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14. ABSTRACT Based on our published data we propose that specific patterns of genomic copy number alterations (CNAs) occur in prostate cancer in African American (AA) men which are associated with different levels of disease aggressiveness. Second, specific patterns of gene expression are associated with disease aggressiveness in AA prostate cancer and these reflect, in part, the specific CNAs at the relevant gene loci. Finally, we propose that 4p16.3, which is lost in 30% of AA prostate cancers, contains one or more tumor suppressor genes that impact prostate cancer initiation and progression in AA men. This proposal will test these hypotheses by carrying out high throughput copy number and expression microarray analysis of DNAs and RNAs from cancer and benign tissues from African American men with prostate followed by an in depth analysis of the 4p16.3 region.					
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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	5-11
Key Research Accomplishments.....	11
Reportable Outcomes.....	11
Conclusions.....	11
References.....	11-12
Appendices.....	N/A

INTRODUCTION

African American (AA) men have both a higher incidence and significantly higher mortality rates from prostate cancer (PCa) than Caucasian (CAU) men¹. To what extent racial differences observed in PCa incidence and mortality are due to socioeconomic or biological factors remains controversial. Several groups have found that AA patients exhibited greater tumor volumes in comparison to similarly staged CAU patients^{2,3}. A major factor that has inhibited understanding the unique biology of PCa in AA men is the lack of clinical and pathological resources focused specifically on this problem. The vast majority of PCa molecular genetic and biological studies do not take differences in race into account when analyzing the results. This reflects the general under-representation of AA men in such studies, which substantially weakens the statistical power of any sub-group analysis. This is exacerbated by the generally lower percentage of AA patients in most tertiary referral centers where most such studies are performed. While some of the difference in mortality due to PCa can be attributed to socioeconomic factors, a number of studies have shown that there is still a higher mortality rate from prostate cancer in AA men even after adjustment for socioeconomic factors⁴. Thus, as concluded by Freedland and Isaacs⁴, that in addition to socioeconomic and cultural factors, biological differences account for some of the disparity in incidence and mortality for prostate cancer in AA men in comparison to CAU men. **The central problem addressed in this project is to understand the biological basis for the more aggressive clinical behavior of PCa in AA men and to develop predictive tools to help manage PCa in AA men.**

We have analyzed 20 PCas from AA men with high density single nucleotide polymorphism arrays⁵ to detect genomic copy number alterations (CNAs). Comparison of our primary tumors with tumors from CAU patients from a previously published cohort with similar pathological characteristics showed higher frequency of loss of at numerous loci, all of which had higher frequencies in metastatic lesions in this previously published cohort. **This difference may in part explain the more aggressive clinical behavior of prostate cancer in AA men and indicates that AA men will need specific prognostic tools based on the biology of their PCa.** Furthermore, when we performed cluster analysis of CNAs with both AA and CAU patients, almost all of the AA patients fell into two clusters, one associated with less aggressive organ confined disease and a second associated with more aggressive, invasive disease. **This is an exciting finding indicating that analysis of CNAs and patterns of CNAs may have prognostic value in AA men with PCa.** Finally, we indentified a novel region on chromosome 4p16.3 that is lost in 30% of AA PCas which has not been previously shown to be lost in PCa. This region has previously shown to be lost in breast, colon and bladder cancer and harbors several potential tumor suppressor genes.

We hypothesize that specific patterns of CNAs occur in AA PCa which are associated with different levels of disease aggressiveness. Second, we hypothesize that specific patterns of gene expression are associated with disease aggressiveness in AA PCa and these reflect in part the specific CNAs at the relevant gene loci. Finally, we hypothesize that 4p16.3, which is lost in 30% of AA PCa, contains one or more tumor suppressor genes that impact PCa initiation and progression in AA men. This proposal will test theses hypotheses by carrying out the tasks outlined below.

BODY

Task 1: High resolution analysis of genomic alterations in African American prostate cancers.

