BRF1 accelerates prostate tumourigenesis and perturbs immune infiltration

Carolyn J. Loveridge1,2 · Sarah Slater2 · Kirsteen J. Campbell1,2 · Noor A. Nam2,5 · John Knight2 · Imran Ahmad1,2 · Ann Hedley2 · Sergio Lilla2 · Peter Repisky2 · Rachana Patel2 · Mark Salji1,2 · Janis Fleming2 · Louise Mitchell2 · Colin Nixon2 · Douglas Stratdhee2 · Matthew Neilson2 · Chara Ntala1,2 · Sheila Bryson2 · Sara Zanivan8 · John Knight2 · Imran Ahmad1,2 · Ann Hedley2 · Sergio Lilla2 · Peter Repisky2 · Rachana Patel2 · Mark Salji1,2 · Janis Fleming2 · Louise Mitchell2 · Colin Nixon2 · Douglas Stratdhee2 · Matthew Neilson2 · Chara Ntala1,2 · Sheila Bryson2 · Sara Zanivan8 · Joanne Edwards8 · Craig N. Robson3 · Carl S. Goodyear4 · Karen Blyth2 · Hing Y. Leung1,2

Received: 21 June 2019 / Revised: 22 October 2019 / Accepted: 6 November 2019 / Published online: 18 November 2019 © The Author(s) 2019. This article is published with open access

Abstract

BRF1 is a rate-limiting factor for RNA Polymerase III-mediated transcription and is elevated in numerous cancers. Here, we report that elevated levels of BRF1 associate with poor prognosis in human prostate cancer. In vitro studies in human prostate cancer cell lines demonstrated that transient overexpression of BRF1 increased cell proliferation whereas the transient downregulation of BRF1 reduced proliferation and mediated cell cycle arrest. Consistent with our clinical observations, BRF1 overexpression in a Pten-deficient mouse (PtenΔ/Δ BRF1Tg) prostate cancer model accelerated prostate carcinogenesis and shortened survival. In PtenΔ/Δ BRF1Tg tumours, immune and inflammatory processes were altered, with reduced tumoral infiltration of neutrophils and CD4 positive T cells, which can be explained by decreased levels of complement factor D (CFD) and C7 components of the complement cascade, an innate immune pathway that influences the adaptive immune response. We tested if the secretome was involved in BRF1-driven tumorigenesis. Unbiased proteomic analysis on BRF1-overexpressing PC3 cells confirmed reduced levels of CFD in the secretome, implicating the complement system in prostate carcinogenesis. We further identify that expression of C7 significantly correlates with expression of CD4 and has the potential to alter clinical outcome in human prostate cancer, where low levels of C7 associate with poorer prognosis.

Introduction

The BRF1 transcription factor functions together with TATA-binding protein and B double-prime 1 in a complex called TFIIB. BRF1 is required to recruit RNA polymerase III (Pol III) to target genes and is rate-limiting for Pol III-mediated transcription [1]. Increased protein synthesis plays a key role in oncogenesis and Pol III products, including transfer RNAs (tRNAs) and other short non-coding RNAs, such as the ribosomal component 5S RNA, are essential for protein synthesis. Major components of the ribosome (ribosomal proteins e.g. RPS-19, -21, -24 and ribosomal RNA) are upregulated in prostate cancer (PCa) [2, 3]. BRF1 expression is elevated and associated with poor prognosis in hepatocellular, breast and gastric cancers [4–6] but its role in PCa remains unclear.

