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PRINCIPAL INVESTIGATOR: Teresa L. Johnson-Pais, Ph.D.

CONTRACTING ORGANIZATION: University of Texas Health Science Center at San Antonio
San Antonio, Texas 78229-3900

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6. AUTHOR(S) :
Teresa L. Johnson-Pais, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Texas Health Science
Center at San Antonio
San Antonio, Texas 78229-3900
paist@uthscsa.edu

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Previous studies using allelic imbalance (AI) have implicated numerous chromosomal regions as potential locations for tumor suppressor genes involved in prostate cancer. In primary prostate cancer the most frequent losses occur at 8p, 10q, 11p, 16q, 17p, 18q, and 21. In addition, in metastatic prostate cancer there is an increased frequency of AI at 18q, compared to primary cancer, and there are two distinct regions of loss (7 and 6 centimorgans in size, respectively) on 18q associated with the metastatic samples. In order to identify a smaller region of 18q that harbors a gene involved in metastatic prostate cancer, we have constructed contigs of bacterial artificial chromosomes (BACs) that encompass the two regions of loss. DNA from these BAC clones has been spotted onto slides by Spectral Genomics. These microarray slides are in the process of being hybridized with DNA from prostate tumors at various stages of cancer progression. These microarray experiments will provide more information about chromosome 18 losses than the previous AI experiments, since there is no limitation for a particular sample to be heterozygous for an 18q marker. These microarray data will help to pinpoint the location of the proposed metastasis suppressor gene.

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Introduction

Previous studies looking at loss of heterozygosity or allelic imbalance (AI) have implicated numerous chromosomal regions as potential locations for tumor suppressor genes involved in prostate cancer. In primary prostate cancer the most frequent allelic losses occur at 8p, 10q, 11p, 16q, 17p, 18q, and 21q (Cunningham et al., 1996; Saric et al., 1999). In addition, in metastatic prostate cancer specimens there is an increased frequency of AI at 18q compared to primary cancer samples, and there are two distinct regions of loss on 18q associated with the metastatic samples (Padalecki et al., 2000). These data imply that loss of chromosome 18q loci is a metastasis-related event. We have found that the introduction of chromosome 18 into the metastatic human prostate cancer cell line PC-3 caused dramatic phenotypic changes, including a suppression of metastatic growth in an *in vivo* model for metastatic potential. The hypothesis for this study is that introduction of chromosome 18 into the PC-3 cell line complements the distal region of loss on 18q found in both metastatic prostate cancer specimens, and in the PC-3 cell line. The overall objective of this research is to identify the gene on 18q22 responsible for suppressing the metastatic potential of PC-3 cells.

Body

The research accomplishments for:

Task 1: Develop efficient in vitro assay to analyze metastatic potential.

We have optimized invasion assays with the PC-3 prostate cancer cells through Matrigel basement membrane matrix (Becton-Dickinson, Bedford, MA) using serum as a chemoattractant. After we have obtained our transfected PC-3 cells from Task 3, we will be able to easily introduce them into this system to characterize any changes in metastatic potential.

Task 2: Narrow the size of the critical region containing the gene involved in metastasis suppression to 1 megabase.

In order to identify a gene on chromosome 18q involved in suppressing metastatic prostate cancer, it is imperative to narrow the sizes of the two critical regions defined using the metastatic prostate cancer specimens. These regions are estimated to be 7 and 6 centimorgans by genetic markers, respectively. To accomplish this task, we have constructed minimum tiling contigs of bacterial artificial chromosomes (BACs) that encompass both the proximal and distal regions of loss on chromosome 18q. This was achieved primarily with data from the human genome draft browser assembly of BAC clones from the University of California – Santa Cruz. However, there were gaps present in these regions that needed to be eliminated. We filled in regions that lacked overlapping clones by screening a BAC library gridded on nylon filters with probes from the ends of BACs that flanked the gaps. This experiment resulted in the identification of additional BAC clones that enabled us to construct complete contigs for the critical regions. The proximal contig spans a region of 4 megabases and consists of 39 BACs, the distal regions spans approximately 2.8 megabases and consists of 24 BACs. DNA

was prepared from all BACs and was submitted to Spectral Genomics of Houston, Texas for spotting onto slides for microarray experiments.

Task 3: Introduce BACs from the region into prostate cancer cells and analyze the phenotype.

In order to introduce BACs into prostate cancer cells and have the DNA be selectively retained, it is necessary to introduce a selectable marker into the BAC DNA. We have obtained the targeting vector pRetroES (Wang et al., 2001) from American Type Tissue Collection (ATCC), which enables us to retrofit the BAC vectors with a mammalian selectable marker that will permit cells containing the BAC to grow in tissue culture medium containing G418. This is a very efficient procedure that will enable us to retrofit numerous BACs in one day with no deletions or recombinations. After the microarray hybridizations and analyses are complete, we will identify key BACs from the regions that will be retrofitted with the mammalian selectable marker and transfected into PC-3 cells.

We are also in the process of identifying immortalized prostate cancer cell lines other than PC-3 that exhibit AI which overlaps with the regions identified from the metastatic tumors. These cells would also be used in the transfection studies with BACs identified from Task 2. There are several other commercially available human metastatic prostate cancer cell lines including NCI-H660 and 22Rv1. These cells were obtained from ATCC and AI analyses for chromosome 18q markers were performed; however, neither of these two cell lines exhibit extended regions of homozygosity overlapping with the critical regions. Thus, we are acquiring other metastatic prostate cancer cell lines that will be tested for extended regions of homozygosity on 18q.

Task 4: Analyze the DNA sequence from the genomic clones for open reading frames.

No work accomplished on this task as of this date.

Key Research Accomplishments

- Constructed a BAC contig for the proximal region of loss on chromosome 18q21.3
- Constructed a BAC contig for the distal region of loss on chromosome 18q22.3
- Isolated DNA from 63 BACs to be spotted onto microarray slides
- Obtained an efficient retrofitting vector for introducing mammalian selectable markers into BACs
- Performed allelic imbalance studies with chromosome 18q markers on two new metastatic prostate cancer cell lines

Reportable outcomes

Funding for this project provided key data for a manuscript that was recently accepted for publication without revision by Urologic Oncology.
"Chromosome 18 suppresses prostate cancer metastases"
Padalecki SS, Weldon KS, Reveles XT, Buller CL, Grubbs B, Cui Y, Yin JJ, Hummer BT, Weissman BE, Dallas M, Guise TA, Leach RJ, and Johnson-Pais, TL.

Conclusions

With the completion of the custom microarray slides containing DNA from the BAC clones covering the proximal and distal critical regions, we are at the point of being able to generate data showing discrete losses of chromosome 18q in different stages of prostate cancer. The microarray experiments will generate more data than the previous allelic imbalance experiments because the samples do not have to be heterozygous for 18q markers. We will be able to generate data from regions that were uninformative in the allelic imbalance experiments. Analysis of the microarray hybridization data should enable us to refine the critical regions originally described for metastatic samples.

References

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Padalecki SS, Troyer DA, Hansen MF, Saric T, Schneider BG, O'Connell P, Leach RJ. 2000. Identification of two distinct regions of allelic imbalance on chromosome 18q in metastatic prostate cancer. Int J Cancer 85:654-658.

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