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Molecular and Clinicopathological Correlates of Wild-Type KRAS Expression in Prostate Cancer

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Abstract

Background The clinicopathological significance of *KRAS* alterations in clinical prostate cancer (PCa) has yet to be comprehensively studied, and the classic *KRAS* somatic mutations are rare in PCa.

Methods The clinico-genomic data of two PCa cohorts were retrieved from the cancer genome databases. *KRAS* expression-based gene enrichment for cell proliferation, apoptosis, and epithelial-mesenchymal transition /invasion programmes, RAS activation, MAPK and PI3K signalling were sought using gene enrichment analyses, and validated with clinicopathologically relevant tumour biology signatures.

Results RAS activation and hallmark tumour biology pathways were enriched in *KRAS*-high PCa subsets. *KRAS* expression also demonstrated significant associations with Gleason score and ISUP prognostic grade groups, pathological tumour stage, overall TNM stage, and treatment outcomes, but not with age, pathological node and metastasis statuses. The study further demonstrated that wild-type *KRAS* expression was deregulated in PCa by a combination of copy number changes, epigenetic/altered transcription factor-expression and microRNA mechanisms.

Conclusion The relevance of *KRAS* expression to clinical PCa biology and therapy outcomes deserves further validation.

Keywords wild-type *KRAS*, enrichment analysis, hallmark tumour biology, prostate cancer

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Introduction

The study of the molecular pathology of prostate cancer (PCa) has significantly improved the understanding of PCa biology [1, 2]. It has also aided the discovery of diagnostic, prognostic and predictive biomarkers in PCa, all of which have promoted the progress of the clinical management of PCa [1, 2]. In spite of this progress, PCa remains the 4th most common cancer diagnosed worldwide, after cancers of the female breast, lung and colorectum. It is also the 8th commonest cause of cancer deaths, in both genders combined, and the 5th most common cause of cancer deaths in men [3, 4]. Therefore, a huge knowledge gap in PCa biology still exists; thus, the pressing need to deepen our understanding of PCa biology further is warranted.

In this study we investigated the clinicopathological and biological correlates of *KRAS* expression in PCa. *KRAS* alterations, in the form of somatic mutations and gene amplification, are commonly found in carcinomas of the lung and digestive tract (pancreas, gastric, colon and rectum). *KRAS* alterations especially have therapeutic relevance in lung cancers in which they are targets of therapy [5], and in gastrointestinal tract cancers in which they are used as predictive biomarkers of anti-EGFR therapy [6-8]. Although the much-valued *KRAS* somatic mutations are rare in PCa [9-11], it has been demonstrated that breast, lung, gastric, colorectal and prostate cancer with wild-type *KRAS* displayed high RAS activation scores and exhibited upregulated RAS signalling, evidence that *KRAS* activation can exist in the absence of somatic *KRAS* gene mutations [12-14]. Interestingly, most of the studies of *KRAS* alterations in PCa has been performed on cell lines [15-23]. However, cell lines may not recapitulate all aspects of tumour biology of any cancer type, inasmuch as culture studies are usually limited to two to seven cell lines per study. Under in vitro conditions, the survival and propagation of specific cancer cell populations and certain cancer states are preferentially supported. Furthermore, it has been observed that additional genetic and epigenetic alterations are introduced in long-term cell cultures, thereby creating systematic differences between cell culture and patients' tumour states [24-27]. For the above reasons, it is pertinent to investigate *KRAS* expression in clinical PCa to verify the findings from cell line studies. A few studies have shown upregulation of *KRAS* signalling pathway in a clinical cohort of primary and metastatic PCa cases [28]. However, the clinicopathological and biological relevance of wild-type *KRAS* expression have yet to be comprehensively interrogated in any clinical PCa cohort.

The paucity of studies that utilize clinical PCa for *KRAS* studies, and the curiosity of active wild-type *KRAS* signalling in cancer, form the rationale for this study.

The aim of this study is to investigate the biological and clinicopathological significance of wild-type *KRAS* expression in clinical cohorts of PCa. The study objectives are to determine (i) whether *KRAS* signalling is active in PCa in the absence of *KRAS* coding mutations, (ii) whether any relationships between wild-type *KRAS* expression and tumour biology indices (cellular proliferation, invasion and apoptosis, etc.) exist in clinical PCa, (iii) whether altered *KRAS* expression in the context of wild-type KRAS can confer specific clinicopathological characteristics on PCa, (iv) to define the mechanisms of *KRAS* deregulation in PCa. The study hypothesis is that wild-type *KRAS* signalling is active in clinical PCa cases, in which altered *KRAS* expression has clinicopathological, molecular and biological significances.

Methods

Prostate cancer cohorts

The study retrospectively analysed the clinicopathological and genomic data of two PCa cohorts, TCGA Firehose [29, 30] and the DKFZ, (or, German Cancer Research Centre) PCa cohorts [31]. All the clinicopathological and genomic data were retrieved from the Genome Data Commons and cBioPortal for Cancer Genomics databases. The mRNA and miRNA quantification were accomplished with RNASeq and miRNASeq, respectively, while methylation data was obtained by methylation array on the Illumina Human Methylation 450 platform. The masked copy number segment data was generated using the Affymetrix SNP 6.0 genotyping array.

Online analyses of *KRAS* expression identified only about 0.3%-0.4% of PCa cases which harboured somatic mutations in *KRAS* in both cohorts [29, 30]. These cases were excluded from this study. Furthermore, while the gene expression dataset of the TCGA PCa cohort contained 60660 records, the DFKZ cohort had 20882. TCGA cohort comprised 500 primary PCa cases with clinicopathological (including prognostic and therapy outcome), RNASeq, chromosomal copy number segment, methylation, and somatic mutation data. The following amount of data was available for this cohort: clinicopathological (between 393 and 497 of the 500 cases for each clinicopathological indices; **Table 1**); mRNA expression (498/500 cases); chromosomal copy number segment (497/500 cases); methylation (between 322 and 498 of 500 cases for individual methylation loci); microRNA expression (498/500 cases) data. The DKFZ cohort included 118 PCa cases with clinicopathological (including biochemical recurrence post-therapy), and mRNA expression data. Data was available for clinicopathological features (93 to 95 of 118 cases with RNASeq data), and RNASeq (118/118 cases).

Bioinformatics analyses

Linux-based codes and scripts were written in the Window-based Ubuntu 20.04 environment in order to retrieved the data of interest from the downloaded materials. Linux-based scripts were also used to prepare gene expression datasets (in txt and gct formats) as per Gene Set Enrichment Analyses (GSEA) [32-34], and DeSeq2 Gene Ontology Enrichment Analyses requirements [35, 36], while the phenotype and derivative gene set files (see below) were prepared in Excel spreadsheet and converted to cls and grp files, respectively.

Study approach

KRAS expression for either PCa cohorts was dichotomised into low and high *KRAS* expression groups using the median expression values as the threshold. This categorization was applied to the GSEA, and to subsequent statistical analyses on SPSS. To determine whether wild-type *KRAS* signalling is active in PCa, we first explored the differential up-regulation of biological pathways (cell proliferation signalling: cell cycle, *KRAS*, *MAPK*, *PI3K-AKT-MTOR*, *E2F* and G2M checkpoint; tumour invasion signalling: TGFB and the epithelial-mesenchymal transition (EMT) signalling; and Apoptosis) between *KRAS*-high and *KRAS*-low cases in the TCGA PCa cohort by using the Molecular Signature Database (MSigDB) Hallmark (*KRAS*, *PI3K-AKT-MTOR*, and G2M checkpoint, *TGFB*, EMT, and Apoptosis) and KEGG (cell cycle and *MAPK* signalling) pathway gene sets [32-34]. Furthermore, we applied the RAS activation gene set described by Barbie et al [37] to the TCGA PCa cohort to confirm *KRAS* activation. Then, we validated the wild-type *KRAS* activation in the DFKZ cohort. To achieve the aforementioned validation, gene sets were created from the core enrichment set obtained from the TCGA dataset analyses and used to examine the DFKZ expression dataset, as per MSigDB recommendations, and to obtain core

Table 1. Clinicopathological features of KRAS expression in TGCA and DFKZ prostate cancer cohorts (one-way ANOVA) (KRAS Expression).

