men experience an aggressive course while many have a slow growing or indolent disease (1-3). Thus, central issues in prostate cancer research are to identify mechanisms which are amenable to prevention and treatment, and to understand pathways that lead to aggressive cancer.

A growing body of epidemiologic, genetic and molecular pathological data points to the role of chronic inflammation in the pathogenesis and progression of prostate cancer (4). The pathways involved in chronic inflammation induce cellular damage and compensatory cellular proliferation (5). Clinical prostatitis, which occurs in approximately 9% of men between the ages of 40 and 79, has been linked to prostate cancer in several epidemiologic studies (6). Moreover, surgical prostate tumor specimens often exhibit histological evidence of prostatitis, although the determinants of this prostatic inflammation are unclear.

Perhaps of greater importance, data also suggests that the degree of inflammation may be a predictor of more aggressive disease. In a study of 161 men undergoing radical prostatectomy (7), 5-year recurrence-free survival was significantly lower among patients with high-grade inflammation in malignant tissue (27%) than in patients with low-grade or no evidence of inflammation (65%), independent of Gleason grade, preoperative PSA level, and pathologic stage.

Infectious agents are likely targets involved in the initiation and exacerbation of chronic inflammation, and infections can lead to increased risk of several cancers (8). Indeed, an estimated 15% of malignancies globally are thought to have an infectious etiology (9, 10). Infectious agents may also have direct effects on carcinogenesis through the transformation of cells via incorporation of active oncogenes into the host genome, inhibition of tumor suppressors, stimulation of proliferation signals, or through immune suppression. Known oncogenic infections are typically highly prevalent within the host population, persistent within the host, and require a variety of co-factors for malignant transformation.

Two recent papers (11, 12) provide emerging evidence suggesting involvement of the newly identified murine-like retrovirus XMRV and the protozoan *T. vaginalis* in prostate cancer; these are the focus of the proposed study. The objective of the proposed study is to evaluate and extend the initial findings on *T. vaginalis* and XMRV, and to more fully characterize the potential role of these infections in the pathogenesis and progression of prostate cancer in a large population-based cohort of Swedish men with prostate cancer who have been followed prospectively for 28 years.

**BODY**

**<u>Aim I.</u> To assess the role of the newly identified XMRV virus in prostate carcinogenesis and progression.**

A major effort of this year has been in the complete pathology review of the tissue specimens. We have now completed construction of the tissue microarrays for all 680 TURP cases at the DFHCC Harvard TMA Core Facility, as well as a test array to be used for pilot studies of the biomarkers. In addition, our pathologist has completed a re-review of the cohort for standardized Gleason grading, and this data is now cleaned and available for analysis.

The pathologist has also completed review of cases for circling areas of benign tissue on the tumor blocks for extraction of DNA for the characterization of RNASEL genotype. In preliminary work, we have shown excellent yields of DNA (100-200 ng) from 3 cores of benign tissue, which will be more than sufficient for our genotyping assays. We will send the tissue blocks for extraction of DNA from the remaining cases during the next three months.

We continue discussions with our colleagues at the Cleveland Clinic regarding the tumor tissue assays for XMRV. Development of the antibody for XMRV has been slower than expected, because the virus elicits a slow immune response. However, our pathology team is ready to undertake immunostaining immediately after the antibody is developed.

We have completed two biomarker studies on the tumor tissue microarrays that are critical to this study: 1-) Tumor apoptosis using a TUNEL assay to assess extent of tumor tissue undergoing apoptosis, and 2-) cellular proliferation assessed by immunohistochemistry with antibody to *ki67*. The data on these two markers have been cleaned and outputted as SAS databases.

Our pathologist has undertaken and completed a comprehensive review of all cases for evidence of atrophy lesions and chronic and acute inflammation. He reviewed all available H&E slides from the 680 men. We have characterize for presence of simple atrophy (SA), simple atrophy with cyst formation (SACF), post atrophic hyperplasia (PAH) and partial atrophy (PA); data on evidence of high grade PIN and perineural invasion, as well as for evidence of acute and chronic inflammation (none, mild, moderate/severe). The data were entered into an ACCESS database, indicated by a Research ID, have been reviewed for quality control, and are now linked together with the clinical database (Gleason grade, T stage, tumor extent, body mass index, date of diagnosis, date and cause of death) as a SAS database.

In preliminary statistical analysis of these data, we have found substantial evidence of atrophy, with 73% of cases showing at least one atrophic lesions, including 20% with evidence of the proliferative atrophic lesion PAH. Both chronic (26%) and acute (14%) atrophy were also commonly evident in the tissue specimens. Among 680 men, 220 died of prostate cancer. We found no overall evidence of PAH and prostate cancer-specific mortality (Odds ratio 1.3, 95% confidence interval 0.8-1.7). However, men who had both evidence of PAH and moderate to severe inflammation were significantly more likely to die of prostate cancer (Odds ratio 2.5, 95% confidence interval 1.3-3.8) suggesting a joint effect of atrophy and inflammation in prostate cancer progression. The data on inflammation and atrophy will play a critical role in our understanding of the infectious pathogenesis of prostate cancer.

