Abstract
Introduction: Despite the widespread use of prostate specific antigen screening for early detection, prostate cancer remains the second leading cause of cancer related death among men in the US. Metastatic, hormone-refractory prostate cancer (HRPC) is the end-stage, fatal form of the disease. Defining the molecular mechanisms underlying the transition of an androgen responsive prostate cancer represents an important clinical problem. Currently, no effective therapies are available to treat hormone-refractory disease. In this study, we investigated the differentially expressed genes in primary prostate cancer vs. locally recurrent, androgen insensitive cancer represents an important clinical problem. Currently, no effective therapies exist for prostate cancer.

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Methods: Formalin-fixed paraffin-embedded human samples were analyzed for the whole genome HumanV250K transcribed microarray analysis using HumanV250K mediated annealing, selection, extension and ligation (GAS) process with the HumanV250K BeadChip (Illumina Inc., San Diego, CA). Additionally, a select number of chemotherapy predictive (theranostics) biomarkers were assessed using immunohistochemical methods.

Results: Transcriptome analysis identified 299 genes highly differentially expressed between the primary and recurrent groups (p-value ≤0.01, Student’s t-test). Using the 299 genes, we performed unsupervised hierarchical clustering and were able to discover 8 clusters corresponding to 5 general patterns: Pattern 1- underexpressed in both groups but more so in recurrent primary (2 clusters of 28 and 32 genes respectively), Pattern 2– slightly overexpressed in primary and underexpressed in recurrent (3 clusters of 7 genes), Pattern 3– overexpressed in primary and unchanged in recurrent (11 clusters of 23 genes), Pattern 4– overexpressed in both groups but more so in recurrent primary (3 clusters with 36, 52 and 57 genes in each cluster). Individual gene analysis revealed upregulation of androgen receptor (AR) and downregulation of the heat shock protein (HSP) and an oncogene encoding beta-catenin (CTNNB1). Consistent down regulation of androgen receptor was found across clusters.

Conclusions: Signature of AR in HSP was associated with down regulation of androgen receptor signaling in recurrent (AR) and HSP, providing a mechanism of androgen independence in prostate cancer. Platinum based drugs may have potential benefit in treating CRPC due to the down regulation of androgen receptor protein expression, while fluoropyrimidines are potentially beneficial due to the lack of TS protein expression in HRPCa. The heat map derived signature readily distinguishes between prostate cancer specimens from men who were treatment naïve vs. hormone refractory cancers with 5 different expression patterns.

Background
Prostate cancer is the most frequently diagnosed cancer other than skin cancer and the second leading cause of death from cancer in men in the United States. (1) Cancer patients are expected to be diagnosed in an estimated 340,000 men and to cause nearly 30,000 deaths. Most men with metastatic prostate carcinoma respond to various types of androgen ablation but progress to androgen resistant disease. Androgen ablation-resistant prostate cancer (CRPC) or hormone-refractory prostate cancer (HRPC) is a serious prognostic concern in men, who were treatment naïve vs. hormone refractory cancers.

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Study Design
Care selection: Twenty-three patients with prostate carcinoma whose tissue samples were analyzed with Caris Target Now™ (Caris Life Sciences, Phoenix, AZ) molecular profiling test were divided into 2 groups: Primary therapy naïve prostate cancer specimens and Recurrent, post-hormone therapy prostate cancer specimens. (2)

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Analysis of the transcriptome from the two groups of patients identified 773 genes highly significantly upregulated between the primary and the recurrent groups (p-value <0.05). Using these 773 genes, we performed two dimensional hierarchical clustering and were able to discover 8 clusters (Figure 2). Cluster 1 – underexpressed in both groups but more so in recurrent primary (85 genes), Cluster 2 – overexpressed in primary and slightly underexpressed in recurrent (62 genes), Cluster 3 – overexpressed in both primary and underexpressed in recurrent (121 genes), Cluster 4 – overexpressed in both primary and recurrent but at much higher levels in primary (148 genes), Cluster 5 – underexpressed in recurrent and no change/lightly repressed in primary (50 genes), Cluster 6 – overexpressed in both groups but more so in primary than recurrent (50 genes), Cluster 7 – overexpressed in primary and no change/lightly repressed in recurrent (46 genes), Cluster 8 – overexpressed in primary and no change/lightly repressed in recurrent (158 genes).

Figure 1

Figure 2

Table 1

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Cluster ID</th>
<th>Primary Gene Description</th>
<th>Recurrent Gene Description</th>
<th>Δ Recurrent/Primary</th>
<th>Control Ratio</th>
<th>T Test p-value</th>
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<th>Control Ratio</th>
<th>T Test p-value</th>
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<td>0.04</td>
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<td>0.03</td>
<td>5.64</td>
<td>0.18</td>
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<td>0.02</td>
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<td>0.60</td>
<td>0.01</td>
<td>6.03</td>
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<td>0.01</td>
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<td>0.18</td>
<td>AF4/FMR2 family, member 3</td>
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</tbody>
</table>

Results

Potential therapy targets include the Ras responsive element binding protein 1 (Ras-responsive element binding protein 1) and the androgen receptor (AR). These molecules may be potential targets for future therapy development.

Conclusions

1. Gene expression analysis shows a total of 8 different clusters which distinguishes the primary prostate carcinoma patients from recurrent/refractory cases. A total of 773 genes were found to be differentially expressed between the primary vs. hormone refractory patients (p≤0.05).

2. Androgen receptor was found to be upregulated both in the AR as well as protein which may indicate the presence of androgen receptor mediated signaling in hormone refractory prostate cancer.

3. All cancers examined (54) were found to be downregulated in hormone refractory prostate carcinomas which may indicate an androgen independent signaling in the absence of androgen through activation of androgen.

4. The cyclin dependent kinase inhibitor p21 was downregulated in 5 of the hormone refractory carcinomas which may indicate unfavorable clinical outcome in this subset of patients as has been reported a variety of solid and hematologic tumors.

5. HLA class I molecules (HLA-A, B, and C) were found to be significantly higher in recurrent tumors compared to primary tumors. This may be associated with therapies benefit from immunotherapy with Spalzik et al. The role of cellular immunotherapy and the first biological approach to treat hormonerefractory prostate carcinoma patients.

References