Differences in gene expression between primary therapy-naïve prostate carcinomas and hormone-refractory prostate carcinomas

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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 (HRPCa) is the end stage, lethal 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 exist for end stage, hormone refractory disease. In this study, we investigated the differentially expressed genes in primary prostate tumor vs. hormone refractory prostate tumor in order to identify potentially important therapeutic targets.

Methods: Formalin fixed paraffin embedded tumor samples from 14 cases (8 primary, chemotherapy-naïve and 6 locally recurrent, hormonal therapy-refractory carcinomas) were analyzed for the whole genome RNA (29,285 transcripts) microarray analysis using Illumina cDNA-mediated annealing, selection, extension and ligation (DASL) process with the HumanHT-12 v4 beadChip (Illumina Inc., San Diego, CA). Additionally, a select number of chemotherapy-predictive (theranostic) biomarkers were analyzed 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 these 299 genes, we performed two dimensional 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 than primary (2 clusters with 28 and 22 genes respectively); Pattern 2- slightly overexpressed in primary and underexpressed or no change in recurrent (1 cluster with 65 genes); Pattern3- slightly under expressed/ no change in primary and overexpressed in recurrent (1 cluster, 12 genes); Pattern 4- overexpressed in primary and no change in recurrent (1 cluster, 25 genes); Pattern 5 - overexpressed in both groups but more so in recurrent than primary (3 clusters with 58, 32, and 57 genes in each cluster).Individual gene analysis revealed upregulation of androgen receptor (AR) mRNA in recurrent HRPCa. This was, however, accompanied by a down regulation of regulator of G-protein signaling 2 (RGS2) and ras responsive element binding protein 1 (RREB1). Consistent down regulation of excision repair cross-complementing group 1 (ERCC1) and thymidylate synthase (TS) proteins were also observed in HRPCa.

Conclusion: Up-regulation of AR in HRPCa is associated with down regulation of androgen receptor signaling co-regulators (RGS2 and RREB1), providing a mechanism of androgen-independent activation of AR in HRPCa. Platinum based drugs may have potential benefit in treating of HRPCa due to the down regulation of nucleotide excision repair protein ERCC1, 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. In 2011, prostate carcinoma is expected to be diagnosed in an estimated 240,000 men and to cause nearly 34,000 deaths. Most men with metastatic prostate carcinoma respond to various types of androgen ablation but progress to castration-resistant disease. Castration-resistant prostate cancer (CRPC) or hormone refractory prostate cancer (HRPCa) has a poor prognosis and remains a significant therapeutic challenge.

Defining the molecular mechanisms underlying the transition of an androgen responsive prostate cancer to androgen insensitive cancer represents an important clinical problem. Currently, no effective therapies exist for end stage, hormone refractory disease. There is a growing body of evidence showing the continued dependence of hormone refractory prostate cancer on androgen receptor signaling even without the absence of androgen. In this study, we investigated the differentially expressed genes in primary prostate cancer vs. locally recurrent, hormone refractory prostate cancer in order to identify important therapeutic targets. In order to maximize treatment benefits in CRPC patients, it is imperative to incorporate predictive biomarkers and develop novel targeted agents.

Study Design

Case selection: Fourteen patients with prostate cancer whose tissue samples were analyzed with Caris Target Now™ (Caris Life Sciences, Phoenix, AZ) molecular profiling test were divided in 2 groups:Primary, therapy-naïve prostate carcinoma samples (n=8), and Recurrent, post hormone - therapy prostate carcinoma samples (locally recurring/progressing cancers at the primary organ site; n=6).

Methods: Formalin fixed, paraffin embedded tumor samples were analyzed for the whole genome RNA (29,285 transcripts) microarray analysis using Illumina cDNA-mediated annealing, selection, extension and ligation (DASL) process with the HumanHT-12 v4 beadChip (Illumina Inc., San Diego, CA).

Additionally, a select number of chemotherapy-predictive (theranostic) biomarkers (Figure 1, Caris Target Now report, IHC summary) were analyzed using immunohistochemical methods.

