Original Article

Multi-omics analysis reveals the *BRCA1* mutation and mismatch repair gene signatures associated with survival, protein expression, and copy number alterations in prostate cancer

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Background: Recent genomic analysis reveals that DNA repair gene mutations can be detected in 15–30% of patients in metastatic castration resistant prostate cancer depending on the population and clinical setting when comparing to a very small fraction in those with indolent localized diseases. The discovery and characterization of function associated with DNA repair gene mutations in prostate cancer patients may increase therapeutic options and lead to improved clinical outcomes.

Methods: To understand the role of DNA repair genes associated with other genomic alteration and signaling pathway, we applied an integrative analysis of multi-omics to The Cancer Genome Atlas (TCGA) prostate cancer dataset which contains 498 patients. We concurrently analyzed gene expression profiles, reverse phase protein lysate microarray (RPPA) data, and copy number alterations to examine the potential genomic mechanisms.

Results: We identified the signature of "chromosome condensation", "*BRCA1* mutation", and "mismatch repair" were associated with disease-free survival in prostate cancer. Through the concurrent analysis of gene expression profiles, reverse RPPA data, and copy number alterations, we found the three signatures are associated with cell cycle and DNA repair pathway and also most events of copy number gains.

Conclusions: This study presents a unique extension from DNA mutations to expressional functions, proteomic activities, and copy numbers of DNA repair genes in prostate cancer. Our findings revealed crucial prognostic markers and candidates for further biological and clinical investigations.

Keywords: Multi-omics; *BRCA1* mutation; mismatch repair; prostate cancer

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Introduction

Prostate cancer demonstrated a higher degree of heritability than other common cancers such as ovarian and breast cancer (1). Molecular profiles are increasingly being untilized for subtyping cancers and guiding treatment selection. Several studies have demonstrated the possibility in diversifying prostate cancer. One of the recent recognition is the germline and somatic mutations. Deleterious mutation in BRCA1 and BRCA2 which are associated with breast, ovarian and pancreatic cancer, have also shown to increase the risk of prostate cancer and some are even associated with aggressive form of the disease (2-4). In addition, early study from The Cancer Genome Atlas (TCGA) found that other genes, *FANCD2*, *CKD12*, and *ATM* classified under DNA repaired genes were also shown a higher prevalence in primary prostat cancer. It is estimated that 7–12% of men with metastatic prostate cancer involved inherited germline mutation in DNA repair genes (5).

The prevalence of somatic and germline mutations in DNA repair genes is becoming better understood. However, disease prognosis and treatment response associated with these mutations are not yet elucidated. Therapeutic management of metastatic castration-resistant prostate cancer (mCRPC) is currently based on new hormonal therapies (abiraterone, enzalutamide) and taxane-type chemotherapy (docetaxel or cabazitaxel). Early reports shown that patients carried genes associated with DNA-repair demonstrated a good response to poly-ADP ribose polymerase (PARP) inhibitors and platinum-based chemotherapy (6,7). Although results are still emerging and need validation, current evidence has suggested a potential subgroup associated with DNA repair genes mutation in prostate cancer may benefit from other treatment strategies.

Normal intracellular metabolism and environmental exposure constantly induce DNA reactivation and damage of the genome of cells in the human body. Various DNA repair mechanisms have been identified which help the cells repairing the many accidental lesions that occur continually in DNA and to protect genome integrity. Inherited and acquired deficiencies in these DNA repair mechanisms can modify cancer susceptibility as well as therapy response. The carcinogenesis of prostate cancer is suggested an accumulation of molecular changes of AR transcription activity, error prone DNA repair, oncogenic replication and changes in chromatin architecture. To further understand the role of DNA repair genes associated with other genomic alteration and signaling pathway, we applied an integrative analysis of multi-omics to TCGA prostate cancer dataset which contains 498 patients. Furthermore, we reveal the signature of "chromosome condensation", "BRCA1", and "mismatch repair" were associated with disease-free survival. Through the concurrent analysis of gene expression profiles, reverse phase protein lysate microarray (RPPA) data, and copy number alterations, we found the three signatures are associated with cell cycle and DNA repair pathway and also lots events of copy number alterations. This finding will support future understanding of how DNA repair processes, and DNA double-strand break repair in particular, are regulated during the cell cycle associated with the disease progression of prostate cancer.