Items	Clinicopathological Features		N	Mean	Std. Deviation Lower Bound	95% Confidence Interval Upper Bound		F	Adjusted P
TCGA Cohort	Age at Diagnosis	60 years and below	223	5.619	2.228	5.325	5.913	0.143	0.705
		61 years and above	274	5.697	2.352	5.417	5.977		
		Total	497	5.662	2.295	5.460	5.864		
	Race/Ethnicity	American Indian	1	4.857	-	-	-	3.323	0.031
		Asian	12	7.240	3.505	5.013	9.466		
		Black	57	6.191	2.630	5.493	6.889		
		White	412	5.548	2.185	5.337	5.760		
		Total	482	5.665	2.296	5.459	5.870		
	Gleason Score	Low Gleason score	292	5.317	2.151	5.069	5.565	16.464	<0.001
		High Gleason score	205	6.153	2.409	5.821	6.485		
		Total	497	5.662	2.295	5.460	5.864		
	ISUP* Prognostic Grade Group	Groups 1 and 2	190	5.241	1.821	4.981	5.502	10.531	0.003
		Groups 3 to 5	307	5.922	2.513	5.640	6.204		
		Total	497	5.662	2.295	5.460	5.864		
	Pathological Tumour Stage	pT2	181	5.107	1.775	4.846	5.367	11.964	<0.001
		pT3	268	6.029	2.541	5.724	6.335		
		pT4	10	7.469	2.864	5.420	9.518		
		Total	459	5.697	2.331	5.483	5.911		

Table 1. Clinicopathological features of KRAS expression in TGCA and DFKZ prostate cancer cohorts (one-way ANOVA) (KRAS Expression) (Continued).

Items	Clinicopathological Features		N	Mean	Std. Deviation Lower Bound	95% Confidence Interval		F	Adjusted P
						Upper Bound			
TCGA Cohort	Lymph Node status	Lymph node negative	320	5.729	2.284	5.478	5.980	2.101	0.197
		Lymph node positive	76	6.159	2.481	5.592	6.726		
		Total	396	5.812	2.326	5.582	6.041		
	Metastasis Status	No distant metastasis	455	5.669	2.282	5.459	5.879	0.336	0.643
		Distant metastasis present	3	6.435	1.699	2.215	10.655		
		Total	458	5.674	2.278	5.465	5.883		
	Overall stage (TNM)	Localised Disease	135	5.335	1.852	5.020	5.650	8.923	0.005
		Advanced Disease	258	6.068	2.515	5.759	6.376		
		Total	393	5.816	2.332	5.585	6.047		
DFKZ Cohort	Age at Diagnosis	32-36 years	1	4.301	-	-	-	0.228	0.877
		37-41 years	4	5.709	2.609	1.558	9.861		
		42-46 years	32	5.861	2.623	4.915	6.806		
		47 - 52 years	58	6.245	3.442	5.340	7.150		
		Total	95	6.073	3.121	5.437	6.708		
	Pathological Tumour Stage	pT2	62	5.539	2.298	4.955	6.122	4.534	0.045
		pT3 and pT4	31	6.872	3.721	5.507	8.237		
		Total	93	5.983	2.901	5.386	6.581		

Table 1. Clinicopathological features of KRAS expression in TGCA and DFKZ prostate cancer cohorts (one-way ANOVA) (KRAS Expression) (Continued).

Items	Clinicopathological Features		N	Mean	Std. Deviation Lower Bound	95% Confidence Interval		F	Adjusted P
						Upper Bound			
DFKZ Cohort	Gleason Score (primary pattern)	Gleason pattern 3	70	5.464	2.187	4.943	5.986	7.478	0.005
		Gleason pattern 4	18	8.455	4.826	6.055	10.854		
		Gleason pattern 5	7	6.029	3.205	3.066	8.993		
		Total	95	6.073	3.121	5.437	6.708		
	ISUP Grade Group	Groups I and II	70	5.464	2.187	4.943	5.986	11.196	0.003
		Groups III-V	25	7.776	4.505	5.916	9.635		
		Total	95	6.073	3.121	5.437	6.708		
	Pre-operative Prostate-Specific Antigen	Low PSA	47	5.325	2.925	4.466	6.183	5.115	0.044
		High PSA	46	6.656	2.747	5.840	7.472		
		Total	93	5.983	2.901	5.386	6.581		

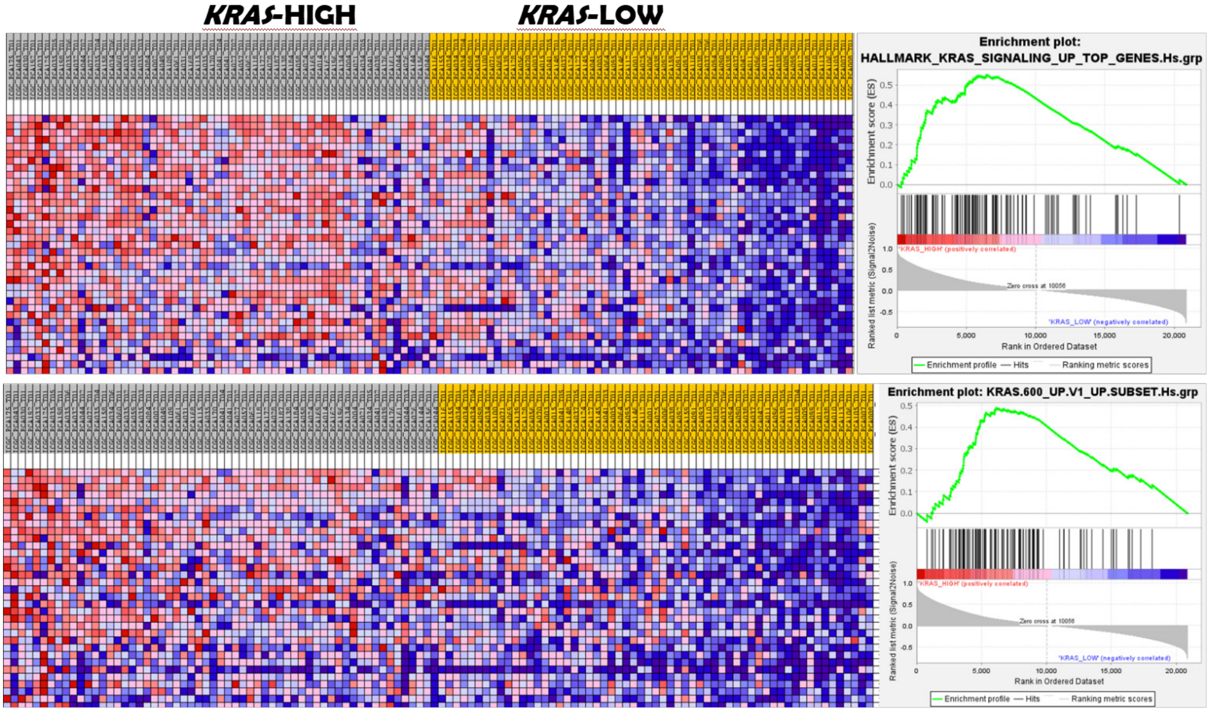

Figure 1. GSEA shows enrichment of *KRAS* signalling in *KRAS*-high prostate cancer subset. The upper panel displays enrichment of the MSigDB hallmark *KRAS* signalling pathway in the *KRAS*-high subset of the prostate cancer subsets. The lower panel shows enrichment of the signature of oncogenic *KRAS*-driven cancers. (GSEA for the DFKZ cohort shown here).

enrichment genes sets that were common to or shared by both PCa cohorts.

Gene Ontology (GO) Enrichment Analysis (https://www.geneontology.org/) [35, 36] was used to confirm the biological processes subserved by the genes in the common core enrichment genes sets. Furthermore, to confirm the biological relevancies of the GSEA results to the cohorts, we generated index scores for all the pathways by using the geometric means [38, 39] of the expression values of the enriched genes in the shared gene sets after filtering out the genes that are duplicated in the shared core enrichment gene sets (see **Supplementary Materials 1: Core Enrichment Genes**). The generated index scores were then validated by clinicopathological and prognostic features of the PCa cohorts. Direct correlation of *KRAS* expression with clinicopathological features such as age, race/ethnicity, pathological tumour, node and metastases statuses, overall tumour stage, Gleason score, prognostic grade groups, treatment outcomes, and follow-up (overall and disease-free survival) profiles was also sought in both the TCGA and DFKZ cohorts. Also, correlations between *KRAS* expression and the expression of androgen deprivation therapy (ADT)-resistance genes, *AR* (androgen receptor), *NR3C1* (glucocorticoid receptor, GCR), and *NR3C2* (mineralocorticoid receptor, MLR) [40-44] were sought in both cohorts. The mechanisms of altered *KRAS* expression – including copy number alterations, promoter methylation/altered transcription factor expression, and miRNA deregulation – were also investigated in the TCGA PCa cohort. For the miRNA deregulation analyses, differential enrichment of miRNA gene expression was sought in the TCGA cohort using DeSeq2 module in the GenePattern computing environment (https://cloud.genepattern.org/) [45]. A list of *KRAS*-relevant miRNA was retrieved from miRTarBase (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/) [46] and used to compare the results of the miRNA gene enrichment analysis. See **Supplementary Materials 2: MirTbase KRAS_targeting miRNAs**. Furthermore, transcription factors which have been experimentally demonstrated to bind to *KRAS* promoter regions were retrieved from TF2DNA_DB (https://www.fiserlab.org/tf2dna_db/search_genes.html) [47]; their expression values were incorporated into correlation and regression analyses to test their relationships with *KRAS* expression, and infer their roles in the deregulation of wild-type *KRAS* expression in PCa.