Subtask 1: Sixty pairs of samples will be obtained from the Baylor Prostate Cancer Tissue Bank. Samples will be from African American (AA) men undergoing radical prostatectomy for treatment of prostate cancer and were collected with informed consent. Prostate cancer (PCa) samples will have 80% tumor and will have a matched benign tissue available from the same patient. DNA and RNA will be extracted by standard methodologies. Assess DNA and RNA integrity by standard techniques. **(Months 1-2)**

Progress: We have extracted DNA and RNA from 69 pairs of prostate cancer and benign tissues. Quality was assessed and is high as shown in Figure 1.

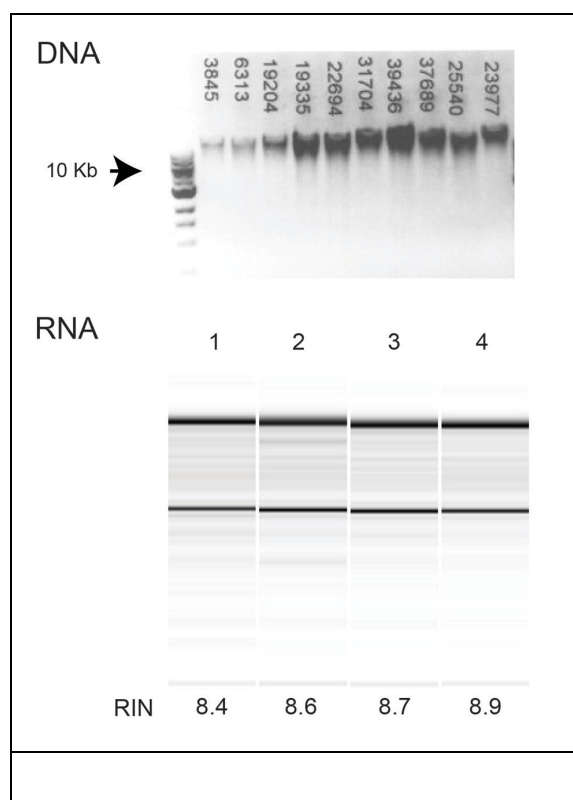


Figure 1. DNA and RNA from African American prostate cancer and prostate tissues.

Top: agarose gel of DNA showing undegraded high molecular weight DNA. The 10 Kb marker is shown on the left. Numbers are sample IDs.

Bottom: Agilent Bioanalyzer analysis of RNA. RNA integrity numbers (RIN) are shown at the bottom of each lane. A RIN of > 7 is required for optimal analysis by expression microarrays.

Subtask 2: Copy number analysis of DNAs from PCa and benign tissues from AA prostate cancers will be carried out as we have described previously except using Affymetrix 6.0 SNP arrays. **(Months 3-18)**