PCa is the third most common cause of cancer-associated death in men worldwide and progression is unpredictable [7]. Patients with similar tumour grade and histology can quickly progress to incurable, advanced metastatic disease
or alternatively they can survive for decades with local, indolent, disease [8]. Evasion of surveillance and recognition by the innate and adaptive immune system are implicated in PCa progression [9] and it has been proposed that oncogenic drivers can contribute to immunoresistance in PCa [10]. There is an unmet need to better understand
BRF1 is a prognostic marker in prostate cancer and mediates effects on cellular proliferation and the cell cycle in vitro. A example images of PCa cores stained for BRF1 with varying Histoscores: negative (score 0), low (score 125), intermediate (score 175) or high (score 300). Higher magnification images show finer detail of staining from the same cores. Scale bars are shown (100 µm for lower magnification images; 10 µm for higher magnification images). B Kaplan–Meier plot for disease-specific survival of PCa patients stratified according to low (below median histoscore; n = 128) versus high (above median histoscore; n = 137) expression of BRF1 within PCa cohort. C Kaplan–Meier plot for progression-free survival of patients in MSKCC (2010) dataset segregated for low and high BRF1 expression as indicated in oncoprint in Fig. S1c. D Kaplan–Meier plot for progression-free survival of patients in TCGA (provisional) dataset segregated for low and high BRF1 expression as indicated in oncoprint in Fig. S1d. Log-rank (Mantel–Cox) Test was performed to compare survival curves; *p < 0.05. E Forest plot summarising WST1 cell proliferation assay results from PC3, PC3M and DU145 (all n = 3) cells which were transiently transfected with HA-BRF1, GFP-BRF1 and their respective HA- and GFP-empty vector controls for 48 h. F Forest plot summarising WST1 cell proliferation assay results from PC3 (n = 4), PC3M, DU145, LNCaP and LNCaP Al (all n = 3) cells which were transiently transfected with three independent siRNAs for BRF1 and control non-targeting (NT) siRNA for 48 h. In presented Forest plots in e and f, each box represents the sample mean [relative to empty vector (e) or control NT siRNA (f)]; error bars represent 95% confidence intervals; the centre of the diamond in summary line represents the collective sample mean; the width of the diamond represents the 95% confidence interval for the collective sample mean. PC3 (g) and PC3M (h) cells were transiently transfected with two independent siRNAs for BRF1 and two different control NT siRNAs for 72 h then subjected to bromodeoxyuridine (BrdU)- and propidium iodide (PI)-labelling followed by flow cytometry analysis to determine cell cycle positions. Data presented are from n = 3 (PC3 cells; NT siRNA 2; BRF1 siRNA’s 2 and 3), n = 4 (PC3 cells; NT siRNA 3; PC3M cells; NT siRNA 3) or n = 5 (PC3M cells: NT siRNA 2; BRF1 siRNA’s 2 and 3) experiments. Individual data points are shown in the presented graphs; long horizontal lines indicate the Mean; error bars represent SEM; 2way ANOVA was used to calculate p values; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 from NT siRNA 2 or 3 or eGFP-BRF1 plasmids, mediated a modest but significant increase in cell proliferation in PC3, PC3M and DU145 cells by WST1 assay (Figs. 1e, S2a and S3a; Table S6). Conversely, downregulation of BRF1 expression in PCa cells (with at least two out of three independent siRNAs) reduced proliferation (Figs. 1f, S2b and S3b; Table S7), with FACS analysis revealing reduced G1 subpopulation and increased G2 phase (Fig. 1g, h). Collectively, manipulation of BRF1 expression alters proliferation in vitro.

Increased BRF1 accelerates prostate tumourigenesis in vivo

An in vivo model with prostate epithelium-specific overexpression of BRF1 was generated by inter-crossing BRF1 mice [18] with the Pbsn (PB)-Cre4 strain [19] to generate PB-Cre4:BRF1 (herein referred to as BRF1Tg) mice (see Supplementary methods; Fig. 2a). Male BRF1Tg mice were aged to over 1 year, and no malignancy or alteration in prostate architecture was observed (n = 12 WT; n = 12 BRF1Tg) (Fig. 2b). We then crossed BRF1Tg with Ptenfl/fl [20] mice to generate prostate epithelium-specific double mutant PB-Cre4:Ptenfl/fl BRF1 (herein referred to as PtenΔ/Δ.
Survival of PB-Cre4:Ptentg (herein referred to as PtenΔ/Δ) control mice was consistent with our previous report [21]. PtenΔ/Δ BRF1tg mice had significantly shorter disease-specific survival compared with PtenΔ/Δ siblings (median 256 vs 316 days, respectively; p < 0.0001) (Fig. 2c, Table S8), with comparable endpoint tumour weights (Fig. 2d, Table S9) despite being harvested earlier. Interestingly, whilst high BRF1 protein levels were observed in all PtenΔ/Δ
BRF1Tg prostate tumours harbour alterations in immune system processes

RNA-Seqencing (RNA-Seq) identified 522 significantly altered genes (fold change > 1.5; p.adj < 0.05) between PtenΔ/Δ and PtenΔ/Δ BRF1Tg endpoint tumours, with 174 coding genes involved in immune processes (see heatmap in Fig. 3a; Table S10)—these are predominantly down-regulated in PtenΔ/Δ BRF1Tg tumours. Gene enrichment analysis further identified immune system processes, inflammatory response and leukocyte chemotaxis amongst the most overrepresented biological processes (Fig. 3b and Table S11).