Statistical analyses

Gene set enrichment analyses were performed with permutations set to 1000, and by using gene-set permutation type, weighted enrichment statistics, and maximum and mini-mum gene-set sizes of 500 and 15, respectively. The nominal P value and false discovery rate (FDR) threshold were both set at 0.05 (or 5%). The clinicopathological and genomic data of interest were output in Excel spreadsheet from the Ubuntu environment and then input into SPSS version 29. Chi square (or Fisher) test was used to define associations between categorical variables, while bivariate correlative analysis was used to test the correlations between continuous variable. Independent Sample, Median k-sample and one-way ANOVA tests were used to measure the mean or median differences of continuous variables between discrete groups. Multiple linear regression analysis was used to predict the relationship between *KRAS* expression and the established mechanisms of altered gene expression (*KRAS* copy number alteration, *KRAS* promoter methylation, altered transcription factor expression and *KRAS*-specific miRNA expression patterns). Kaplan Meier and Cox regression analyses were used to define the prognostic significance of the generated tumour biology signatures and of *KRAS* expression. A P value of <0.05 was used as the threshold for significant test while the Benjamini-Hochberg correction was used to correct for multiple testing at an FDR of 0.05.

Results

Wild-type KRAS signalling is active in PCa

TCGA and the DFKZ cohorts were probed for *KRAS* activation using GSEA. The results showed that *KRAS* signalling was upregulated in the *KRAS*-high relative to *KRAS*-low subsets in both PCa cohorts (**Supplementary Materials 2: GSEA Results**). Furthermore, differential enrichment of the Barbie et al RAS activation gene set was observed in the *KRAS*-high subsets of both PCa cohorts (**Figure 1**), thereby validating the results obtained with the *KRAS* signalling pathway enrichment analysis.

Differential expression of tumour biology pathways in KRAS expression groups

Gene set enrichment analyses showed differential enrichment of tumour biology pathways between the *KRAS*-high and *KRAS*-low groups in both PCa cohorts. Differential enrichment was observed in all the Hallmark and KEGG gene sets that were interrogated (**Figure 2** and **Supplementary Materials 2: GSEA_ Results**), at nominal P value and false discovery rate (FDR) of less than 0.05. GO Enrichment Analysis confirmed that the core enrichment genes common to both PCa expression datasets included established members of the following pathways and biological functions: G2M transition genes (*CHEK1*, *RAD21*, *HUS1*, *BARD1*, *AURKA*, *FBXO5*, *CENPF*, *CDC7*, *CDKN3*, *STIL*, *CUL3*, *CUL4A*, *EZH2*, *PAFAH1B1*, *BRCA2*), regulation of cell cycles (*RACGAP1*, *STIL*, *TPX2*, *SMARCC1*, *ATRX*, *TTK*, *PRPF4B*, *BRCA2*, *EZH2*, *CDC27*, *KIF15*, *HNRNPU*, *AURKA*, *MAD2L1*, *SMC4*, *KIF11*, *FBXO5*, *CENPE*, *CHEK1*, *DBF4*, *PAFAH1B1*, *PLK4*, *BUB3*, *RAD21*, *BUB1*, *CENPF*, *SMC2*, *CUL3*, *DR1*, *CDC7*), regulation of cell population proliferation (*SMARCC1*, *TTK*, *BRCA2*, *EZH2*, *HNRNPU*, *PDS5B*, *FBXO5*, *CHEK1*, *HIF1A*, *E2F3*, *SQLE*, *CUL3*, *CDC7*, *CDKN3*, *NOLC1*, *SS18*, *KIF20B*, *CCNA2*, *CUL4A*), cell cycle and G1/S transition of mitotic cycle (*RB1*, *RBL1*, *CDK2*, *CDKN1B*, *CCNH*, *CDK1*, *CDK7*, *CDK6*, *TFDP1*, *CCNB1*), *TGFB* signalling genes (*BMPR2*, *MAP3K7*, *BMPR1A*, *TGFBR1*, *SMURF2*, *ACVR1*), PI3K signalling pathway (*PDK1*, *PTEN*, *GSK3B*), RAS and EGF signalling pathways (*PAK2*, *SOS2*, *MAP3K1*, *SOS1*, *MAPK14*, *MAPK1*, *CDC42*, *RPS6KA6*, *MAP2K1*, *NRAS*, *RPS6KA3*, *PAK1*, *ATF2*, *BRAF*, *MAPK9*, *RAF1*, *MAPK8*, *MAP2K6*, *MAP2K4*, *AKT3*), MAPK signalling (*RRAS2*, *SOS2*, *MAP3K1*, *MAP3K5*, *SOS1*, *NF1*, *MAPK14*, *MAPK1*, *NRAS*, *RASA1*, *RASA2*, *BRAF*, *MAPK9*, *MAP3K2*, *RAF1*, *MAPK8*, *MAP2K6*, *MAP2K4*) and MAPK pathway-interacting genes (*PAK2*, *CRK*, *CHUK*, *ATF2*, *TRAF6*, *PAK1*), Apoptosis signalling (*BCL10*, *CASP3*, *XIAP*, *FAS*, *CASP8*, *CASP2*, *CYLD*, *MCL1*, *BCL2L11*, *CASP7*, *DAP3*, *BIRC3*), and extracellular matrix pathways (*ITGAV*, *PFN2*, *PLOD2*, *ECM2*, *SGCB*, *TPM4*, *SPOCK1*, *GREM1*, *MATN3*, *TNFRSF11B*, *NT5E*, *ITGB1*, *PRRX1*, *CADM1*, *ITGA2*, *EDIL3*, *COL11A1*, *COL5A2*, *INHBA*, *VCAN*, *VEGFC*, *SNTB1*, *LAMC1*, *MFAP5*, *ADAM12*, *BASP1*, *TGFBR3*, *POSTN*, *DCN*, *SPP1*, *VCAM1*, *COL3A1*, *CTHRC1*, *LUM*, *SERPINE2*, *COL4A1*, *IGFBP3*) and EMT pathway genes (*CDH6*, *WNT5A*, *ID2*, *NOTCH2*) (**Supplementary Materials 2: GO_Analysis**).

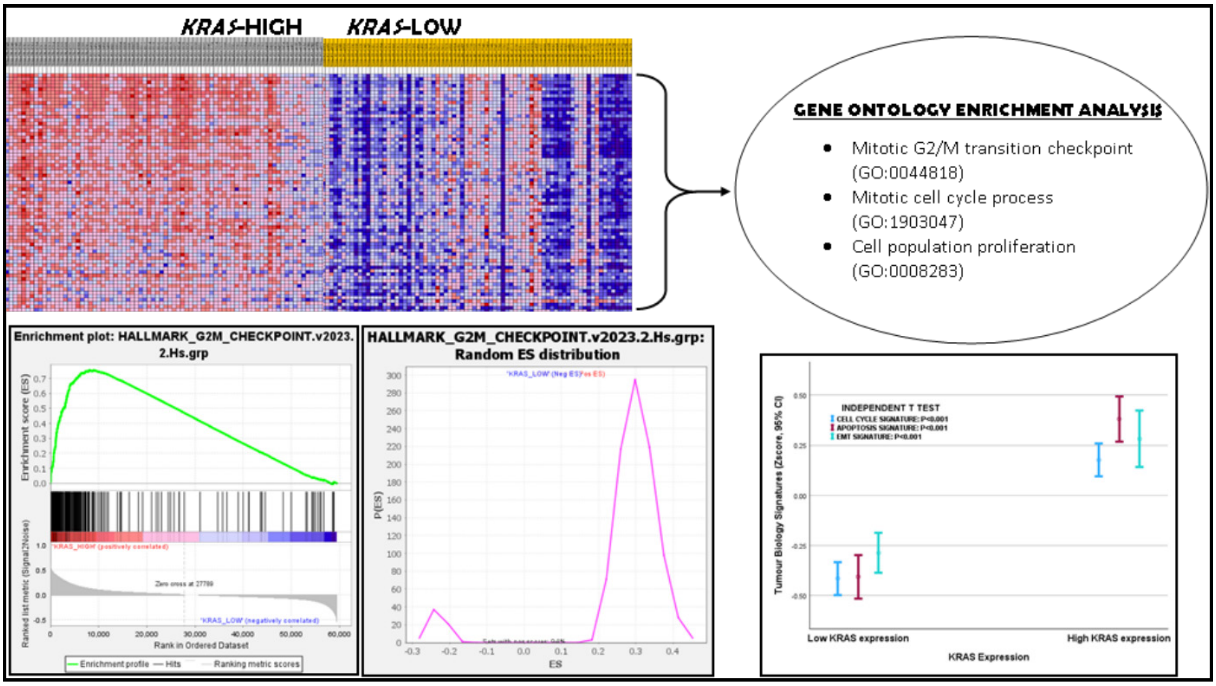

Figure 2. Gene Set Enrichment Analysis showed enrichment of G2M checkpoint gene set in *KRAS*-high prostate cancer. Gene Ontology Enrichment Analysis confirmed that G2M checkpoint, mitotic cell cycle process and cell population proliferation processes, among many others, were enriched in the *KRAS*-high subset of prostate cancer. The Error Bar graph shows that the Cell cycle, Apoptosis, Apoptosis and EMT/Invasion Signatures were relatively higher in the *KRAS*-high prostate cancer subset.