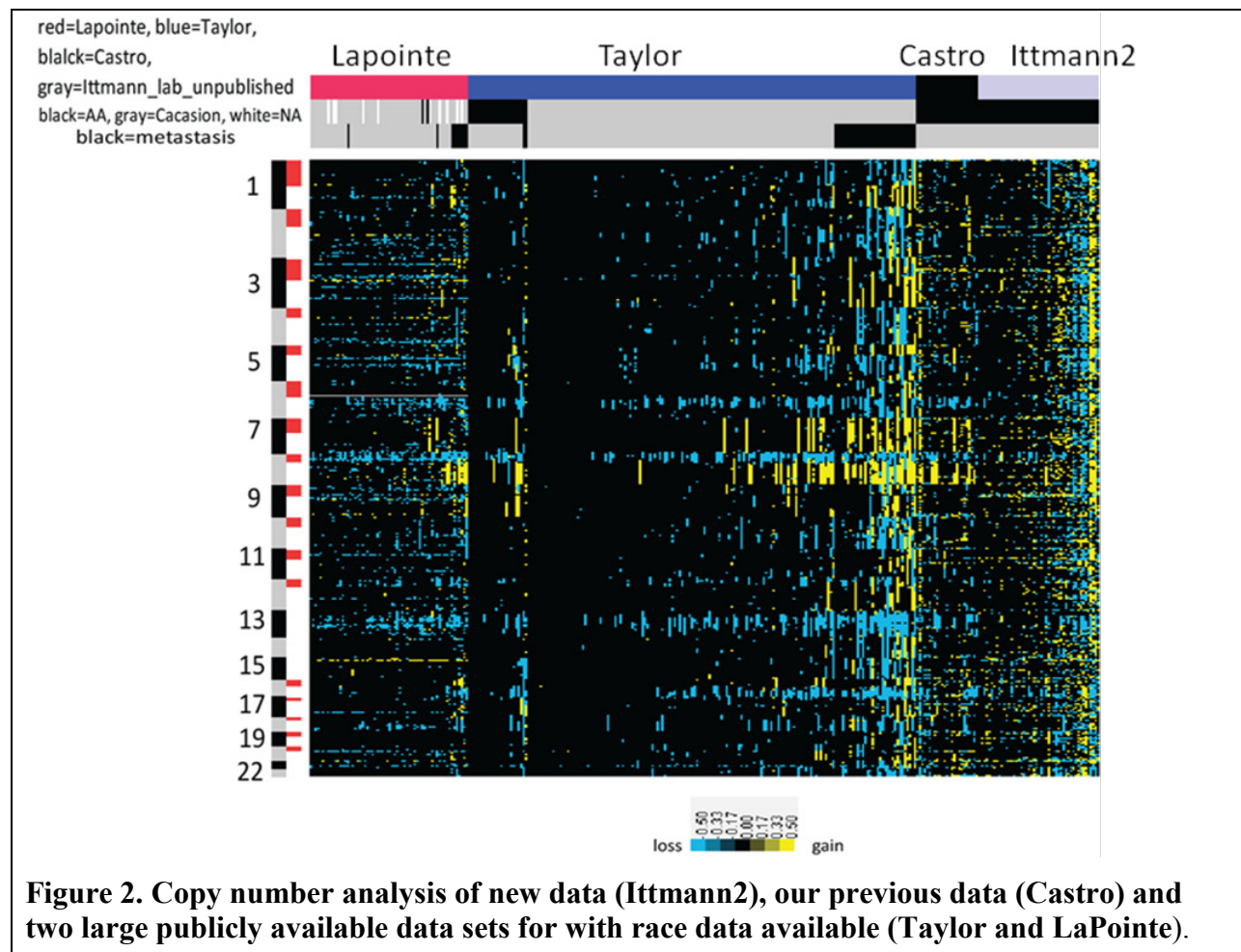
Progress: A total of 98 Affymetrix 6.0 Arrays have been completed (49 cancer/benign pairs).