Methods

Signature score of a gene set

To evaluate the activities of the gene sets examined in the study, the scoring method of Tian *et al.* (8) was used. Suppose there are N genes in a given gene set. Let $\mathbf{x}_l = \{x_{1,l}, \ldots, x_{N,l}\}$, where $x_{j,l}$ is the $\log_2$-transformed expression level of gene j in the expression profile of sample l. For a given gene set, the signature score of a sample is defined as

$$s_l = \frac{1}{N}\sum_{j=1}^{N} z_{j,l} \quad [1]$$

where $z_{j,l}=(x_{j,l}-\mu_j)/\sigma_j$, and μ_j and σ_j are the mean and standard deviation of the expression level of gene j in all expression profiles.

Gene sets

Total 10,236 gene sets were collected from 4 sources: Gene Ontology (GO), chemical and genetic perturbations (CGP), hallmark gene sets, and motif gene sets were used. The gene sets were downloaded from Molecular Signature database (MSigDB v3.1, http://www.broadinstitute.org/gsea/msigdb/index.jsp).

TCGA and The Cancer Protein Atlas (TCPA) data sets

Multiple genomics data of 498 prostate tumors were downloaded from the The Cancer Genome Altas (TCGA) website (http://cancergenome.nih.gov/) and also cBioPortal for Cancer Genomics (http://www.cbioportal.org/). Data includes gene expression profiles, the status of DNA copy number alternation and reverse phase protein array (RPPA) data were analyzed. The Reverse phase protein array (RPPA) data of lung adenocarcinoma datasets were downloaded

from The Cancer Protein Atlas (TCPA) website (9). Data used in this study are publicly available which do not constitute research involving human subjects.

Results

Gene expression signatures of DNA mismatch repair, BRCA1 mutations, and mitosis are intercorrelated in prostate cancer

We studied the prognostic roles of biological functions (represented by gene signatures) in prostate cancer. We re-analyzed expression profiles of 498 prostate tumors of TCGA and calculated per-sample gene signature scores of curated gene sets. Signature scores of the collected 10,236 gene sets were analyzed by a Cox hazard regression. A total of 285 gene sets were significantly associated with relapse-free survival (RFS). Heatmap of signature scores of the 285 gene sets is shown in *Figure 1A*. Among these gene sets, 6 gene sets were associated with mitosis and 24 were associated with cell cycle. "GO CHROMOSOME CONDENSATION" was significantly associated with RFS ($P=1.5\times10^{-11}$). Chromosome condensation is an essential process during mitosis phase of cell cycle and thus may mark tumor progression and aggressiveness. A sub gene set "GO MITOTIC CHROMOSOME CONDENSATION", which contains 14 out of its 31 member genes, was ranked as the 6th significant gene set ($P=2.0\times10^{-6}$). The results indicated that cell cycle is highly associated with cancer progression in prostate cancer. Chromosome condensation in the mitotic phase is the most prognostic gene set.

We further investigated the role of the DNA repair pathway in cancer progression. Six prognostic gene sets were correlated with DNA repair ("MATZUK MEIOTIC AND DNA REPAIR", "GO DNA REPAIR", "KAUFFMANN DNA REPAIR GENES", "GO REGULATION OF DOUBLE STRAND BREAK REPAIR VIA HOMOLOGOUS RECOMBINATION", "GO DNA SYNTHESIS INVOLVED IN DNA REPAIR", and "GO MISMATCH REPAIR"). We also observed a *BRCA1* mutation associated gene set "PUJANA BREAST CANCER WITH BRCA1 MUTATED UP" which was originally derived from differential expressed genes associated with *BRCA1* mutation in breast cancer. We selected the three gene sets, "GO CHROMOSOME CONDENSATION", "GO MISMATCH REPAIR", and "PUJANA BREAST CANCER WITH BRCA1 MUTATED UP", in representation of the signatures of chromosome condensation (and mitosis), mismatch repair, and *BRCA1* mutation pathway for further investigation. Although member genes were rarely overlapped among the three gene sets (*Figure 1B*), their signature scores were highly correlated (*Figure 1B*). The results indicate the functional cooperation and interplay among these functions. Kaplan–Meier plots showed that patients with higher signature score of the gene set had worse prognosis (*Figure 1C,D,E*).