Untargeted proteomics analysis on the PtenΔ/Δ and PtenΔ/Δ BRF1Tg tumours revealed that the abundance of 300 proteins was significantly (fold change > 1.5; p.adj < 0.05) altered, with 52 of these being related to immune processes (Fig. 3c, Table S12). Pathway enrichment analysis subsequently revealed that 7 of the top 10 most overrepresented biological processes related to immune responses (Fig. 3d and Table S13).

Finally, analysis of the top 10 overrepresented pathways (Table S14) in both RNA-Seq and proteomics again pointed towards altered immune status as a key difference between PtenΔ/Δ and PtenΔ/Δ BRF1Tg prostate tumours, highlighting aspects of both the innate immune response and the adaptive immune response.

Infiltration of neutrophils, CD3 and CD4 positive T lymphocytes is significantly reduced in PtenΔ/Δ BRF1Tg (compared with PtenΔ/Δ) tumours

Analysis of innate immune cells showed no significant difference in the level of macrophages but neutrophils were significantly reduced (p = 0.0421) in the stroma (defined in Fig. S5a) of PtenΔ/Δ BRF1Tg versus PtenΔ/Δ prostate tumours (Figs. 3e, f and S5c). Consistent with our previous report [21] and clinical PCa [22], stromal tertiary lymphoid aggregates were present in murine tumours (Fig. S5b), neither the total area of these structures nor markers of B-cell response were unaltered (Figs. S6 and S7a, b). PtenΔ/Δ BRF1Tg tumours contained significantly fewer stromal CD3 and CD4 T cells (p = 0.0438, 0.0394, respectively) but stromal CD8 or FOXP3 (a marker for regulatory T cells) T cells were not altered (Figs. 3g–i, S5d, c).

Collectively, we identified reduced infiltration of neutrophils and CD4 positive T lymphocytes in PtenΔ/Δ BRF1Tg prostate tumours, in keeping with reduced levels of immune/inflammatory response genes (Fig. 3a) and expression of Cdx4 (RNA-Seq data: log2FoldChange = −1.8203; p.adj = 0.0067; Table S8) in prostate tumours from PtenΔ/Δ BRF1Tg mice.

Fig. 2 Double mutant PtenΔ/Δ BRF1Tg mice have reduced survival compared with PtenΔ/Δ mice. a Illustration of strategy for targeting overexpression of human BRF1 gene. The mouse Hprt genomic locus, the Hprt targeting vector (including the human BRF1 transgene) and the restored Hprt locus (with transgene inserted on 5’ side) are shown. b Representative micrographs of H and E and BRF1 IHC staining in anterior prostate tissue from WT and BRF1Tg mice (n=5 for each genotype). Scale bars are shown (100µm for all images). Kaplan–Meier plot representing disease-specific survival of BRF1Tg (n = 12), PtenΔ/Δ (n = 12) and PtenΔ/Δ BRF1Tg (n = 14) mice. Log-rank (Mantel–Cox) Test was performed to compare PtenΔ/Δ and PtenΔ/Δ BRF1IΔ survival curves; ***p < 0.0001. d Representative images of isolated prostate tumours in WT mice that were found to be histologically identical (Fig. S5a) of PtenΔ/Δ and PtenΔ/Δ BRF1Tg mice. Illustration of strategy for targeting overexpression of human BRF1 gene. The mouse Hprt genomic locus, the Hprt targeting vector (including the human BRF1 transgene) and the restored Hprt locus (with transgene inserted on 5’ side) are shown. e Whole-cell lysates prepared from prostate tumour tissue obtained from PtenΔ/Δ (n = 4) and PtenΔ/Δ BRF1Tg (n = 4) mice were subjected to SDS-PAGE, followed by western blotting using an anti-BRF1 antibody. HSP70 served as a loading control. f qPCR analysis of RNA isolated from prostate tumour tissue obtained from PtenΔ/Δ (n = 3) and PtenΔ/Δ BRF1Tg (n = 3) mice specifically for the human BRF1 transgene. Cas3 was used as a reference gene for normalisation. g Representative micrographs of H and E staining in anterior prostate tissue from PtenΔ/Δ and PtenΔ/Δ BRF1Tg mice (n=5 for each genotype). Red box in lower magnification images in upper panel highlights region shown in higher magnification images in lower panel. Scale bars are shown (1mm for upper panel images; 100µm for lower panel images). h Representative micrographs of BRF1, Ki67, Cleaved Caspase-3 and p21 IHC staining in anterior prostate tumour tissue from PtenΔ/Δ and PtenΔ/Δ BRF1Tg mice (n=5 for each genotype). Yellow arrows highlight p21 staining. Scale bars are shown (100µm for all images). i Scoring of p21 IHC staining. p21 IHC staining was analysed in 25 manually annotated areas of prostate tumour epithelium per slide from PtenΔ/Δ (n = 5) and PtenΔ/Δ BRF1Tg (n = 5) mice using HALO software (see methods). j, k, l Individual data points are shown in the presented graphs; long horizontal line indicates the Mean; error bars represent SEM; *p value; *p < 0.05
\textit{Pten}^{Δ/Δ} \textit{BRF1}^{Tg} \textit{tumours have reduced complement pathway activation}