Figure 1

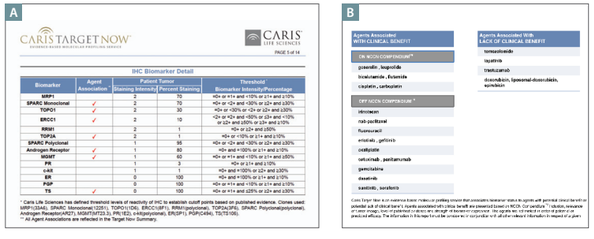

Figure 1 – **A)** Select biomarkers tested on all prostate cancer patients using immunohistochemistry. **B)** Drug associations based on results of the markers tested by IHC and expression array, which are parts of the Caris Target Now report. Drugs are separated out as agents associated with potential benefit that are ON or OFF NCCN compendia. Further the table on the right lists the drugs associated with a lack of potential benefit.

Results

Figure 2

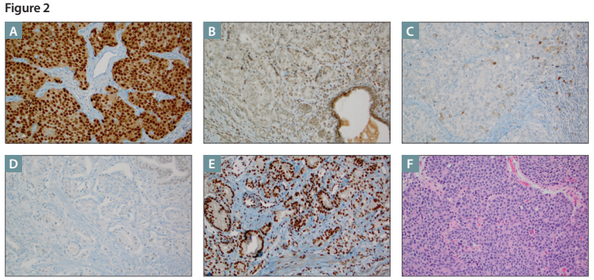

Figure 2 – AR, ERCC1 and TS expression analyzed by immunohistochemical staining in prostate cancer samples. **A)** AR over expression in recurrent prostate cancer; **B)** ERCC1 under expression in recurrent prostate cancer; **C)** TS under expression in recurrent prostate cancer; **D)** AR under expression in primary prostate cancer; **E)** ERCC1 over expression in primary prostate cancer; **F)** H&E stained high grade recurrent prostate cancer (20x). Under expression of ERCC1 and TS in hormone refractory cancers maybe associated with a response to platinum agents and fluropyrimidines respectively.

Figure 3

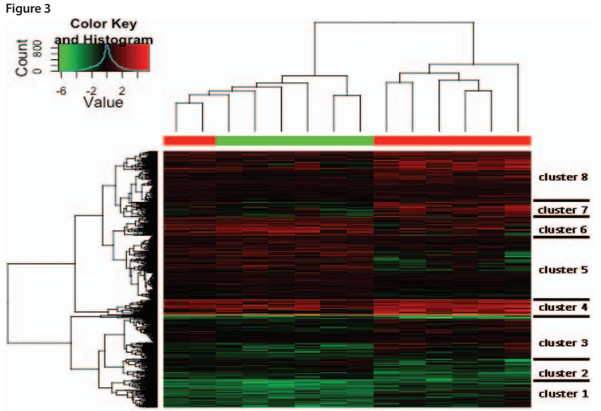

Figure 3 – The heat map derived signature between treatment naïve (Primary – red bar) vs. hormone refractory prostate cancers (Recurrent – green bar)

Analysis of the transcriptomes from the 2 groups of patients identified 773 genes highly significantly expressed 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 (heat map, Figure 3): **Cluster 1** - underexpressed in both groups but more so in recurrent than primary (85 genes); **Cluster 2 -** underexpressed in primary and slightly underexpressed or no change in recurrent (62 genes); **Cluster 3 -** overexpressed in primary and underexpressed in recurrent (121 genes); **Cluster 4 -** overexpressed in both primary and recurrent but at much higher levels in primary (48 genes); **Cluster 5 -** overexpressed in recurrent and no change/slightly repressed in primary (193 genes); **Cluster 6 -** overexpressed in both groups but more so in primary than recurrent (56 genes); **Cluster 7 -** overexpressed in primary and no change/slightly repressed in recurrent (40 genes); **Cluster 8 -** overexpressed in primary and no change/slightly over expressed in recurrent (158 genes). (Ten genes did not show a pattern consistent with any of the 8 clusters).

Figure 4

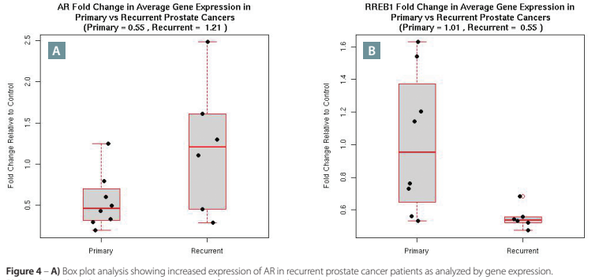

Figure 4 – **A)** Box plot analysis showing increased expression of AR in recurrent prostate cancer patients as analyzed by gene expression. **B)** Box plot analysis showing decreased expression of ras Responsive element binding protein 1 in recurrent prostate cancer patients.

