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Numerous chromosomal regions have been implicated as potential locations for tumor suppressor genes involved in prostate caner including 8p, 10q, 11p, 16q, 17p, 18q, 21 and Y. In addition, in metastatic prostate cancer there is increased frequency of loss of genetic material from chromosome 18q. We have previously identified two distinct regions on chromosome 18q as the site of a tumor suppressor gene involved in metastasis suppression. In order to identify the genes on 18q involved in metastasis suppression, we have created custom bacterial artificial chromosome (BAC) microarrays containing genomic DNA form the two regions of 18q that are lost at a high frequency in metastatic prostate cancer. We are in the process of analyzing the data obtained form the hybridization of prostate cancer DNA to the microarray slide. We have also analyzed the expression of known genes/expressed sequence tagged sites (ESTs) form these town regions on 18q in normal prostate epithelium and in metastatic prostate cancer cell lines. Interestingly, two of the ESTs in the distal region exhibit altered expression when comparing less aggressive prostate cancer to highly-metastatic prostate cancer. The data generated form this proposal will pinpoint the location of the proposed metastasis suppressor gene.

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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..8

Appendices..

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.

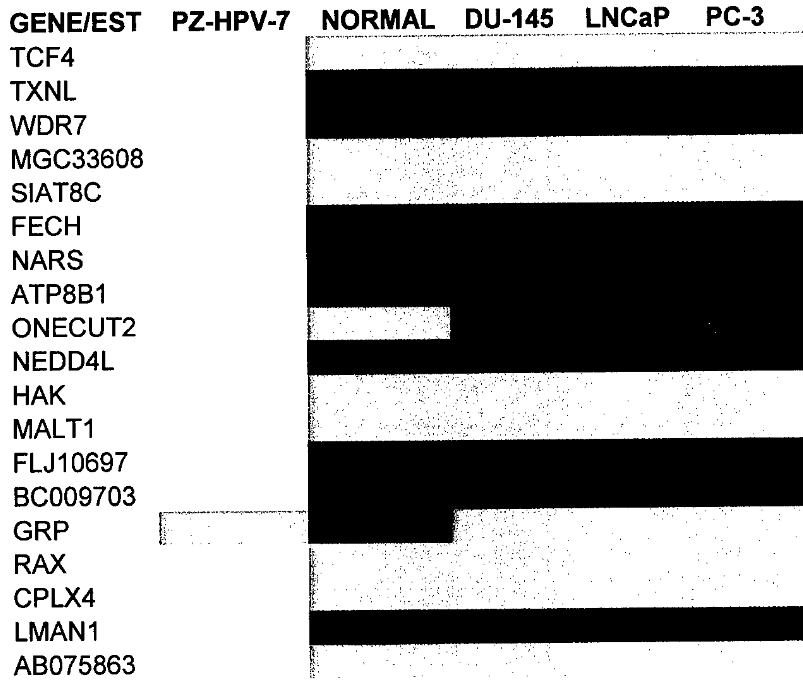
In order to identify a gene on chromosome 18q involved in suppressing metastatic prostate cancer, it is imperative to narrow the sizes of the two critical regions defined using the metastatic prostate cancer specimens. These regions are approximately 7 and 6 centimorgans by genetic markers, respectively. 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. 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. We have received the custom microarray slides and after optimization of the hybridization/washing conditions, we are currently in the process of hybridizing DNA from prostate cancer specimens to the microarray slides and analyzing the results using software developed by Spectral Genomics.

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 vector. 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 enables us to retrofit numerous BACs in one day with no deletions or recombinations. We have retrofitted BACs from the distal region, and we will be starting transfections of these BACs into the prostate cancer cell line PC-3.

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

With the data available from the Human Genome Project, we have identified known genes and expressed sequence tagged sites (ESTs) from both the proximal and distal region. We have designed primer sets for these genes and ESTs and have analyzed the expression levels of these genes/ESTs by reverse transcription/PCR in a normal prostate epithelial line and in six commercially available metastatic prostate cancer cell lines. Interestingly, two of the ESTs demonstrated a differential pattern of expression in the metastatic prostate cancer cell lines, and are being further investigated. A summary of the results is presented in Figure 1.



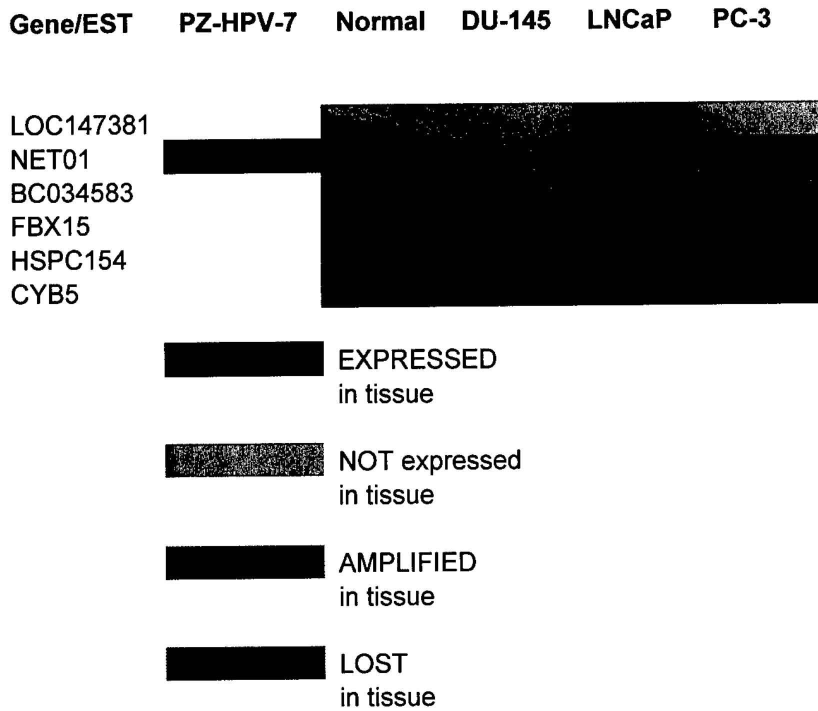


Figure 1: Gene/EST expression in metastatic prostate cancer cell lines
A. Proximal region B. Distal region

Key Research Accomplishments

- Performed hybridizations with DNA isolated from prostate cancer specimens/cell lines onto our chromosome 18 microarray slides and are in the process of analyzing the data

- Identified two ESTs in the distal region (LOC 147381 and BC034583) which show differential levels of expression between less aggressive and metastatic prostate cancer cell lines

- Retrofitted BAC vectors with a selectable marker to aid in the selective retention of the BACs following transfection into the prostate cancer cell line PC-3.

Reportable outcomes

Due to the time it has taken to optimize the chromosome 18 custom microarray hybridization/washing conditions, we are not yet to the point of being able to submit an abstract/manuscript. However, within the next several months we will have all the hybridizations completed with the prostate cancer DNA and will be in an excellent position for the preparation of an abstract/manuscript.

Conclusions

With the completion of the custom microarray slides containing DNA from the BAC clones covering the proximal and distal critical regions, we are in the process of generating 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. Analysis of the microarray hybridization data should enable us to refine the critical regions originally described for metastatic samples. We have identified two ESTs that demonstrated differential levels of expression between less aggressive and highly-aggressive metastatic cell lines and are in the process of characterizing the ESTs.

Since the prostate specific antigen (PSA) is such a poor tool for the identification and characterization of aggressive prostate cancer, the identification of genes whose loss or gain could serve as molecular markers to identify highly aggressive prostate cancer which would be an extremely useful clinical tool. The data generated from these tasks should provide such a biomarker.

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