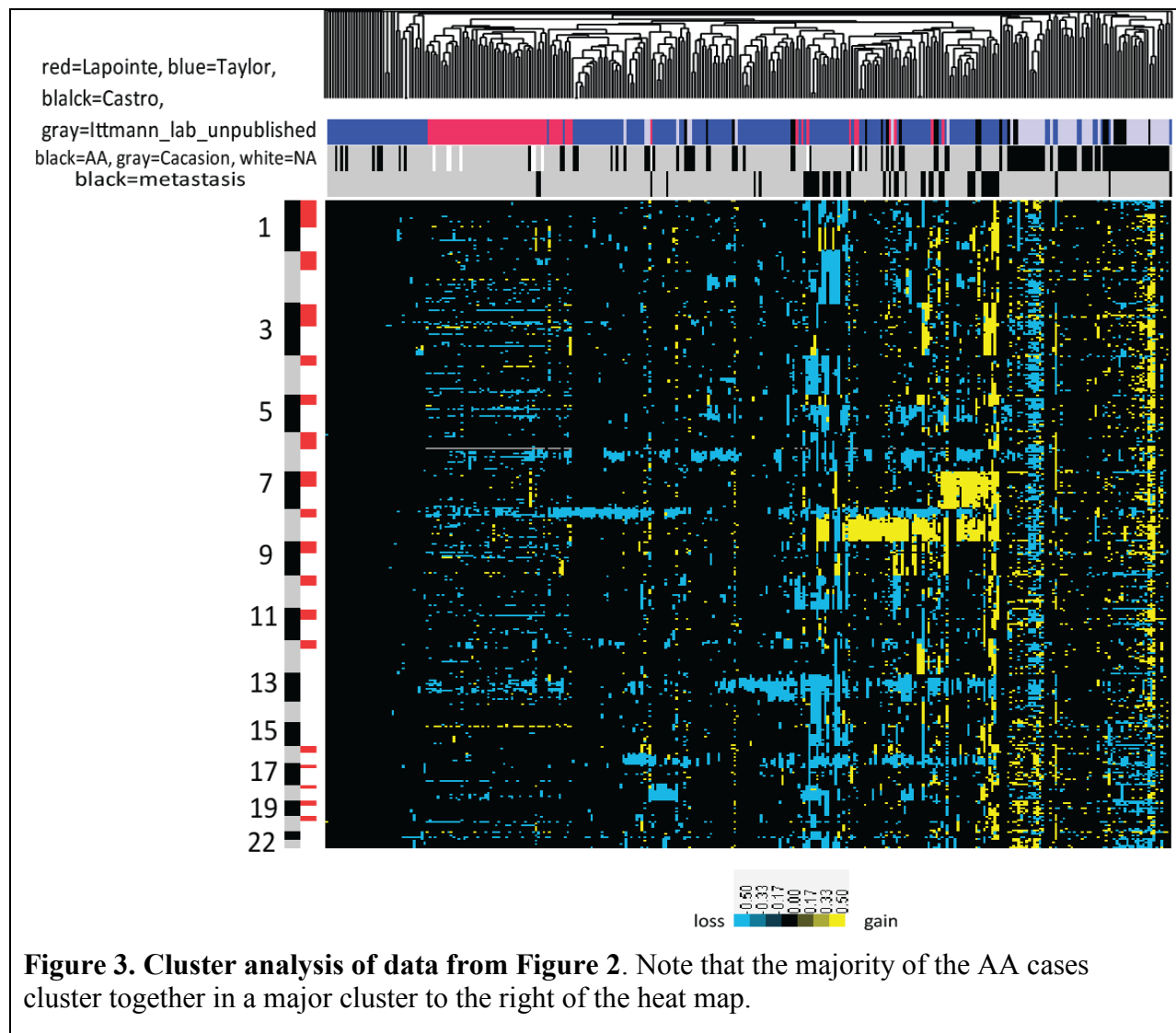
Subtask 3: Continuous quality control of data **(Months 3-18)**

Progress: We are carrying out continuous quality assurance.

Subtask 4: Data analysis for copy number alterations in PCas from AA. Hierarchical clustering (complete linkage method) of copy gain/loss profiles of prostate tumors from: AA PCa (new dataset); all Baylor AA PCa (new and old dataset); all AA PCa (our datasets and published AA datasets). We will also compare our AA cases (new and old) and the published CAU datasets. (Months 18-36)

Progress: We are carrying extensive data analysis of the copy number changes in the new data set and comparing it to our previous dataset as well as published datasets. We are using both the LaPointe dataset⁶ and the Taylor dataset⁷. The data is shown in Figure 2 with our new data labeled Ittmann2. Results of cluster analysis are shown in Figure 3. As in our previous analysis with a more limited data set, the majority of the AA cases are clustering together (right cluster). To better understand the basis for this clustering we have identified the statistically significant differences in gain or loss of specific cytobands between AA and CAU cases. This analysis is shown is summarized in Figure 4. Data analysis is still ongoing but it should be noted that are the loss of 4p16.3 is confirmed in this new analysis. In addition, it appears that the AA cases cluster into 4 major groups. From left to right there is a group without changes in the differentially lost loci; a group with widespread gains in these loci; a group with widespread losses and a group on the far right with more focal gains. Of note, the middle two groups cluster adjacent to CAU cases with metastasis. Data analysis is still ongoing but overall the data indicates that AA and CAU PCa have differences in CNAs and these differences are associated with aggressive disease.