Table 1

Table A – Top 15 genes over-expressed in primary prostate cancer vs. recurrent prostate cancer patients

Names	Cluster ID	Primary Over Control Ratio	Recurrent Over Control Ratio	T Test pvalues	Delta Primary/ Recurrent	Delta Recurrent/ Primary	Gene Description
SNORA39	cluster_4	12.10	0.99	0.04	12.17	0.08	small nucleolar RNA, H/ACA box 39
OR56A4	cluster_4	8.38	1.10	0.01	7.60	0.13	olfactory receptor, family 56, subfamily A, member 4
SOD2	cluster_1	1.17	0.16	0.05	7.14	0.14	superoxide dismutase 2, mitochondrial
UHRF1	cluster_4	7.91	1.16	0.01	6.83	0.15	ubiquitin-like with PHD and ring finger domains 1
SNORA3	cluster_4	5.56	0.83	0.05	6.71	0.15	small nucleolar RNA, H/ACA box 3
RBM3	cluster_7	2.73	0.41	0.02	6.65	0.15	RNA binding motif (RNP1, RRM) protein 3
SNORA71A	cluster_4	7.32	1.12	0.04	6.53	0.15	small nucleolar RNA, H/ACA box 71A
ZC3HAV1	cluster_3	1.18	0.19	0.04	6.28	0.16	zinc finger CCCH-type, antiviral 1
TAF13	cluster_8	3.64	0.60	0.01	6.03	0.17	TAF13 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 18kDa
SPAG1	cluster_7	3.67	0.62	0.05	5.91	0.17	sperm associated antigen 1
TAF13	cluster_8	3.71	0.64	0.01	5.81	0.17	TAF13 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 18kDa
PABPC1L2B	cluster_4	4.35	0.75	0.01	5.80	0.17	poly(A) binding protein, cytoplasmic 1-like 2B
CDKN1A	cluster_7	2.87	0.51	0.03	5.64	0.18	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
AFF3	cluster_3	1.86	0.33	0.01	5.62	0.18	AF4/FMR2 family, member 3
ZNF48	cluster_3	1.37	0.26	0.02	5.36	0.19	zinc finger protein 48

Table B – Top 10 genes under-expressed in primary prostate cancer vs recurrent prostate cancer patients

Names	Cluster ID	Primary Over Control Ratio	Recurrent Over Control Ratio	T Test pvalues	Delta Primary/ Recurrent	Delta Recurrent/ Primary	Gene Description
HLA-DRB1	no_cluster_membership	0.03	0.57	0.04	0.06	17.02	major histocompatibility complex, class II, DR beta 1
GCK	cluster_6	0.63	5.43	0.04	0.12	8.57	glucokinase (hexokinase 4)
AHNAK2	cluster_5	0.45	2.78	0.03	0.16	6.15	AHNAK nucleoprotein 2
RFXANK	cluster_5	0.26	1.34	0.01	0.19	5.26	regulatory factor X-associated ankyrin-containing protein
RABIB	cluster_2	0.11	0.57	0.02	0.19	5.13	RABIB, member RAS oncogene family
TRIM19	cluster_5	0.37	1.72	0.02	0.22	4.60	T-box 19
PFKFB2	cluster_2	0.23	1.00	0.05	0.23	4.43	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2
ADSL1	no_cluster_membership	0.09	0.36	0.03	0.24	4.14	adenylosuccinate synthase like 1
MIPEP	cluster_2	0.17	0.68	0.03	0.26	3.89	mitochondrial intermediate peptidase
CALCB	cluster_6	1.17	4.55	0.02	0.26	3.87	calcitonin-related polypeptide beta

Conclusions

1. Gene expression analysis shows a total of 8 different clusters which distinguishes the primary prostate cancer patients from refractory carcinomas. 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 at the RNA as well as protein level which may indicate the presence of androgen receptor mediated signaling in hormone refractory prostate tumors.
3. AR coregulator, RREB1 gene was found to be down regulated in hormone refractory prostate carcinomas which may be indicative of androgen independent AR signaling in the absence of androgen through activation of ras pathway.
4. The cyclin-dependent kinase inhibitor p21 was down regulated 5.6 fold in the hormone refractory tumors 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 II molecule mRNA (HLA-DRB1) was found to be significantly higher in recurrent tumor samples when compared to primary tumors. This may be associated with therapeutic benefit from immunotherapy with Sipuleucel-T, the first active cellular immunotherapy and the first biologic approved to treat hormone refractory prostate cancer patients.

References

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