Investigations of member genes of the three prognostic gene signatures of mitosis and DNA repair

There were 30, 55, and 28 member genes of the "chromosome condensation", "mismatch repair" and "*BRCA1* mutation" gene sets, respectively. Cox regression showed that 61% (19/31), 69% (38/55), and 32% (10/31) of them had significant adverse effects in RFS [hazard ratio (HR) >0 and P<0.05; *Figure 2*]. Of note, *NCAPD3* of the "chromosome condensation" signature was the only with significant protective hazard ratio (HR=0.76; *Figure 2*). In the "chromosome condensation" signature, NUSAP1, a coding gene of a nucleolar-spindle-associated protein, had the highest HR (HR=1.50) and *TOP2A*, a well-studied oncogene that encodes a DNA topoisomerase, was the second (HR=1.47). The two genes have been reported with roles in chromosome condensation and mitosis (10,11). The top 2 genes of the "*BRCA1* mutation" signature were *CDC20* and *RAD54L*. *CDC20* is a protein of the anaphase-promoting complex which could inhibit *BRCA1* facilitated homologous recombination (12). *RAD54L* is involved in the *BRCA1* mediated homologous recombination repair (13). In the "mismatch repair" gene set, *LIG1* (1st ranked) is DNA Ligase 1 that functions in DNA replication and recombination. *POLD1* (DNA polymerase delta 1) also plays a critical role in DNA replication and repair (14). Overall, analysis of member genes brought biologically meaningful results that supported our findings at the gene signature level.

Proteomics validation of the activities of mitotic and DNA repair pathways

At both the gene and gene set levels, our data suggested that DNA repair and the *BRCA1* pathway are prognostic and correlated with mitosis. We used the RPPA data of TCPA (9) to confirm such findings in the protein level. We calculated correlation coefficients between a signature