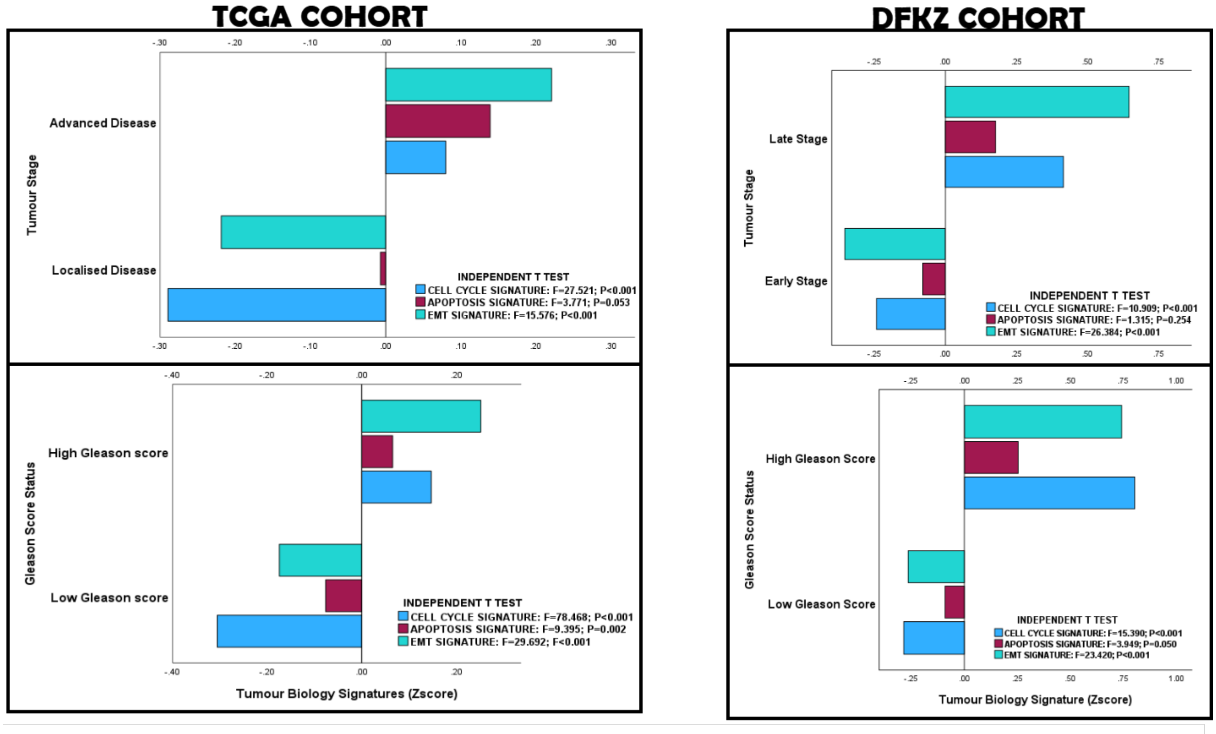

Figure 3. ***KRAS*-based Tumour Biology Signature Validation in the TCGA and DFKZ PCa cohorts. The correlation of the clinicopathological features of the prostate cancer cohort with tumour biology validates the activation of wild-type *KRAS* signalling in prostate cancer.**

Validation of GSEA results using tumour biology signatures and clinicopathological indices

The Cell Cycle, Apoptosis and EMT/invasion index scores for the PCa cohorts were generated by calculating the geometric means of the expression values of the core enrichment gene sets common to both PCa cohorts (Hallmark G2M_checkpoint, Apoptosis and EMT gene sets; *KRAS* expression was excluded in the generation of the tumour biology signatures). One-way ANOVA test demonstrated that the *KRAS* expression-based tumour biology signatures in the TCGA PCa cohort showed significant correlation with Gleason score (Cell Cycle, EMT/invasion and Apoptosis signatures), ISUP prognostic grade group (Cell Cycle and EMT/invasion signatures), pathological T stage (Cell Cycle, EMT/invasion and Apoptosis signatures), pathological metastasis stage (EMT/invasion signatures), TNM stage (Cell Cycle and EMT/invasion signatures), biochemical recurrence (Cell Cycle signature), and treatment outcomes (Cell Cycle, EMT/invasion and Apoptosis signatures). Cox regression analysis demonstrated that the tumour biological processes predicted disease-free survival (Cell Cycle and Apoptosis signatures), and 10 years overall survival (Cell Cycle and Apoptosis signatures) (see **Figure 3** and **Supplementary Materials 2: Signature_Validation.TCGA**). In the DFKZ cohort, significant correlation was found between the tumour biology signatures and pathological T stage (Cell Cycle, EMT/Invasion and Apoptosis signatures), Gleason score (Cell Cycle and EMT/Invasion signatures), ISUP Grade Group (Cell Cycle and EMT/Invasion signatures), and pre-operative prostate-Specific Antigen levels (Cell Cycle and EMT/Invasive signatures) (see **Figure 3** and **Supplementary Materials 2: Signature_ Validation.DFKZ**). This is evidence that the *KRAS* expression-based differential gene set enrichment observed with the GSEA have biological relevancies in both PCa cohorts. It also validated our hypothesis of an active wild-type KRAS signalling in PCa.

Clinicopathological correlates of wild-type KRAS expression

Having demonstrated wild-type *KRAS* activation in subsets of PCa expressing high *KRAS* mRNA, we sought to directly examine the clinicopathological significance of *KRAS* expression in PCa. The relationships between *KRAS* expression and clinicopathological indices such as age, race/ethnicity, pathological tumour, node and metastases statuses, overall tumour stage, Gleason score, prognostic grade groups, and follow-up (overall survival and disease-free) profiles were sought in the PCa cohorts. One-way ANOVA tests showed that *KRAS* expression showed significant relationships with race/ethnicity, pathological tumour stage, overall tumour stage, Gleason score, and Gleason prognostic grade groups (**Table 1**). However, no significant relationships were found for age, pathological node and metastasis statuses. Furthermore, no relationships were found between *KRAS* expression and disease-free or overall survival.

Therapy resistance correlates of wild-type KRAS expression

In both PCa cohorts a direct relationship was observed between *KRAS* signalling and ex-pression of AR, GCR and MLR. High levels of expression of these therapy resistance-associated genes were found in PCa cases with high *KRAS* expression compared to cases with low *KRAS* expression (**Figure 4**). The results suggested that *KRAS* signalling may play a role in determining therapeutic outcome through its interactions with these therapy resistance-associated genes. Next, we compared the primary therapeutic out-comes (TCGA cohort) and biochemical recurrences (TCGA