We generated an in vitro model system by engineering PC3 clones to stably express eGFP-tagged \textit{BRF1} (BRF1 CL4, 5, 6), or eGFP empty vector (Ctrl CL1, 2) as controls. Parental PC3 and CL1 and CL2 cells exhibited similar protein synthesis (Fig. S8a) and so CL2 was thereafter used as a representative control. Overexpression of BRF1 (Fig. 4a) resulted in significantly increased protein synthesis \( p \text{adj} = 0.0132 \) (CL4); 0.0024 (CL5); 0.0047 (CL6) (Fig. 4b), demonstrating BRF1 expression promoted Pol III function as expected.

Stable isotope labelling by amino acids in cell culture followed by mass spectrometry (Fig. S8b) on culture media from BRF1 overexpressing PC3 cells revealed 33 common
proteins which had >1.2 fold change in abundance in the same direction in CL4, CL5 and 6 compared with Ctrl CL2 (Fig. 4c; Table S15). Reduced levels of complement factor D (CFD) in the secretome of BRF1 overexpressing cells was validated (Fig. 4d) and this was particularly interesting because the complement pathway, including C7, an ‘endpoint’ component of the MAC, was highlighted from RNA-Seq and proteomic data (Tables S10 and S14). Reduced C7 mRNA (p = 0.0244) (Fig. 4e) and CFD and C7 protein expression (Fig. 4f) was validated in murine prostate tumours. Taken together, these data indicate that complement pathway activation is reduced in PtenΔ/Δ BRF1Tg tumours.

Low C7 levels are clinically relevant in human prostate cancer

In clinical PCa cBioPortal data, alterations in BRF1 and C7 significantly co-occurred when interrogating all (p = 0.002) and metastatic (p < 0.0001) tumour cases in MSKCC (2010) dataset (Fig. 4g, h). Segregating cases revealed that patients with low C7 expression (Fig. S8c, d) have a poorer prognosis in both datasets (Fig. 4i, j; Tables S16 and 17), in keeping with mouse model data. Furthermore, an inverse correlation between BRF1 and C7 mRNA expression and a positive correlation between CFD and C7 mRNA expression was found in both MSKCC (2010) and TCGA (provisional) datasets (BRF1 vs C7; Pearson correlation coefficient, r = −0.307; p = 0.0001 and r = −0.173; p = 0.0001, respectively; C7 vs CFD: Pearson correlation coefficient, r = 0.490; p < 0.0001 and r = 0.495; p < 0.0001, respectively) (Figs. 4k, S8e–g).

Complement activation can enhance the differentiation and activation of T lymphocytes [12]. In both MSKCC (2010) and TCGA (provisional) datasets, a positive relationship between C7 and the status of either CD3E [MSKCC: Pearson correlation coefficient, r = 0.324; p < 0.0001; TCGA: Pearson correlation coefficient, r = 0.139; p = 0.0019] or CD4 [MSKCC: Pearson correlation coefficient, r = 0.356; p < 0.0001; TCGA: Pearson correlation coefficient, r = 0.205; p < 0.0001] (Fig. S8h–k) was observed, consistent with mouse model data.

In summary (Fig. 4l), BRF1 expression is elevated in clinical PCa and its overexpression promotes prostate carcinogenesis in an established GEM model in vivo. Aberrant complement pathway activation, as indicated by altered secretion of CFD in vitro and reduced levels of CFD and C7 in vivo, is mechanistically consistent with the observed altered immune infiltrates, including neutrophils and CD4 positive T cells, within the tumour microenvironment upon elevation of BRF1.