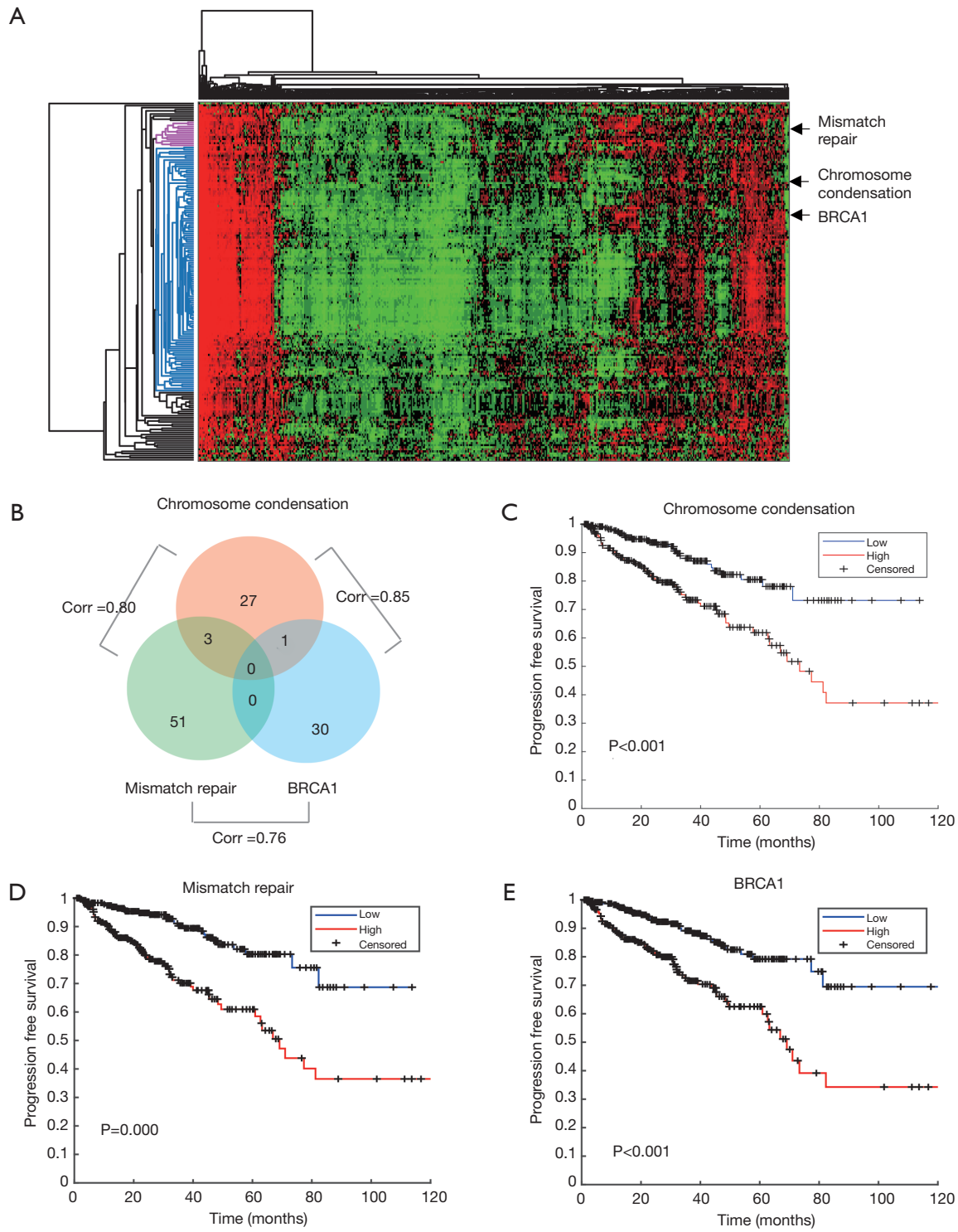

Figure 1 Associations among chromosome condensation, BRCA1, mismatch repair signature scores and relapse-free survival in prostate cancer patients. (A) The heatmap of signature scores of the 285 prognostic gene sets. Three of them were selected to represent the function of chromosome condensation, BRCA1, mismatch repair; (B) Pearson correlations show that the three gene-signatures were mutually interdependent in prostate cancer. All coefficients were >0.76. The number of overlapping genes between any two gene set are less than 3; (C,D,E) the Kaplan-Meier analysis shows the three gene sets are prognostic.