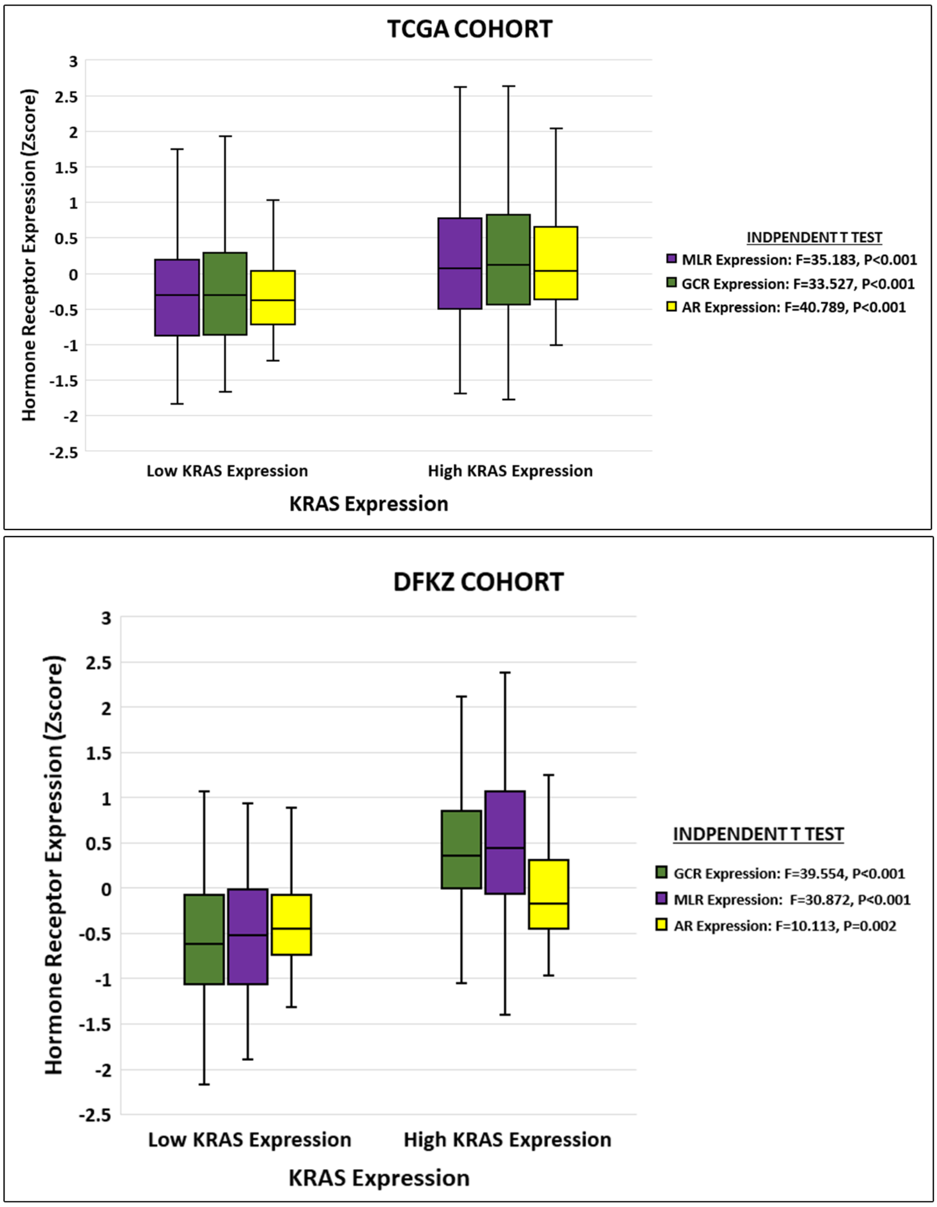

Figure 4. Relationships between *KRAS* expression and steroid hormone receptor expression in PCa. High expression of *KRAS* is associated with high expression of *AR*, GCR and MLR in TCGA and DFKZ cohorts.

and DFKZ cohorts) between cases with high and low *KRAS* expression. Chi square test showed that patients with high *KRAS* expression significantly exhibited less than complete outcomes compared to those with low *KRAS* expression in the TCGA cohort (**Figure 5A**). However, binary logistic regression analyses showed that the relationship between KRAS expression levels and therapeutic outcome was not independent of AR expression in the TCGA cohort (data not shown). Moreover, Kaplan-Meier test showed that no significant correlation exists be-tween time to biochemical recurrence and *KRAS* or *AR* expression levels, even though the pre-operative pSA levels were significantly higher in the DFKZ patients with high *KRAS* expression (**Figure 5B & 5C,** and **Table 1**).

Deregulation of KRAS expression in PCa

The TCGA PCa cohort was used to investigate the deregulation of *KRAS* expression in PCa as it has comprehensive data on mRNA expression, copy number segment, methylation and miRNA expression. *KRAS* copy number alteration (CNA) status was derived from the copy number segment data by using the segment mean thresholds of -0.3 and 0.3. Based on these thresholds there were 38/498, 456/498 and 6/498 losses/deletions, wild-types/neutrals and gains/amplifications, respectively. There was a significant *KRAS* copy number-expression correlation (one-way ANOVA test: F=25.181, degree of freedom=2, P<0.001, **Figure 6**). Furthermore, we investigated the role of *KRAS*-targeting transcription factors in the deregulation of *KRAS* expression in PCa using known *KRAS*-targeting transcription factors obtained via analysis from the transcription factor database TF2DNA_DB. Seven transcription factors, including *NKX3-1*, *HMGA1*, *NHLH1*, *SMAD2*, *MECP2*, *ZNF219*, and *ESRRB*, were identified from

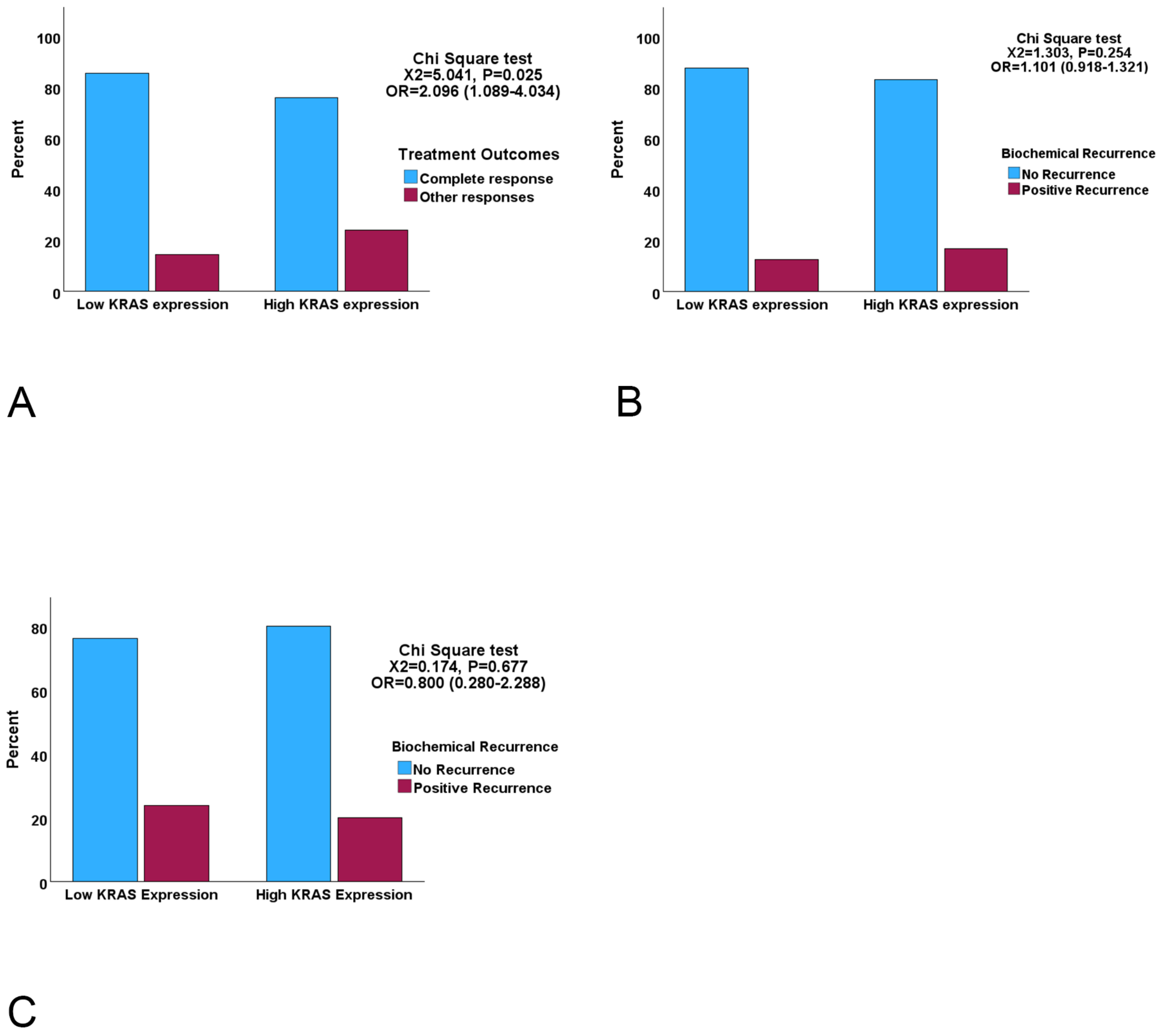

Figure 5. Clustered bar charts showing the relationships between *KRAS* expression and treatment outcome indices A. High *KRAS* expression cases are significantly more likely to have incomplete response to ADT than low *KRAS* cases (OR=Odds Ratio) B. & C. Biochemical recurrence status for TCGA (B) and DFKZ (C) cohorts showing that *KRAS* expression did not display significant associations with biochemical recurrence.