Discussion

For the first time, we show that BRF1 expression drives prostate carcinogenesis in vitro and in vivo. Recent work has highlighted that the repertoire of tRNAs expressed in oncogenic contexts specifically supports the production of proteins for a proliferative program [23]. Increases in specific tRNAs such as the initiator methionine tRNA^{Met} are sufficient to promote migration and invasive behaviour in melanoma cells [24] and for fibroblasts to secrete collagen, which in turn enhanced tumour growth and angiogenesis [25], suggesting that BRF1 may play a major role in orchestrating altered programs of tRNA and thus protein synthesis. In support of this, we found that increased expression of BRF1 upregulated global protein synthesis in vitro and mediated a switch in cellular programme, manifesting with an altered secretome.

To our knowledge, this is the first study to report effects of BRF1 on the immune response in an in vivo model. A previous study identified a direct effect of Pol III activity in enhancing macrophage phagocytic function in vitro [26]. Our study did not target Pol III activity directly in immune cells, but instead we show that elevated expression of BRF1 is associated with altered immune cell infiltration in an in vivo model of prostate carcinogenesis.
It is recognised that immune infiltration can have a significant influence upon clinical outcome in cancer patients [22]. Our previous study found that improved survival upon genetic loss of Erk5 in Pten<sup>Δ/Δ</sup> PCa model was related to increased levels of the T cell recruiting cytokines Ccl5 and Cxcl10 and enhanced T cell infiltration (predominantly
Figure 4 Complement pathway activation is (i) reduced when BRF1 is overexpressed in vitro, (ii) reduced in prostate tumours from Pten<sup>ΔΔ2</sup> BRF1<sup>Tg</sup> mice and (iii) inversely correlates with BRF1 expression in human PCa. a Western blotting of whole-cell lysates prepared from PC3 Ctrl CL2 and BRF1 CL4, 5 and 6 cells using anti-BRF1 antibody and HSC70 as a loading control. Blot shown is representative of three independent experiments. b Comparison of incorporation of <sup>35</sup>S-methionine label, which was measured by scintillation counting, between PC3 stable clones (n = 3; 2-3 wells per clone were used per each individual experiment). Data from BRF1 CL4, 5 and 6 cells were normalised to the mean of PC3 Ctrl CL2 cells. Individual data points are shown in the presented graph; long horizontal line indicates the Mean; error bars represent SEM; Welch’s t test (unpaired, two tailed) with Bonferroni correction for multiple testing was used to calculate p adj values; *p adj < 0.05; **p adj < 0.01. c Venn diagram outlining numbers of proteins that had >1.2-fold change in abundance in the same direction in PC3 BRF1 overexpressing clones CL4, 5 and 6 compared with Ctrl CL2. d Western blotting of purified secreted proteins prepared from PC3 Ctrl CL2 and PC3 BRF1 overexpressing CL4, 5 and 6 using an anti-CFD antibody. PonceauS staining was used as a loading control. e qPCR analysis of isolated RNA prepared from prostate tumour tissue obtained from Pten<sup>ΔΔ2</sup> (n = 5) and Pten<sup>ΔΔ2</sup> BRF1<sup>Tg</sup> (n = 5) mice for C7. Ccasc3 was used as a reference gene for normalisation. Individual data points are shown in the presented graph; long horizontal line indicates the Mean; error bars represent SEM; Welch’s t test (unpaired, 2 tailed) was used to calculate p value; *p < 0.05. f Western blotting of whole-cell lysates prepared from prostate tumour tissue obtained from Pten<sup>ΔΔ2</sup> (n = 5) and Pten<sup>ΔΔ2</sup> BRF1<sup>Tg</sup> (n = 5) mice using anti-C7 and anti-CFD antibodies. HSC70 served as a loading control. Oncoprint from cBioPortal illustrating mRNA expression in MSKCC (2010) prostate adenocarcinoma dataset. Kaplan–Meier plots for progression-free survival in MSKCC (2010) (i) and TCGA (provisional) (j) prostate adenocarcinoma datasets of patients segregated for low and high C7 expression as indicated in the Oncoprints presented in Fig. S8c, d, respectively. Log-rank (Mantel–Cox) Test was performed to compare survival curves; *p < 0.05; ***p < 0.001. k Scatter plot showing the correlation of C7 and BRF1 mRNA expression in MSKCC (2010) prostate adenocarcinoma dataset (n = 150; all tumours with mRNA expression data). Pearson correlation coefficient and p value for the comparison is stated in the main text. l Schematic summary of key observations as described in the main text.