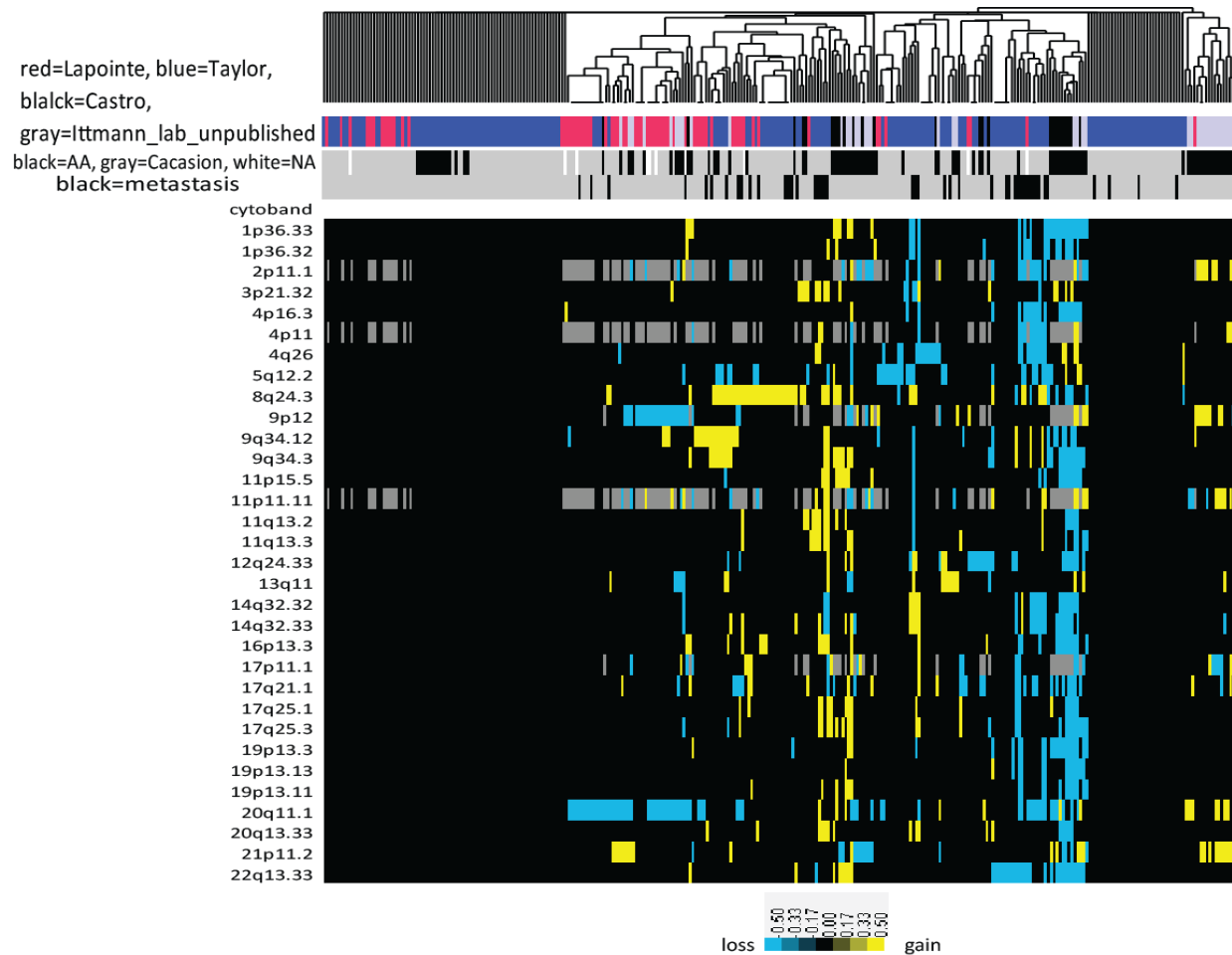


Figure 4. Cytobands lost or gained at significantly higher rates in AA Vs CAU prostate cancers ($p < .001$)

Subtask 5: We will determine the extent to which any CNA or pattern of CNAs is associated with PSA recurrence using both the new dataset and the combined dataset (new plus prior study) using Cox proportional hazard regression modeling of biochemical recurrence to develop multivariate survival models with specific CNA cluster groups, specific CNAs and/or groups of CNAs. (Months 18-36)

We have initiated this analysis. To date we have not identified specific single CNAs that are present in aggressive AA PCa but we have only examined a limited number of the potentially relevant alterations to date and the grouped analysis is underway.

Task 2: Whole genome expression array analysis in African American prostate cancers.

Subtask 1. Expression array analysis of prostate cancers from AA men using RNAs extracted from PCa tissues containing 80% or more tumor from AA men in Task 1 above and matching benign tissues. We will use human whole genome arrays from Agilent for expression microarray analysis as described previously. Each of these arrays contains 60-mer oligos that can detect 41,000 transcripts corresponding to the known human transcriptome. **(3-18 months)**

Progress: RNA expression analysis has been carried out on 96 RNAs (48 matched pairs)

Subtask 2. Continuous quality control of data **(3-18 months)**

Progress: We are carrying out continuous quality assurance.

Subtask 3. Initial data analysis using unsupervised cluster analysis of the AA expression dataset. Compare our data to existing publically available datasets available on the Web for both AA and CAU men. **(18-24 months).**

