Award Number: W81XWH-04-1-0268

TITLE: The Role of HCDC4 in Prostate Tumorigenesis

PRINCIPAL INVESTIGATOR: Charles H. Spruck, Ph.D.

CONTRACTING ORGANIZATION: Sidney Kimmel Cancer Center
San Diego, CA 92121-1123

REPORT DATE: August 2005

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and
should not be construed as an official Department of the Army position, policy or decision
unless so designated by other documentation.

20060525025
This study investigated the role of a newly identified gene called hCDC4 in prostate cancer. The hCDC4 gene encodes a protein that functions in a cellular process called proteolysis, or protein degradation. hCdc4 degrades a protein called cyclin E, which is a central component of the cell division machinery. Cyclin E is involved in initiating DNA replication in cells. However, in many types of human tumors cyclin E protein levels are aberrant and this phenotype has been shown in vitro and in vivo to be oncogenic. Very little is known regarding cyclin E/hCdc4 in prostate tumors.

We explored whether hCDC4 functions as a tumor suppressor gene in prostate cancer. We have completed a genetic screen of prostate tumors and found an hCDC4 gene mutation. We are currently determining whether hCDC4 functions as a haplo-insufficient tumor suppressor through LOH and expression analysis. We are also exploring whether the mutant hCDC4 allele we have found in a prostate tumor functionally inactivates the hCdc4 protein.
# Table of Contents

Cover ..............................................................................................................1

SF 298 ...........................................................................................................2

Table of Contents ....................................................................................3

Introduction ..............................................................................................4

Body ............................................................................................................4-5

Key Research Accomplishments ...............................................................5

Reportable Outcomes ...............................................................................6

Conclusions ..............................................................................................6

References .................................................................................................6
A. Introduction

This study investigated the role of a newly identified gene called hCDC4 in prostate cancer. The hCDC4 gene encodes a protein that functions in a cellular process called proteolysis, or protein degradation. hCdc4 degrades a protein called cyclin E\textsuperscript{3}, which is a central component of the cell division machinery\textsuperscript{4}. Cyclin E is involved in initiating DNA replication in cells\textsuperscript{4}. However, in many types of human tumors cyclin E protein levels are aberrant and this phenotype has been shown \textit{in vitro} and \textit{in vivo} to be oncogenic\textsuperscript{5-7}. In this proposal we explore whether hCDC4 functions as a tumor suppressor gene in prostate cancer through its role in cyclin E proteolysis.

B. Body

1. \textit{Identify and collect fresh-frozen and corresponding archival prostate tumor specimens from the tissue bank at The Sidney Kimmel Cancer Center (Months 1-2).}

We have obtained 40 prostate tumor specimens from the SKCC Tumor Bank. Four sections of 10 μm thickness were obtained for each specimen.

2. \textit{Isolate DNA, RNA and protein from fresh frozen prostate tumor specimens (Months 2-3).}

Two 10 μm sections of each specimen were used for DNA isolations using the QiaAmp DNA Isolation Kit (Qiagen). Approximate total yield of DNA for each sample was 20 μg. Each DNA sample was diluted to yield a 20 μg/ml stock solution.

3. \textit{Microdissect matching normal DNA tissue from paraffin-embedded archival tissue specimens (Months 2-3).}

Normal tissue for each tumor specimen was marked by microscopic examination and dissected. DNA was isolated by proteinase K digestion. DNA will be used as control in loss of heterozygosity (LOH) determinations.

4. \textit{Screen prostate tumors for hCDC4 gene mutations by SSCP (Months 3-6).}

We have screened 40 prostate tumor specimens for hCDC4 gene mutations by SSCP. Eighteen different PCR reactions were used to cover the 13 different exons of the hCDC4 gene. An aberrant SSCP banding pattern was detected for a single prostate tumor specimen corresponding to the alpha-exon of hCDC4 (Fig. 1).

![Figure 1. hCDC4 gene mutation in a prostate tumor. SSCP analysis of the b-exon of hCDC4 demonstrated an aberrant banding pattern for tumor in lane 5 (arrows). DNA sequencing revealed the mutated allele contains a three base pair insertion (CCG) introducing an in-frame proline residue in the hCdc4 protein.](image-url)
5. **Sequence hCDC4 gene mutations** (Months 5-6).
We cloned the hCDC4 alpha-exon for the prostate tumor containing an aberrant SSCP banding pattern in pCR-TOPO (Invitrogen). DNA sequencing revealed a three base pair insertion in the gene. This sequence is predicted to introduce an in-frame proline residue in the hCdc4 protein.

6. **Real-time PCR and Northern blot analysis of hCDC4 gene expression in prostate tumors** (Months 6-8).
We have isolated RNA from 40 prostate tumor specimens using the RNaseasy Kit (Qiagen). Materials required for Real-Time PCR analysis have been ordered and received. To date, we have yet to analyze the samples due high usage of the Real-time PCR machine at SKCC. We estimate that the Real-time PCR analysis will be performed within the next 60 days.

7. **Clone mutant hCDC4 alleles into expression vector for functional analysis** (Months 7-8).
The mutant hCDC4 cDNA found in a prostate tumor specimen was isolated by site-directed mutagenesis of the wild-type cDNA. The mutant cDNA was cloned into the pCDNA3.1 mammalian expression vector (Invitrogen). We have transfected this plasmid into 293T cells and confirmed its expression. The wild-type hCDC4 cDNA was also cloned as control.

8. **Western blot analysis of cyclin E and hCdc4 protein in prostate tumor specimens** (Months 8-10).
We have isolated protein from 40 fresh frozen prostate tumor specimens. Approximate yield of protein for each sample was 30 μg. We have performed Western blot analysis on these samples for cyclin E protein and scaled the results from 0 (absent) to 4 (highly expressed). hCdc4 protein has not been analyzed due to the lack of an anti-hCdc4 antibody that gives a sufficiently low background by Western blot analysis. These results have necessitated our undertaking of LOH and Real-time PCR analysis to substitute for hCdc4 western blot analysis in hCdc4 expression determinations.

9. **Immunohistochemical staining of archival prostate tumor specimens for cyclins E, A and B1** (Months 9-12).
We have immuno-histochemically analyzed the prostate tumor containing the hCDC4 mutation or wild-type alleles. Archival paraffin-embedded specimens were analyzed for cyclin E and cyclin A expression. Slides were analyzed microscopically for the percentage of positive staining nuclei. Determinations for cyclin E were found to be difficult to interpret due to high background. We are currently exploring alternative fixation and detection methods in order to limit background staining.

C. **Key research Accomplishments**

1. The hCDC4 gene is mutated in a prostate cancer.
2. Inactivation of the hCDC4 alpha-isoform is present in prostate cancer.
D. Reportable Outcomes

The data obtained from this project will undoubtedly necessitate the publication of a manuscript.

The results of this study have prompted us to apply for a DOD Prostate Award to fund a continuation of this research. This proposal will analyze hCDC4 defects in more detail and determine the role of hCdc4 in genetic instability and androgen-independent proliferation in prostate tumorigenesis.

E. Conclusions

We have discovered that the hCDC4 gene functions as a tumor suppressor in prostate cancer. hCDC4 inactivation/cyclin E deregulation has been related to genetic instability and androgen-independent proliferation of prostate cells. Therefore, drugs that target hCdc4 function could be used to prevent prostate tumor progression.

G. References