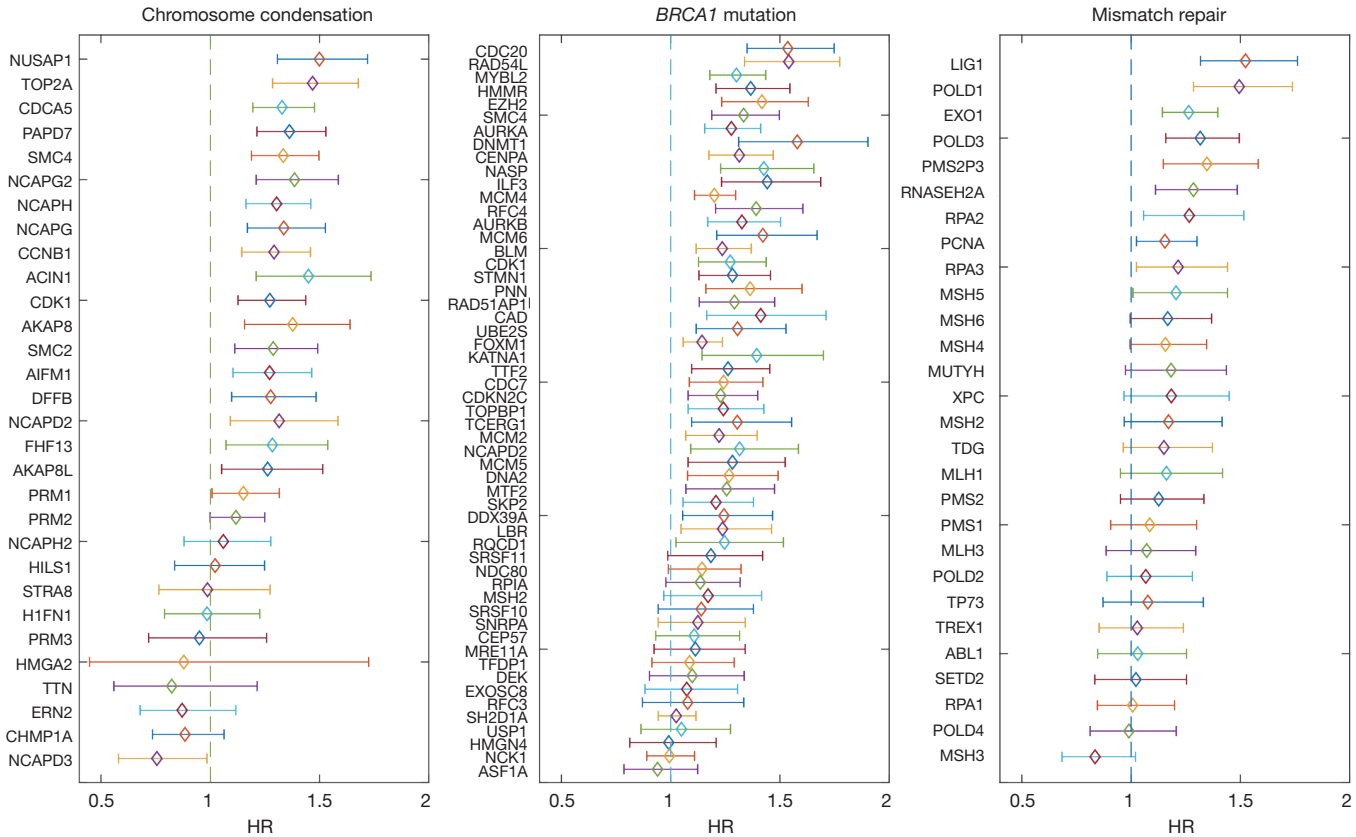

Figure 2 Cox regression analysis of relapse-free survival associated with the member genes in three gene signatures. Association between the relapse-free survival and 30, 55, and 28 member genes of "chromosome condensation", "BRCA1", "DNA repair" signatures. Hazard ratios and 95% confidence intervals are shown.

score and proteomic activities of its members. Proteins with high correlation coefficients are showed in *Table 1*. The correlation coefficients of Cyclin B1 are 0.675 (ranked 1st), 0.651 (ranked 2nd), 0.495 (ranked 1st) to the three signatures, respectively. Cyclin B1 has been reported as a regulatory protein involved in mitosis. Chek2, a serine-threonine kinase involved in DNA repair, was correlated with both "chromosome condensation" and "*BRCA1* mutation" signatures. Our data also showed Brca2 was negatively correlated to "mismatch repair" signature (coefficient =–0.317). The results again confirmed the association of BCRA1 and DNA repair pathways with mitosis.

Copy number alterations are associated with the signature scores

To identify survival associated CNAs, copy number status of each gene was applied to Cox regression model. A total of 198 genes with copy number gains and 337 genes with copy number losses were identified with survival association. Then samples were categorized into two groups based on scores of each gene signature, and chi-square test was used to estimate the proportional difference of copy number changes between the two groups. For the 198 prognostic copy number gains, 197 genes showed significant proportional differences in all three gene sets (P<0.001). RNA5SP73 was the only gene with a proportional difference in the "*BRCA1* mutation" signature, but with no statistical difference in the other two. The top 5 prognostic genes are listed in the *Table 2*. Copy number gains of *RN7SL815P* was associated with worse survival. We also discovered the proportion of samples with copy number gains in the high signature score groups are higher than those in the low score groups. Comparing with 20% of samples have copy number gains in the high signature score groups, <5% of samples in the low score groups have copy

Table 1 Pearson correlation coefficient of the signature score and protein expression using the TCGA and TCPA prostate cancer datasets