We are carrying out extensive data analysis of the expression array data. We are using the Taylor data⁷ set as our main comparator since it contains both AA and CAU patients, although mainly the latter. We have focused on identifying all genes with significant increased or decreased expression in AA PCa and have compared this data set to the Taylor CAU dataset to identify genes whose alterations may be unique to AA PCa. We have identified 97 genes that are increased in AA PCa in our dataset but are not increased in the CAU cancers in the Taylor dataset. We have prioritized this gene list to identify the most potentially interesting genes for validation and are undertaking initial validation using independent sets of AA and CAU PCa RNAs.

We have also identified genes that are significantly associated with aggressive pathological features (high Gleason score, seminal vesicle invasion and extracapsular extension). Again, a subset of these are being validated in an independent set of AA PCa RNAs using Q-RT-PCR.

Subtask 4: Correlate expression and CNA analysis **(18-36 months)**

This has been completed and genes in which there are significant correlations of CNA and expression have been identified. We are correlating this gene list with the list of cytobands significantly differentially altered in AA PCa (Fig 4) to identify potential targets of these losses and gains in AA PCa

Subtask 5: Carry out pathway analysis of expression data **(18-36 months)**

These studies are currently underway

Subtask 6: Validation of key gene expression changes in PCa identified during data analysis using quantitative RT-PCR **(18-36 months)**

This is underway (see Subtask 3 above)

Task 3: Identification of potential tumor suppressor gene(s) on 4p16.3 in AA PCa.