TF2DNA_DB; their expression levels were incorporated into a bivariate analysis which revealed that the expression of 4/7 of the transcription factors displayed correlations with *KRAS* expression (*MECP2*: R=0.290, P<0.001; *NKX3-1*: R=0.325, P<0.001; *SMAD2*: R=0.395, P<0.001; *ZNF219*: R= -0.224, P<0.001). Differential Expression Analysis using DeSeq2 identified the top 40 differentially expressed miRNAs between the *KRAS*-high and *KRAS*-low PCa subsets. Bivariate correlation analysis demonstrated that 28/40 of the miRNA exhibited direct and indirect correlations with *KRAS* expression (see **Supplementary Materials 2: MicroRNA Enrichment Analyses and MicroRNA-KRAS Correlation**). However, only four of the miRNAs matched the list of *KRAS*-relevant miRNAs retrieved from the miRTarBase database (see **Supplementary Materials 2: MirTbase KRAS_targeting miRNAs**). Furthermore, bivariate correlation analysis showed that 7/28 *KRAS* methylation loci, cg06891455, cg10569807, cg13085893, cg17197538, cg25763538, cg27174311, cg27550152, correlated with *KRAS* expression (see **Supplementary Materials 2: KRAS Methylation-Expression**).

The *KRAS* copy number data, beta and expression values, respectively, of the positive *KRAS* methylation loci and the identified transcription factors and miRNAs from the bi-variate analyses above were incorporated into a multiple linear regression to test whether they independently predict *KRAS* expression. The analysis identified *MIR30C-1* (P=0.003), *KRAS* Copy Number Alterations (P<0.001), *MIR4664* (P<0.001), *MIRLET7A3* (P=0.040), *MIR5001* (P=0.027), *MIR1224* (P=0.024), *SMAD2* (P<0.001), *NKX3-1* (P<0.001), *MECP2* (P=0.001), *ZNF219*

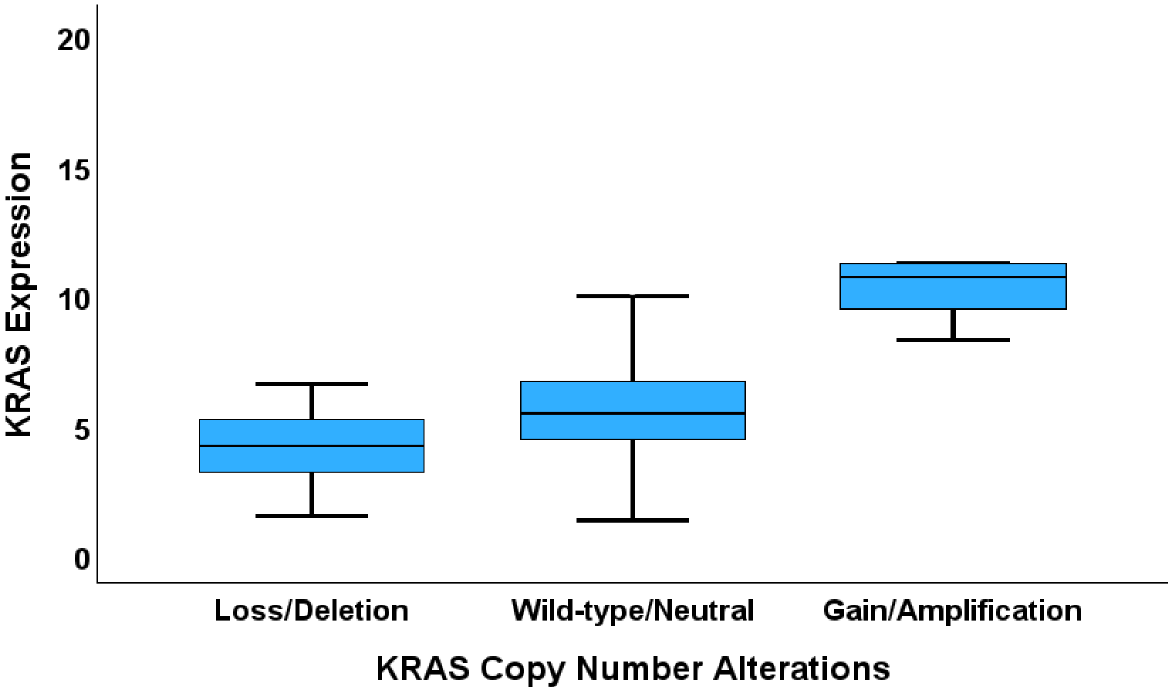

Figure 6. A Box plot showing correlation between *KRAS* CNA and expression.

(P<0.001) as independent predictors of *KRAS* expression in the regression model (F=26.66, R2=0.383, P<0.001). The methylation loci were also significant predictors of *KRAS* expression in the regression analysis (cg17197538, P=0.041 and cg25763538, P=0.002), but in the absence of the transcription factors. This is evidence that the transcription factors exhibited collinearity with methylation loci, and thus may bind at those specific loci. Overall, the results showed that *KRAS* expression in PCa is deregulated by copy number changes, miRNA and epigenetic/altered transcription factor-expression mechanisms.

Discussion

One of the motivations for this study is the paucity of research that have utilized primary cohort to verify whether wild-type *KRAS* signalling is active in clinical PCa cases, as it is in PCa cell lines [15-23, 28, 48]. Researching clinical PCa for *KRAS* activity is pertinent because cancer cell line biology, for the reasons mentioned in the introduction, do not always recapitulate the biology of clinical cancers [24-27]. This study demonstrated, using GSEA, that the *KRAS* expression status has important biological implications for clinical PCa. This study analysed two prostate cancer (PCa) cohorts using a gene set associated with RAS activation identified in a previous study [37]. Surprisingly, it revealed that RAS activation occurs in clinical PCa even without *KRAS* mutations. Moreover, gene sets enriched for genes related to *KRAS* expression showed associations with clinicopathological features in both cohorts, suggesting their potential relevance to PCa tumour biology. The existence of active wild-type *KRAS* signalling in clinical PCa has far-reaching implications for PCa biology, clinicopathological presentations and drug response profile.

This study validated previous cell line studies in two ways. First, the enrichment of the *KRAS* activation gene sets in the PCa cohorts confirmed that *KRAS* is activable in cancer in the absence of *KRAS* somatic mutations. This is in consonance with the study by Mita et al, which found that knockdown of *KRAS* in gastric cell lines which overexpressed wild-type *KRAS* resulted in inhibition of tumour growth and suppression of p44/42 MAP kinase (*MAPK1* or *ERK2*) and *AKT* activity [12]. It also concurs with the Valtorta et al study which demonstrated resistance of anti-EGFR therapy in a colorectal cancer cell line with *KRAS* overexpression (via gene amplification) [13]. Furthermore, the Laboda et al study demonstrated wild-type *KRAS* activation in breast cancer (which have low frequency of somatic *KRAS* mutations), and in lung and colorectal cancer subsets with wild-type *KRAS* expression, providing evidence that even in cancer types with high rates of somatic *KRAS* mutations, wild-type *KRAS* signalling can be active [14]. Secondly, this study confirmed the results of cell line studies which have demonstrated active wild-type *KRAS* signalling in PCa cell lines, and upregulation of *KRAS* expression in primary PCa [15-23, 28, 48-51]. According to these studies, *KRAS* expression regulates the fundamental tumour biology of PCa, including cell proliferation, EMT, metastasis and stem cell activities [12, 18-23, 49, 50], and in cooperation with other signalling pathways including PI3K, WNT, β-catenin, and hedgehog signalling [16, 18, 20, 21, 48, 51, 52]. In consonance with these previous results, this study demonstrated enrichment of cell cycle and G1/S transition of mitotic cycle, G2/M transition, regulation of cell population proliferation, regulation of cell cycles, regulation of cell population proliferation, RAS and *EGF* signalling pathways, *MAPK* signalling, *TGFB* signalling, PI3K signalling, extracellular matrix pathways, and EMT pathway genes in the *KRAS*-high subset of both PCa cohorts.

In keeping with the patterns of enrichment of tumour-promoting gene sets in the PCa cohorts, the study found adverse clinicopathological features of the PCa in the *KRAS*-high subsets. For example, the *KRAS*-high subsets displayed higher stage, Gleason score, ISUP prognostic grade groups and poorer therapy outcomes. There is paucity of studies which have investigated the clinicopathological features of *KRAS* in PCa, hence only a small frame of reference for the clinicopathological relevance of *KRAS* expression in PCa exists for comparison. However, Yin et al [19] and Yang et al [50] showed that high *KRAS* expression is associated with bone metastasis. Plus, cell line studies have demonstrated some reverse correlation between *KRAS* expression and response to therapy. Activation of the *KRAS* signalling is associated with resistance to anti-androgen and other therapies in PCa sell lines [16, 17, 51].