Gene set	Protein	Corr	Gene set	Protein	Corr	Gene set	Protein	Corr
Chromosome condensation	Cyclin B1	0.675	*BRCA1* mutation	Cyclin B1	0.651	Mismatch repair	Cyclin B1	0.497
	TFRC	0.454		TFRC	0.468		Chk2	0.370
	Caspase-7cleavedD198	0.385		RBM15	0.423		ACC_pS79	0.326
	PCNA	0.375		Syk	0.419		Bim	0.325
	Chk2	0.359		Bim	0.404		XRCC1	0.320
	Rictor	–0.330		DJ-1	–0.392		PKC-alpha_pS657	–0.301
	ACVRL1	–0.349		ACVRL1	–0.426		PKC-alpha	–0.315
	PKC-alpha_pS657	–0.355		PKC-alpha_pS657	–0.428		BRCA2	–0.317
	Caveolin-1	–0.356		PKC-alpha	–0.432		ACVRL1	–0.353
	PKC-alpha	–0.358		Caveolin-1	–0.445		Caveolin-1	–0.353

TCGA, The Cancer Genome Atlas; TCPA, The Cancer Protein Atlas.

Table 2 Cox regression analysis of the gene copy number status and the chi-square test in the groups of high and low signature score

Gene	HR	95% CI	p value	Chromosome condensation			*BRCA1* mutation			Mismatch repair		
				High	Low	P value	High	Low	P value	High	Low	P value
Gain												
RN7SL815P	2.93	(1.84–4.67)	5.49E-06	38/195 (19.5%)	9/195 (4.6%)	9.30E-06	40/193 (20.7%)	7/226 (3.1%)	3.00E-07	37/198 (18.7%)	10/221 (4.5%)	4.32E-05
RN7SL159P	2.52	(1.60–3.97)	6.17E-05	42/189 (22.2%)	14/189 (7.4%)	4.85E-05	46/182 (25.3%)	10/233 (4.3%)	4.93E-08	38/195 (19.5%)	18/220 (8.2%)	4.15E-03
LINC00963	2.52	(1.60–3.96)	6.36E-05	43/189 (22.8%)	14/189 (7.4%)	2.94E-05	47/182 (25.8%)	10/233 (4.3%)	2.76E-08	39/195 (20.0%)	18/220 (8.2%)	2.83E-03
GAP43	2.72	(1.66–4.45)	7.28E-05	37/196 (18.9%)	9/196 (4.6%)	1.62E-05	39/194 (20.1%)	7/225 (3.1%)	5.55E-07	36/199 (18.1%)	10/220 (4.5%)	7.41E-05
XPO6	2.56	(1.61–4.09)	7.75E-05	30/194 (15.5%)	9/194 (4.6%)	3.28E-04	33/193 (17.1%)	6/225 (2.7%)	2.84E-06	27/202 (13.4%)	12/216 (5.6%)	1.81E-02
Loss												
NDUFAF2	0.52	(0.39–0.70)	8.03E-06	65/172 (37.8%)	25/172 (14.5%)	2.10E-06	66/170 (38.8%)	24/219 (11.0%)	3.55E-07	60/177 (33.9%)	30/212 (14.2%)	4.08E-04
SMIM15	0.52	(0.39–0.70)	8.03E-06	65/172 (37.8%)	25/172 (14.5%)	2.10E-06	66/170 (38.8%)	24/219 (11.0%)	3.55E-07	60/177 (33.9%)	30/212 (14.2%)	4.08E-04
KIF2A	0.53	(0.40–0.71)	1.30E-05	66/170 (38.8%)	23/170 (13.5%)	1.71E-07	66/169 (39.1%)	23/220 (10.5%)	1.53E-07	59/177 (33.3%)	30/212 (14.2%)	4.14E-04
IPO11	0.53	(0.40–0.71)	1.30E-05	66/170 (38.8%)	23/170 (13.5%)	1.71E-07	66/169 (39.1%)	23/220 (10.5%)	1.53E-07	59/177 (33.3%)	30/212 (14.2%)	4.14E-04
CKS1B	0.54	(0.41–0.72)	2.32E-05	65/171 (38.0%)	22/171 (12.9%)	1.31E-07	65/170 (38.2%)	22/221 (10.0%)	1.17E-07	57/179 (31.8%)	30/212 (14.2%)	8.96E-04