Subtask 1: Identify minimal deleted region on 4p16.3 by analysis of CNA data from Task 1. Identify any homozygous deletions. **(Months 18-21).**

Subtask 2: Further define minimal deleted region by analysis of gene expression data from Task 2 **(Months 18-21).**

Progress on Subtasks 1 and 2:

We have analyzed the 4p16 region in detail for the presence of heterozygous and homozygous deletions, loss of expression in AA PCa and correlation of gene copy number changes with gene expression. We have identified a single region of interest and the genes in this region are shown in Table 1. This region encompasses approximately 400 Kbp and is located at 4p16.3,

Gene	Het Loss	Homo Loss	r value	p value
HTT	1	0	-0.0455	0.86714
C4orf44	13	3	0.35822	0.17308
RGS12	4	0	0.59163	0.01578

Table 1. Genes in region of interest at 4p16.2/16.3 boundary

RGS12 is a negative regulator of G-protein coupled signaling and also regulates RAS/Raf/ERK signaling^{8,9}. Its role in cancer has not been studied in detail but it might potentially function as a tumor suppressor. Of note, RGS12 shows decreased expression in AA PCa (relative to matched benign tissue; $p < .0005$, t-test). Expression is correlated with gene copy number as shown in Table 1. No other gene on 4p16 shows both loss in AA PCa and correlation of copy number with expression. C4orf44 is a gene of unknown function adjacent to RGS12 and shows high levels of LOH as well as homozygous deletions but copy number is not correlated with expression and its expression is not decreased in AA PCa. We hypothesize that deletion in C4orf44 may impact expression of RGS12 since it is only 50 Kbp upstream of RGS12. Alternatively, C4orf44 might encode a novel tumor suppressor. HTT does not appear to be target in this region.

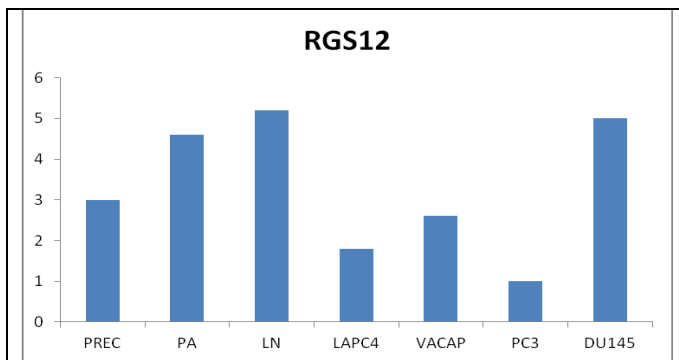


Fig 5. Expression of RGS12 mRNA in primary prostate epithelial cells (PREC) or immortalized normal prostate epithelium (PN: PNT1a). LN: LNCaP

Our initial studies show that RGS12 shows decreased expression by Q-RT-PCR in 3 of 5 PCa cell lines compared to primary prostate epithelial cells and PNT1a immortalized normal prostate epithelial cells. This is consistent with a potential tumor suppressor function for this gene.

Progress:

Subtask 3: Exome capture of minimal deleted region defined in Sub-tasks 1 and 2 above and perform next generation sequencing to identify mutations (**Months 21-26**)

Progress: Given that we identified a gene of interest as described above we are focusing on mutation analysis of this gene (RGS12) rather than exome sequencing of the entire region.

Subtask 4: In vitro functional studies of potential tumor suppressor genes identified based on homozygous deletion, mutation or decreased expression with relevant known or predicted biological functions. (**26-36 months**)

Progress: We are initiating functional studies of RGS12 using both overexpression and gene knockdown approaches.

KEY RESEARCH ACCOMPLISHMENTS

- We have identified and extracted high quality DNAs and RNAs from matched cancer and benign tissues from 69 radical prostatectomies.
- Copy number and expression array analysis has been performed and generated large amounts of data relevant to the biology and clinical behavior of AA PCa.
- We have identified RGS12 as potential tumor suppressor gene on 4p16.3. Further analysis, including mutation and functional analysis has been initiated.

REPORTABLE OUTCOMES

- None to date

CONCLUSION

We have carried out large scale CNA and expression array analysis of AA PCa to provide a comprehensive picture of the genomic landscape in African American prostate cancer. This data will yield important insights into the biology of AA PCa as well as potential biomarkers of disease aggressiveness.

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