Further on therapy resistance, this study demonstrated a significant relationship between *KRAS* expression and the therapy-resistance markers *AR*, GCR and MLR [40-44] in both PCa cohorts. However, no relationship could be established between treatment outcomes and *KRAS* expression independent of *AR* expression, a result that suggested that the relationship between *KRAS* expression and clinical response to ADT may be AR-dependent, at least in the TCGA PCa cohort. Barkin et al. [13] demonstrated that attenuation of RAS signalling restored sensitivity to hormone-refractory PCa cell lines. Also, an *AR-KRAS* axis signalling, which mediates malignant transformation and induction of stem cells characteristics, has been described for PCa cells [52, 53]. The role of cancer stem cells in therapy resistance is established in oncology [54]. It is therefore plausible that *KRAS* signalling acts upstream of AR signalling in the development of resistance to ADT, hence the relationship between *KRAS* expression and therapy outcome would be dependent on AR expression. Whilst this study did not find an independent link between *KRAS* expression and treatment outcome, the fact of high *KRAS* expression being associated with enrichment of PI3K signalling in this and other studies [13, 18, 19, 20, 48, 51] suggests an indirect association of *KRAS* expression with treatment response. The prospect of targeting *KRAS* for the circumvention of ADT resistance is an attractive one [13, 55]. Considering that tumours can have RAS activation independent of *KRAS* somatic mutations [12, 13], it is plausible that some therapies which are designed to target *KRAS* mutation-driven tumours would also find application in treating wild-type *KRAS*-driven tumours. *KRAS*- and *KRAS* signalling-specific drugs, whose targets reside outside the *KRAS* mutations hotspots, such as those that target *KRAS* membrane association [40], inhibit *KRAS* processing and activation [55-57], target downstream mediators of intracellular signalling [56], and target *KRAS* synthetic lethal partnerships [56, 58], may find utility in the treatment of PCa cases with high *KRAS* expression.

The study also showed that *KRAS* deregulation may be predominantly transcriptional and/or translational via epigenetic and miRNA mechanisms. This finding is supported by a previous preclinical study which demonstrated transcriptional deregulation of *KRAS* expression at the promoter site [50]. Copy number alteration of *KRAS* has been demonstrated to be a mechanism of *KRAS* deregulation in gastric, lung and colorectal cancers [12, 13]. Therefore, testing *KRAS* promoter methylation, *KRAS*-targeting microRNA expression or *KRAS* copy number changes may be useful in the diagnosis of active *KRAS* signalling in PCa. Furthermore, whilst structural variants such as *KRAS* fusions [59] may lead to the upregulation of *KRAS* expression in PCa, as would *KRAS* gain or amplification, no gene fusion data were available for the TCGA or DFKZ PCa datasets utilized in this study. Hence, the level of contribution of *KRAS* gene fusions to *KRAS* expression levels in primary PCa could not be determined in this study.

Conclusions

In conclusion, this study has demonstrated evidences of active wild-type *KRAS* signalling in clinical PCa, and the associations of high *KRAS* expression with tumour biology and the adverse clinicopathological features of PCa. The study has also shown that *KRAS* expression relationship with therapy response may be dependent on AR signalling. The results from this study lend credence to the findings from previous preclinical and translational researches on *KRAS* in PCa. Furthermore, we propose that high *KRAS* expression impacts PCa clinicopathological presentations, and that targeting wild-type *KRAS* or its activation mechanisms may be a suitable therapeutic strategy for circumventing drug resistance in clinical PCa. The proposed relationships between *KRAS* expression and therapy resistance in clinical PCa need further validation.

Supplementary materials

Supplementary materials 1

Supplementary materials 2

Ethical policy

Not applicable.

Author contribution

Conception or design of the work was done by H.O.E.; Data collection was performed by H.O.E. and P.A.A.; Data analysis and interpretation were done by S.A.O., U.S.E., P.A.A., A.E.A., O.I., C.A.E., J.C.A., N.C., A.O.A, C.U.A., M.I.M., I.D.N.; Drafting the article was undertaken by H.O.E., S.A.O.; Critical revision of the article performed by U.S.E., P.A.A., A.E.A., O.I., C.A.E., J.C.A., N.C., A.O.A, C.U.A., M.I.M., and I.D.N. Final approval of submitted manuscript (All the authors).

Competing interest

All the authors declare no conflict of interest.

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References

1. Vlajnic T, Bubendorf L: Molecular pathology of prostate cancer: a practical approach. Pathology 2021, 53(1): 36-43.
2. Faisal FA, Lotan TL: The Genomic and Molecular Pathology of Prostate Cancer: Clinical Implications for Diagnosis, Prognosis, and Therapy. Adv Anat Pathol 2020, 27(1): 11-19.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021, 71(3): 209-249.
4. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F: Cancer statistics for the year 2020: An overview. Int J Cancer 2021, 149: 778-789.
5. O'Sullivan É, Keogh A, Henderson B, Finn SP, Gray SG, Gately K: Treatment Strategies for KRAS-Mutated Non-Small-Cell Lung Cancer. Cancers 2023, 15(6): 1635.
6. Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au

HJ, Berry SR, Krahn M, Price T, Simes RJ, et al: Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007, 357(20): 2040-2048.