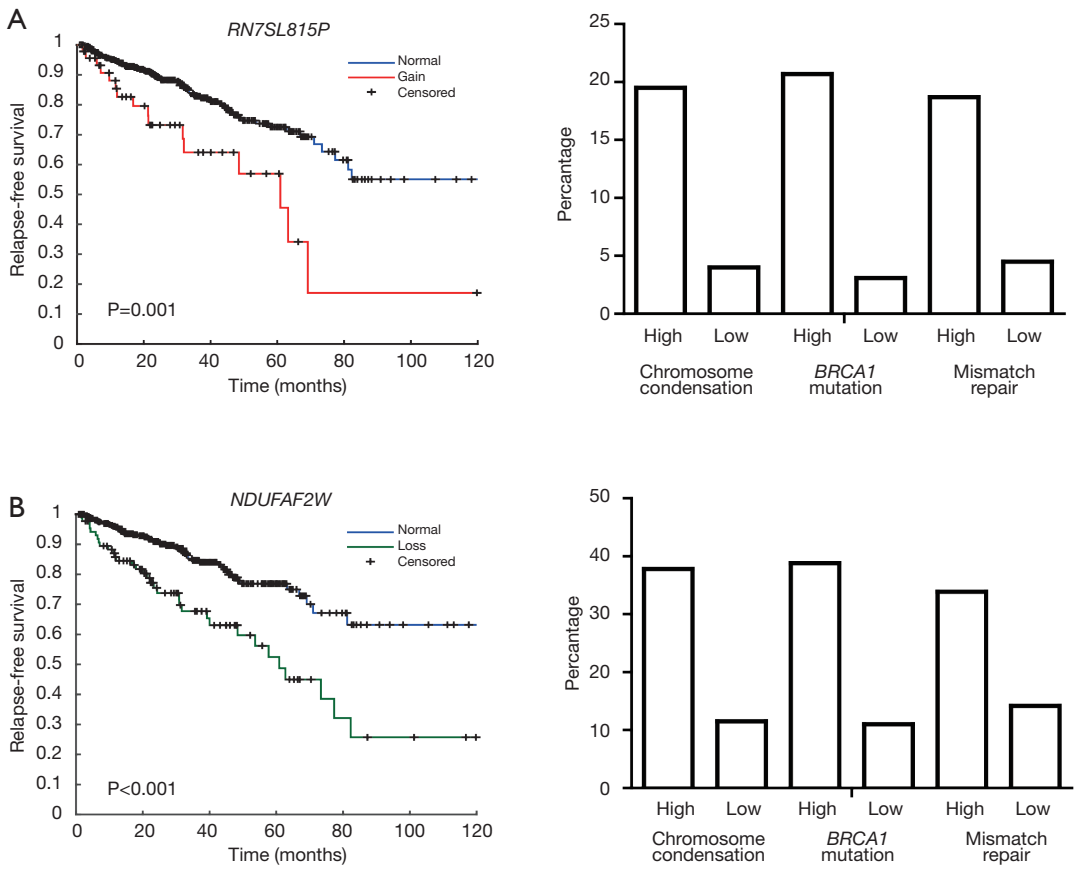

Figure 3 High proportion of copy number change in patient groups with high signature scores. (A) Copy number status of *RN7SL815P* is survival association. Patients with copy number gain have worse survival. About 20% of patients with high signature scores have *RN7SL815P* copy number gain. In the groups with low signature score, less than 5% has *RN7SL815P* copy number gain; (B) copy number loss status of *NDUFAF2* is prognosis. More than 30% of patients with high signature scores have *NDUFAF2* copy number loss.

