TITLE: Prostate Cancer Aggressiveness Genes in Hereditary Prostate Cancer

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INTRODUCTION: The EZH2 transcriptional regulator, recently shown by to be overexpressed in prostate cancer specimens that are more likely to recur, maps to 7q35 and represents a candidate gene for inherited prostate cancer susceptibility. This statement is based on the identification of prostate cancer linkage to distal 7q markers in a recently completed genome-wide scan using hereditary prostate cancer families from the University of Michigan Prostate Cancer Genetics Project (PCGP). Relatively little is known about the molecular basis of EZH2 function or its DNA specificity. The major hypothesis of this proposal is that germline mutations in the EZH2 gene will predispose to more clinically aggressive forms of prostate cancer and the characterization of these mutations will provide more information about the function of the EZH2 molecule in prostate cancer and metastasis. To address this hypothesis, the following two specific aims were proposed: 1) to identify germline mutations in EZH2 that predispose to aggressive prostate cancer in prostate cancer families, and 2) to characterize the functional consequences of EZH2 mutations specifically focusing on the role of EZH2 in transcriptional regulation.

BODY: To address Specific Aim 1, we have revised our current IRBMED approved research project to add this DOD-funded research project. The revised proposal is under review by our University of Michigan IRB. We expect that this will be approved by April 2005. In the interim, we have designed sequencing primers to span all of the EZH2 coding regions. The primers have been tested on control DNA samples and conditions for amplification have been standardized.

To address Specific Aim 2, we have modified a molecular biological technique first developed in yeast, known as the TAP (tandem affinity purification) system, which has allowed us to express a tagged form of EZH2 in human cells at physiological concentrations, and subsequently to purify EZH2-associated proteins from cells. We have identified several EZH2-binding proteins by mass spectrometry, and our lead candidate is a recently described factor designated REA (repressor of estrogen activity). We have isolated full-length cDNA clones encoding REA, and generated a range of mammalian expression vectors, both with and without epitope tags. Through coprecipitation experiments, we have confirmed the validity of the interaction, and our studies have now moved to the next phase, namely the functional characterization of the two proteins to determine the physiological consequences of the interaction.

REA has been shown to bind directly to the estrogen receptor (ER), and can function to suppress ER-mediated activity. We therefore are taking several approaches to examine the functional consequences of the EZH2-REA interaction. Firstly, we have established a system described recently by the laboratory of Danny Reinberg, in which the transcription suppressing activity of EZH2 is examined through the use of a Gal4-EZH2 chimera. By monitoring the expression of a Gal4-dependent luciferase reporter construct, we are in the process of examining the effects of either ectopic expression, or removal by RNA interference, of REA on EZH2 activity.

We have obtained several monoclonal antibodies specific to REA, and we are taking an immunohistochemical approach to examine a panel of histological samples to determine whether REA expression is altered in prostate cancer.

Finally, we are testing the reciprocal possibility, namely that EZH2 is involved in REA function, and to this end we have established a similar luciferase-based reporter
system to that described above to examine the effect of \textit{EZH2} on estrogen receptor-mediated transcription. The hypothesis being tested is that REA-mediated inhibition of ER activity may be modulated by, or even mediated by \textit{EZH2}.

**KEY RESEARCH ACCOMPLISHMENTS:**

Specific Aim 1  
Completion and approval of IRB protocols.  
Design and validation of exon-specific primers

Specific Aim 2  
Establishment of \textit{EZH2}-TAP system  
Purification and indentification of \textit{EZH2}-associated proteins  
Validation of REA as a physiologically relevant \textit{EZH2}-associated protein  
Characterization of REA-specific antibodies for Immunohistochemistry  
Establishment of reporter systems to analyze \textit{EZH2} and REA Activity  
Design and validation of small interfering RNAs (siRNAs) for REA and XIAP

**REPORTABLE OUTCOMES:** None to date.

**CONCLUSIONS:** We are now very excited about the ability to proceed with Specific Aim 1, having, we believe, updated and amended the IRB protocols in a manner which is satisfactory to the DOD. Specific Aim 2 has also seen significant progress, with the identification and validation of REA as a previously undescribed \textit{EZH2}-interacting protein. In summary, we feel that we are well underway in the successful undertaking of our aims.