CD4<sup>+</sup>) within the tumours [21]. Here, we report an association between reduced levels of CD4<sup>+</sup> T lymphocytes, and also neutrophils, with poorer survival in Pten<sup>ΔΔ2</sup> BRF1<sup>Tg</sup> compared with Pten<sup>ΔΔ2</sup> mice.

The complement pathway has been reported to have both pro- and anti-tumourigenic effects [11]. We observe that a low complement C7 environment is associated with poorer survival in our transgenic mouse model and human PCa. Furthermore, in both murine and human PCa, tumoral CD4<sup>+</sup> T cell level positively correlated with complement C7 level. These data significantly contribute to our understanding of how the complement system may contribute to tumourigenesis.

In conclusion, our data highlight that elevated BRF1 expression promotes prostate carcinogenesis. We implicate a non-cell autonomous role for high Brf1 expression in the prostatic epithelium as impacting on the tumour micro-environment with altered immune infiltrates. Given the rapid pace of development of cancer therapies targeting the immune system, further research in this area is warranted.

**Materials and methods**

**Experimental methodology**

Detail for the following procedures is provided in Supplementary Materials and Methods: IHC; ISH; image analysis of IHC and ISH staining; histoscore; use and analysis of clinical datasets; culture of human PCa cell lines; transient transfection of plasmids and siRNA; generation of PC3 eGFP empty vector and eGFP-PRF1 stable clones; western blotting; WST-1 cell proliferation assay; cell cycle analysis from BrdU labelling; generation of conditional human BRF1 expressing mice; mouse strains and breeding; quantitative real-time PCR (Table S18); isolation and quantification of RNA (Table S19); RNA-sequencing and bioinformatic analysis; SILAC labelling and preparation of secretome samples; proteomics; comparison of RNA-Seq and proteomics data and <sup>35</sup>S-Methionine incorporation assay.

**Human tissue microarray**

Tissue microarrays (TMAs) consisting of cores of formalin-fixed, paraffin-embedded (FFPE) tissue from a cohort of 134 individuals with BPH and 516 patients with primary PCa were analysed for expression of BRF1 by immunohistochemistry. See Table S20 for demographic data at diagnosis: Age—median = 69.5 years (interquartile range (IQR) 63–75.5); Gleason sum score—median = 7 (IQR 6–8) (60.8% cases ≥ 7); Serum PSA levels—median = 15.55 ng/ml (IQR 7.025–44).