number gains (*Figure 3A*). For the top 5 genes, *RN7SL815P* and *RN7SL159P* are pseudogenes affiliated with the antisense RNA class, and *LINC00963* is a long non-coding gene. *GAP43* is considered as a neuron growth-associated protein. *XPO6* is a member of the importin-beta family. For the 5 genes, the proportions of samples with copy number gains were all above 15% in groups of samples with high scores. On the other hand, the proportions were below 9% in the low-score groups. For the 337 genes with prognostic copy number losses, a total of 185, 273, 156 genes were identified with proportional differences (P<0.001) in the three gene signatures, respectively. The significant percentage in copy number losses is much lower than copy number gain. For the top 5 prognostic genes with copy number losses, the proportion of sample with copy number loss are more than 37% in the groups of high score and only around 10% in the low score groups. Taking NDAUFAF as an example, the percentage of sample with copy number losses was 37.8%, 38.8%, and 33.9% in the high score groups for "chromosome condensation", "mismatch repair" and "*BRCA1* mutation", respectively. The percentage in low score groups was only 11.5%, 11%, and 14.2%, respectively (*Figure 3B*).

Discussion

The usage of PARP inhibitors to treat tumors harboring DNA repair defects, especially with homologous recombination deficiency (HRD), is one of the earliest successes of precision oncology (15,16). In prostate cancer, germline and/or somatic mutations of DNA repair genes are closely associated with patients' response to not only

PARP inhibitor (6,17), but also chemotherapy (18,19) and androgen deprivation therapies (20,21). The emerging technology of liquid biopsies, such as circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), enables a least-invasive assay for these mutations and promises wide clinical applications (22). However, the role of genomic mechanisms other than mutations of the DNA repair genes remains an uncharted territory. In fact, integrative analysis of genomic and proteomic profiles of prostate tumors is realized using next-generation sequencing and RPPA. For instance, integrations of DNA- and RNA-Seq revealed novel classification beyond Gleason score (23) and clinically actionable alterations (24) of prostate cancer. However, such analysis has not been focused on DNA repair genes that can bring biological insights to the observed clinical significance and reveal novel experiment and therapeutic targets. Here, we re-explored the multi-omics profiles of TCGA and carried out a comprehensive analysis of gene expression, functional proteomics, and CNAs of DNA repair genes. Our analysis reached several major findings. First, we conducted a gene set analysis of expression profiles of DNA repair genes. Such analyses have been shown to achieve higher interpretability and tolerance to data noise than investigations at the single-gene level (25-27). As a result, we demonstrated that activities of DNA repair-related functions, including chromosome condensation, mismatch repair, and signaling of *BRCA1* mutation, were predictive of progression-free survival of prostate cancer. Our data are in line with previous studies showing that activations of a cell cycle marker and a cell proliferation signature were predictive of adverse survival of prostate cancer (28,29). Second, we demonstrated a concordant prognostic value in protein activities of these genes. Such concordance indicates a meaningful effect of expressional changes in DNA repair genes to really perturb protein functions and affect survival outcomes. Our data underlined many proteins for further investigations. For instance, CDC20 was the top survival predictor among *BRCA1* mutation-regulated genes. It is a regulatory protein functioning at several points in the cell division cycle. High expression of *CDC20* is associated with high Gleason score, biochemical recurrence, tumor cell growth, and chemoresistance of prostate cancer (30,31). Our data confirmed the unfavorable role of its protein activity. Among proteins associated with chromosome condensation, several non-SMC condensin complexes (NCAPD2, NCAPD3, NCAPH, NCAPH2, NCAPG, and NCAPG2) were associated with adverse survival. These complexes are players in chromosome assembly and segregation, and may be crucial in tumor cell proliferation and tumor progression. Our data warrant further investigations on these genes. At last, CNAs in some DNA repair genes, including two previously unexplored genes *NDUFAF2* and *RN7SL815P*, were indicative of poor survival. *NDUFAF2* encodes a mitochondrial protein that is moderately to strongly expressed among cancers according to the Human Protein Atlas (32,33). Our data were the first to demonstrate the prognostic effect of its CNA in prostate cancer. Altogether, the study presents a unique extension from DNA mutations to expressional functions, proteomic activities, and copy numbers of DNA repair genes in prostate cancer. Our findings revealed crucial prognostic markers and candidates for further biological and clinical investigations.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.07.05). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was waived. This study used public available data which do not require ethics approval.

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