7. Janani B, Vijayakumar M, Priya K, Kim JH, Prabakaran DS, Shahid M, Al-Ghamdi S, Alsaidan M, Othman Bahakim N, Hassan Abdelzaher M, et al: EGFR-Based Targeted Therapy for Colorectal Cancer-Promises and Challenges. Vaccines (Basel) 2022, 10(4): 499.
8. Maron SB, Xu J, Janjigian YY: Targeting EGFR in Esophagogastric Cancer. Front Oncol 2020, 10: 553876.
9. Mustachio LM, Chelariu-Raicu A, Szekvolgyi L, Roszik J: Targeting KRAS in Cancer: Promising Therapeutic Strategies. Cancers 2021, 13: 1204.
10. Simanshu DK, Nissley DV, McCormick F: RAS Proteins and Their Regulators in Human Disease. Cell 2017, 170(1): 17-33.
11. Saito Y, Koya J, Araki M, Kogure Y, Shingaki S, Tabata M, McClure MB, Yoshifuji K, Matsumoto S, Isaka Y, et al: Landscape and function of multiple mutations within individual oncogenes. Nat Cell Biol 2020, 582(7810): 95-99.
12. Mita H, Toyota M, Aoki F, Akashi H, Maruyama R, Sasaki Y, Suzuki H, Idogawa M, Kashima L, Yanagihara K, et al: A novel method, digital genome scanning detects KRAS gene amplification in gastric cancers: involvement of overexpressed wild-type KRAS in downstream signaling and cancer cell growth. BMC Cancer 2009, 9: 198.
13. Valtorta E, Misale S, Sartore-Bianchi A, Nagtegaal ID, Paraf F, Lauricella C, Dimartino V, Hobor S, Jacobs B, Ercolani C, et al: KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. Int J Cancer 2013, 133(5): 1259-1265.
14. Loboda A, Nebozhyn M, Klinghoffer R, Frazier J, Chastain M, Arthur W, Roberts B, Zhang T, Chenard M, Haines B, et al: A gene expression signature of RAS pathway dependence predicts response to PI3K and RAS pathway inhibitors and expands the population of RAS pathway activated tumors. BMC Med Genomics 2010, 3: 26.
15. Butler DE, Marlein C, Walker HF, Frame FM, Mann VM, Simms MS, Davies BR, Collins AT, Maitland NJ: Inhibition of the PI3K/AKT/mTOR pathway activates autophagy and compensatory Ras/Raf/MEK/ERK signalling in prostate cancer. Oncotarget 2017, 8(34): 56698-56713.
16. Barkin RE, Gioeli D, Bissonette EA, Weber MJ: Attenuation of Ras Signalling Restores Androgen Sensitivity to Hormone-refractory C4-2 Prostate Cancer Cells. Cancer Res 2003, 63(8): 1975-1980.
17. Erlich S, Tal-Or P, Liebling R, Blum R, Karunagaran D, Kloog Y, Pinkas-Kramarski R: Ras inhibition results in growth arrest and death of androgen-dependent and androgen-independent prostate cancer cells. Biochem Pharmacol 2006, 72(4): 427-436.
18. Suzuki A, Nakano T, Mak TW, Sasaki T: Portrait of PTEN: Messages from mutant mice. Cancer Science 2008, 99: 209-213.
19. Yin J, Pollock C, Tracy K, Chock M, Martin P, Oberst M, Kelly K: Activation of the RalGEF/Ral pathway promotes prostate cancer metastasis to bone. Mol Cell Biol 2007, 27(21): 7538-7550.
20. Mulholland DJ, Kobayashi N, Ruscetti M, Zhi A, Tran LM, Huang J, Gleave M, Wu H: Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells. Cancer Res 2012, 72(7): 878-1889.
21. Jefferies MT, Cox AC, Shorning BY, Meniel V, Griffiths D, Kynaston HG, Smalley MJ, Clarke AR: PTEN loss and activation of K-RAS and β-catenin cooperate to accelerate prostate tumourigenesis. J Pathol 2017, 243: 442-456.
22. Rybak AP, Bristow RG, Kapoor A: Prostate cancer stem cells: deciphering the origins and pathways involved in prostate tumorigenesis and aggression. Oncotarget 2015, 6(4): 1900-1919.
23. Jeong JH, Wang Z, Guimaraes AS, Ouyang X, Figueiredo JL, Ding Z, Jiang S, Guney I, Kang GH, Shin E: BRAF activation initiates but does not maintain invasive prostate adenocarcinoma. PLoS One 2008, 3(12): e3949.
24. Noorbakhsh J, Vazquez F, McFarland JM: Bridging the gap between cancer cell line models and tumours using gene expression data. Br J Cancer 2021, 125(3): 311-312.
25. Wilding JL, Bodmer WF: Cancer Cell Lines for Drug Discovery and Development. Cancer Res 2014, 74 (9): 2377-2384.
26. Roschke AV, Tonon G, Gehlhaus KS, McTyre N, Bussey KJ, Lababidi S, Scudiero DA, Weinstein JN, Kirsch IR: Karyotypic complexity of the NCI-60 drug-screening panel. Cancer Res 2003, 63: 8634-8647.
27. Daniel VC, Marchionni L, Hierman JS, Rhodes JT, Devereux WL, Rudin CM, Yung R, Parmigiani G, Dorsch M, Peacock CD, et al: A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. Cancer Res 2009, 69(8): 3364-3373
28. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, et al: Integrative genomic profiling of human prostate cancer. Cancer Cell 2010, 18(1): 11-22.
29. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, et al: The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012, 2(5): 401-404.
30. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, et al: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013, 6(269): pl1.
31. Gerhauser C, Favero F, Risch T, Simon R, Feuerbach L, Assenov Y, Heckmann D, Sidiropoulos N, Waszak SM, Hübschmann D, et al: Molecular Evolution of Early-Onset Prostate Cancer Identifies Molecular Risk Markers and Clinical Trajectories. Cancer Cell 2018, 34(6): 996-1011.e8.
32. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, et al: Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. PNAS USA 2005, 102(43): 15545-15550.
33. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP: Molecular signatures database (MSigDB) 3.0. Bioinformatics 2011, 27(12): 1739-1740.
34. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P: The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst 2015, 1(6): 417-425.
35. Gene Ontology Consortium; Aleksander SA, Balhoff J, Carbon S, Cherry JM, Drabkin HJ, Ebert D, Feuermann M, Gaudet P, Harris NL, et al: The Gene Ontology knowledgebase in 2023. Genetics 2023, 224(1): iyad031.
36. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al: Gene Ontology: tool for the unification of biology. Nat Genet 2000, 25(1): 25-29.
37. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C, et al: Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature 2009, 462(7269): 108-112.
38. Vallania F, Zisman L, Macaubas C, Hung SC, Rajasekaran N, Mason S, Graf J, Nakamura M, Mellins ED, Khatri P: Multicohort Analysis Identifies Monocyte Gene Signatures to Accurately Monitor Subset-Specific Changes in Human Diseases. Front Immunol 2021, 12: 659255.
39. Feys S, Heylen J, Carvalho A, Van Weyenbergh J, Wauters J, Variomic Study Group: A signature of differential gene expression in bronchoalveolar lavage fluid predicts mortality in influenza-associated pulmonary aspergillosis. Intensive Care Med 2023, 49(2): 254-257.
40. Yehya A, Ghamlouche F, Zahwe A, Zeid Y, Wakimian K, Mukherji D, Abou-Kheir W: Drug resistance in metastatic castration-resistant prostate cancer: an update on the status quo. Cancer Drug Resist

2022, 5(3): 667-690.
41. Kushwaha PP, Gupta S: New insights for drug resistance in metastatic castration-resistant prostate cancer. Cancer Drug Resist 2022, 5(3): 846-849.
42. Kumar R: Emerging role of glucocorticoid receptor in castration resistant prostate cancer: A potential therapeutic target. J Cancer 2020, 11(3): 696-701.
43. Kaochar S, Mitsiades N: Glucocorticoids mediate adverse events of deep androgen receptor axis inhibition in prostate cancer patients. Ann Oncol 2020, 31(3): 323-325.
44. Shiota M, Fujimoto N, Higashijima K, Imada K, Kashiwagi E, Takeuchi A, Inokuchi J, Tatsugami K, Kajioka S, Uchiumi T, et al: Mineralocorticoid receptor signalling affects therapeutic effect of enzalutamide. Prostate 2018, 78: 1045-1052.
45. Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP: GenePattern 2.0. Nat Genet 2006, 38(5): 500-501.
46. Huang HY, Lin YC, Cui S, Huang Y, Tang Y, Xu J, Bao J, Li Y, Wen J, Zuo H, et al: miRTarBase update 2022: an informative resource for experimentally validated miRNA-target interactions. Nucleic Acids Res 2022, 50(D1): D222-D230.
47. Pujato M, Kieken F, Skiles AA, Tapinos N, Fiser A: Prediction of DNA binding motifs from 3D models of transcription factors; identifying TLX3 regulated genes. Nucleic Acids Res 2014, 42(22): 13500-13512.
48. Shorning BY, Dass MS, Smalley MJ, Pearson HB: The PI3K-AKT-mTOR Pathway and Prostate Cancer: At the Crossroads of AR, MAPK, and WNT Signaling. Int J Mol Sci 2020, 21(12): 4507.
49. Ngalame NN, Tokar EJ, Person RJ, Waalkes MP: Silencing KRAS overexpression in arsenic-transformed prostate epithelial and stem cells partially mitigates malignant phenotype. Toxicol Sci 2014, 142(2): 489-496.
50. Yang Q, Lang C, Wu Z, Dai Y, He S, Guo W, Huang S, Du H, Ren D, Peng X: MAZ promotes prostate cancer bone metastasis through transcriptionally activating the KRas-dependent Ral-GEFs pathway. J Exp Clin Cancer Res 2019, 38(1): 391.
51. Bouali S, Chrétien A, Ramacci C, Rouyer M, Becuwe P, Merlin J: PTEN expression controls cellular response to cetuximab by mediating PI3K/AKT and RAS/RAF/MAPK downstream signaling in KRAS wild-type, hormone refractory prostate cancer cells. Oncol Rep 2009, 21(3): 731-735.
52. Wu M, Ingram L, Tolosa EJ, Vera RE, Li Q, Kim S, Ma Y, Spyropoulos DD, Beharry Z, Huang J, et al: Gli Transcription Factors Mediate the Oncogenic Transformation of Prostate Basal Cells Induced by a Kras-Androgen Receptor Axis. J Biol Chem 2016, 291(49): 25749-25760.
53. Cai H, Memarzadeh S, Stoyanova T, Beharry Z, Kraft AS, Witte ON: Collaboration of Kras and androgen receptor signaling stimulates EZH2 expression and tumor-propagating cells in prostate cancer. Cancer Res 2012, 72(18): 4672-4681.
54. Kushwaha PP, Verma S, Kumar S, Gupta S: Role of prostate cancer stem-like cells in the development of antiandrogen resistance. Cancer Drug Resist 2022, 5(2): 459-471.
55. Hillig RC, Sautier B, Schroeder J, Moosmayer D, Hilpmann A, Stegmann CM, Werbeck ND, Briem H, Boemer U, Weiske J, et al: Discovery of potent SOS1 inhibitors that block RAS activation via disruption of the RAS-SOS1 interaction. PNAS USA 2019, 116(7): 2551-2560.
56. Merz V, Gaule M, Zecchetto C, Cavaliere A, Casalino S, Pesoni C, Contarelli S, Sabbadini F, Bertolini M, Mangiameli D, et al: Targeting KRAS: The Elephant in the Room of Epithelial Cancers. Front Oncol 2021, 11: 638360.
57. Wong GS, Zhou J, Liu JB, Wu Z, Xu X, Li T, Xu D, Schumacher SE, Puschhof J, McFarland J, et al: Targeting wild-type KRAS-amplified gastroesophageal cancer through combined MEK and SHP2 inhibition. Nat Med 2018, 24(7): 968-977.
58. Liu P, Wang Y, Li X: Targeting the untargetable KRAS in cancer therapy. Acta Pharm Sin B 2019, 9(5): 871-879.
59. Wang XS, Shankar S, Dhanasekaran SM, Ateeq B, Sasaki AT, Jing X, Robinson D, Cao Q, Prensner JR, Yocum AK, et al: Characterization of KRAS rearrangements in metastatic prostate cancer. Cancer Discov 2011, 1(1): 35-43.