**<u>Aim II.</u> To characterize the role of the infectious protozoa *T. vaginalis* in prostate carcinogenesis and progression.**

Much of the preliminary work summarized in Aim I is directly relevant to Aim II of the project, including the clinical data review, tissue retrieval, histologic evaluation, TMA construction, and atrophy/inflammation assessment.

Relevant for this aim, we have also undertaken pilot work on our test array to work up antibody concentrations for the T Vaginalis antibody, that was retrieved from the lab of Dr. Alderete. The pathologist is reviewing those slides now. In addition, we are also in the process of working with clinical colleagues to identify positive controls to be used for this project, namely known T vaginalis positive samples.

We are planning a meeting in May with the Swedish research team to discuss the preliminary findings and move forward on the project.

**Related work**

Related to this project, we have completed an analysis of *T vaginalis* serostatus measured in prediagnostic blood and prostate cancer risk and progression in the Physicians' Health Study among 673 incident prostate cancer cases and 673 matched controls. The bloods were collected and stored prospectively, and cases were diagnosed from 1982-2000. We found a suggestion of *T. vaginalis* seropositivity and overall prostate cancer risk (Odds ratio 1.23; 95% confidence interval (CI): 0.94-1.61). Moreover, seropositive men had a 2.2-fold increase in risk of extraprostatic prostate cancer (95% CI: 1.08-4.37) and a 2.7-fold increase in risk (95% CI: 1.37-5.28) of cancer that would ultimately progress to distant metastases or prostate cancer-specific death. These data provide compelling evidence of a role of this infectious protozoan in the progression of prostate cancer. A manuscript of these data is currently under peer-review.

*Propionibacterium acnes* is a bacterium which has previously been shown to infect prostatectomy specimens and is demonstrated to elicit an inflammatory response. Within the Swedish Watchful Waiting Cohort, we have completed an evaluation of the tissue specimens for evidence of *P acnes* with collaborators at the University of Orebro, Sweden using a FISH assay. Almost 20 percent of men had evidence of *P acnes* infection in the tissue. We are now undertaking statistical analyses to evaluate the association of *P acnes*, extent of atrophy and inflammation, and cancer-specific mortality.

## KEY RESEARCH ACCOMPLISHMENTS

- Completed standardized review of cohort for Gleason grading, and finalized construction of all tumor tissue microarrays
- Completed pathologic review of cohort on extent of inflammation, atrophy, high grade PIN, and perineural invasion on all cases
- Completed biomarker studies on tumor tissue microarrays to assess extent of tumor apoptosis and cellular proliferation
- Created a merged clinical and tissue SAS database, including clinical information, atrophy and inflammation data, and biomarker data for statistical analyses
- Completed review of tissue specimens for indentifying histologically normal tissue for DNA extraction; extracted DNA from benign tissue in pilot study of 92 cases and found excellent DNA yields
- Undertook statistical analyses linking data on inflammation and atrophy in relation to prostate cancer-specific mortality
- Completed laboratory assessment of *P acnes* on the tumor tissue microarrays
- Submitted manuscript on serostatus for *T vaginalis* and prostate cancer risk and progression based on the Physicians' Health Study

## REPORTABLE OUTCOMES

- Student working on this project (Jennifer Stark) was promoted to post-doctoral fellow
- Dr. Stark received a Career Development Award from the Dana Farber/Harvard Cancer Center Prostate Cancer SPORE based on an extension of this project
- Recruited collaborator Dr. Katja Fall from Karolinska Institutet as Visiting Scientist at Harvard School of Public Health
- Dr. Fall received funding from the Swedish Cancer Foundation to study *P acnes* and prostate cancer progression based on preliminary work from this project
- Dr. Mucci received a Young Investigators Award from the Prostate Cancer Foundation based on experience supported by this award
- Development of prostate tumor tissue repository of TURP specimens and clinical-tumor database
- Submitted related manuscript to peer-reviewed journal

## CONCLUSION

We have demonstrated our ability to undertake this large cohort and collect archival tumor specimens from 680 men. We have demonstrated a proven working relationship with the pathology team, as shown by substantial progress on construction of the tissue microarrays, standardized Gleason grading, evaluation of atrophy and inflammation, and successful completion of two biomarkers on the tissue microarrays. Moreover, our preliminary statistical analyses on atrophy-inflammation and prostate cancer-specific mortality, combined with the findings of serologic evidence of *T vaginalis* and prostate cancer mortality provide supportive evidence for the study hypothesis.

During the next year, we will complete the remaining biomarker analyses and undertake statistical analyses to successfully complete the study aims. Ultimately, this project has the potential to provide strong evidence (for or against) in assessing the role of infectious agents and inflammation in prostate pathogenesis. Moreover, the tumor tissue repository we are establishing is a unique resource in which to test future hypothesis. Given the substantial biologic heterogeneity of prostate cancer, the proposed project would ultimately have exciting implications for prevention and potentially treatment of prostate cancer.