**Statistics**

Statistical analyses, including: Mann–Whitney, Log-rank (Mantel–Cox), Pearson correlation coefficient, Welch’s t test (unpaired, 2 tailed) and Kaplan–Meier survival analysis, were performed using GraphPad Prism v7.02 or IBM SPSS Statistics 25 software. Bonferroni correction for multiple testing was performed as indicated. For all graphs, mean ± standard deviation (SD) or standard error of mean (SEM) (error bars) are presented and these are defined for each figure. In Forest plots, in both the upregulated and down-regulated cases, the data were pooled (across all cell-lines), log-transformed, and a one-sample t-test was performed.
Acknowledgements This work was funded by Cancer Research UK (A15151, A10419 and A17196; HYL) and Prostate Cancer UK (PG10-10; HYL). SS’s PhD was supported by Medical Research Council Clinical Research Training Fellowship. NANN’s PhD was sponsored by Ministry of Higher Education, Malaysia and SLAI Fellowship from University of Malaya. We thank the Cancer Research UK Beatson Institute core research services, including the biological services unit and the histology department. We acknowledge Arnaud Blomme for helpful discussions and commenting on the manuscript. We are grateful to Bob White for involvement and useful discussions in the initial stage of this project, and to Owen Sansom for general support in the development of the BRF1 mouse model.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Goodfellow SJ, Innes F, Derblay LE, MacLellan WR, Scott PH, White RJ. Regulation of RNA polymerase III transcription during hypertrophic growth. EMBO J. 2006;25:1522–33.
2. Arthurs C, Murtaza BN, Thomson C, Dickens K, Henrique R, Patel HRH, et al. Expression of ribosomal proteins in normal and cancerous human prostate tissue. PLoS ONE. 2012;7:e0186047.
3. Uemura M, Zheng Q, Koh CM, Nelson WG, Yegnasubramanian S, De Marzo AM. Overexpression of ribosomal RNA in prostate cancer is common but not linked to rDNA promoter hypomethylation. Oncogene. 2012;31:1254–63.
4. Zhang Y, Wu H, Yang F, Ning J, Li M, Zhao C, et al. Prognostic value of the expression of DNA repair-related biomarkers mediated by alcohol in gastric cancer patients. Am J Pathol. 2018;188:367–77.
5. Zhong Q, Xi S, Liang J, Shi G, Huang Y, Zhang Y, et al. The significance of BRF1 overexpression in human hepatocellular carcinoma. Oncotarget. 2016;7:6243–54.
6. Fang Z, Yi Y, Shi G, Li S, Chen S, Lin Y, et al. Role of BRF1 interaction with ERAlpha, and significance of its overexpression, in human breast cancer. Mol Oncol. 2017;11:1752–67.
7. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68:7–30.
8. Moschini M, Carroll PR, Eggener SE, Epstein JJ, Graefen M, Montironi R, et al. Low-risk prostate cancer: identification, management, and outcomes. Eur Urol. 2017;72:238–49.
9. Thakur A, Vaishampayan U, Lumb LG. Immunotherapy and immune evasion in prostate cancer. Cancers Basel. 2013;5:569–90.
10. Bryant G, Wang L, Mulholland DJ. Overcoming oncogenic mediated tumor immunity in prostate cancer. Int J Mol Sci. 2017;18:E1542.
11. Kourtzelis I, Rafail S. The dual role of complement in cancer and its implication in anti-tumor therapy. Ann Transl Med. 2016;4:265.
12. Afshar-Kharghan V. The role of the complement system in cancer. J Clin Invest. 2017;127:780–9.
13. Warzner R. Modulation of complement membrane attack by local C7 synthesis. Clin Exp Immunol. 2000;121:8–10.
14. Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: pathophysiological mechanisms. J Immunol. 2013;190:3831–8.
15. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18:11–22.
16. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:p11.
17. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Disco. 2012;2:401–4.
18. Liko D, Mitchell L, Campbell KJ, Ridgway RA, Jones C, Dudek K et al. BRF1 loss and not overexpression disrupts tissues homeostasis in the intestine, liver and pancreas. Cell Death Differ. 2019;26:2353–50.
19. Wu X, Wu J, Huang J, Powell WC, Zhang J, Matusik RJ, et al. Generation of a prostate epithelial cell-specific Cre transgenic mouse model for tissue-specific gene ablation. Mech Dev. 2001;101:61–69.
20. Lesche R, Groszer M, Gao J, Wang Y, Messing A, Sun H, et al. Cre/loxP-mediated inactivation of the murine Pten tumor suppressor gene. Genesis. 2002;32:148–9.
21. Loveridge CJ, Mui EJ, Patel R, Tan EH, Ahmad I, Welsh M, et al. Increased T-cell infiltration elicited by Erk5 deletion in a pten-deficient mouse model of prostate carcinogenesis. Cancer Res. 2017;77:3158–68.
22. Garcia-Hernandez ML, Uribe-Uribé NO, Espinosa-Gonzalez R, Kast WM, Khader SA, Rangel-Moreno J. A unique cellular and molecular microenvironment is present in tertiary lymphoid organs of patients with spontaneous prostate cancer regression. Front Immunol. 2017;8:563.
23. Gingold H, Theiler D, Christoffersen NR, Nielsen MM, Asmar F, Kooistra SM, et al. A dual program for translation regulation in cellular proliferation and differentiation. Cell. 2014;158:1281–92.
24. Birch J, Clarke CJ, Campbell AD, Campbell K, Mitchell L, Liko D, et al. The initiator methionine tRNA drives cell migration and invasion leading to increased metastatic potential in melanoma. Biol Open. 2016;5:1371–9.
25. Clarke CJ, Berg TJ, Birch J, Ennis D, Mitchell L, Cloix C, et al. The initiator methionine tRNA drives secretion of type II collagen from stromal fibroblasts to promote tumor growth and angiogenesis. Curr Biol. 2016;26:755–65.
26. Graczyk D, White RJ, Ryan KM. Involvement of RNA Polymerase III in Immune Responses. Mol Cell Biol. 2015;35:1848–59.