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## APPENDICES

None

**SUPPORTING DATA**

**Table 1. Prevalence of atrophy and inflammation in the Swedish Watchful Waiting Cohort, overall and by Gleason grade**

| | | Gleason grade | | | |
|---|---|---|---|---|---|
| | **Overall** N=619 | **2-6** N=288 | **3+4** N=121 | **4+3** N=90 | **8-10** N=120 |
| **Focal Prostate Atrophy** | | | | | |
| Proliferative atrophic hyperplasia | 125 (20.2%) | 18.4% | 25.6% | 21.1% | 18.3% |
| Simple atrophy | 367 (59.3%) | 61.8% | 59.5% | 56.7% | 55.0% |
| Simple atrophy with cyst formation | 38 (6.1%) | 7.6% | 1.7% | 8.9% | 5.0% |
| Partial atrophy | 11 (1.8%) | 2.8% | 2.5% | 0% | 0% |
| **Chronic inflammation** | | | | | |
| None | 164 (26.5%) | 24.3% | 22.3% | 28.9% | 34.2% |
| Mild | 296 (47.8%) | 51.0% | 52.1% | 40.0% | 41.7% |
| Moderate/Severe | 159 (25.7%) | 24.7% | 15.6% | 31.1% | 24.2% |
| **Acute inflammation** | 84 (13.6%) | 16.7% | 14.1% | 12.2% | 6.7% |
| **Perineural invasion** | 43 (7.0%) | 0.7% | 3.3% | 10.0% | 23.3% |
| **High grade PIN** | 81 (13.1%) | 6.6% | 16.5% | 21.1% | 19.2% |

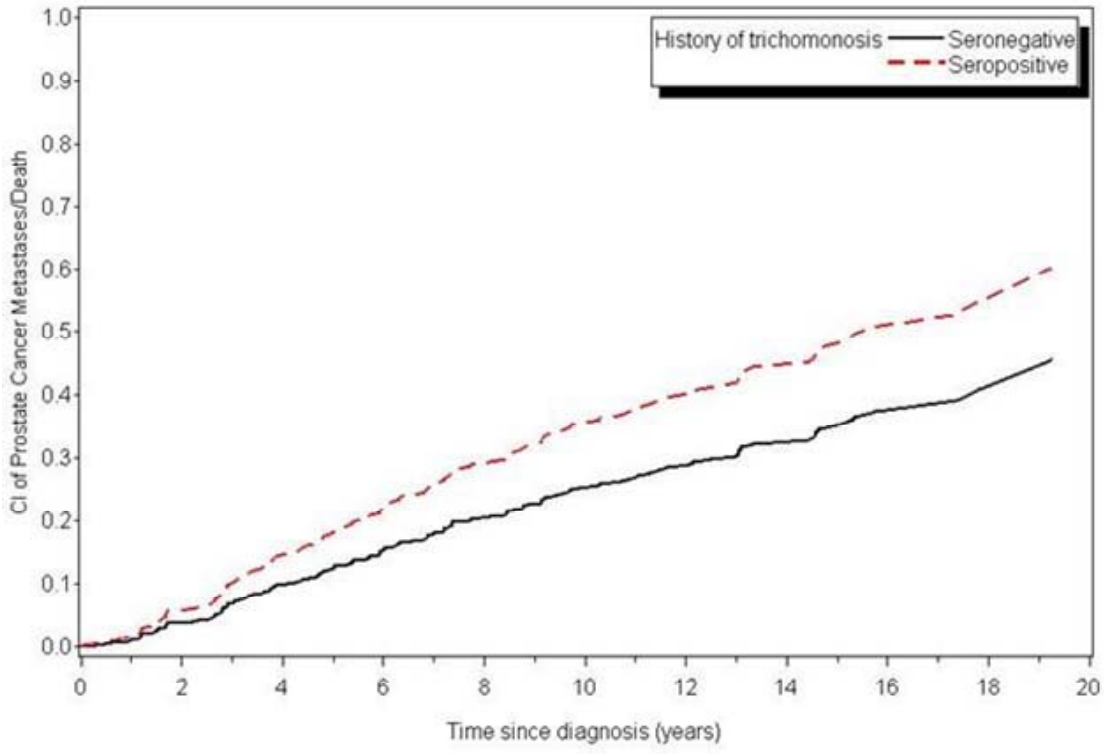


**Figure 1. Cumulative incidence of lethal prostate cancer among 673 PCa cases according to *T. vaginalis* serostatus, Physicians' Health Study 1982-2008**. Cumulative incidence curves estimated from proportional hazards models controlling for age at diagnosis, tumor aggressiveness, baseline BMI, smoking status, and aspirin randomization arm (placebo).