Received:
9 March 2017

Revised:
14 April 2017

Accepted:
3 May 2017

<https://doi.org/10.1259/bjr.20170174>

Cite this article as:

Robinson JL, Tzou KS, Parker AS, Heckman MG, Wu KJ, Hilton TW, et al. GATA2 expression and biochemical recurrence following salvage radiation therapy for relapsing prostate cancer. *Br J Radiol* 2017; **90**: 20170174.

FULL PAPER

GATA2 expression and biochemical recurrence following salvage radiation therapy for relapsing prostate cancer

¹JESSICA L ROBINSON, PhD, ²KATHERINE S TZOU, MD, ³ALEXANDER S PARKER, PhD, ³MICHAEL G HECKMAN, MS, ⁴KEVIN J WU, MD, ⁴TRACY W HILTON, BS, ⁵THOMAS M PISANSKY, MD, ⁶STEVEN E SCHILD, MD, ²JENNIFER L PETERSON, MD, ²LAURA A VALLOW, MD and ²STEVEN J BUSKIRK, MD

¹Department of Biology, Edgewood College, Madison, WI, USA

²Department of Radiation Oncology, Mayo Clinic, Jacksonville, FL, USA

³Department of Health Sciences Research, Mayo Clinic, Jacksonville, FL, USA

⁴Department of Pathology, Mayo Clinic, Jacksonville, FL, USA

⁵Department of Radiation Oncology, Mayo Clinic, Rochester, MN, USA

⁶Department of Radiation Oncology, Mayo Clinic, Scottsdale, AZ, USA

Address correspondence to: Dr Katherine S Tzou

E-mail: tzou.katherine@mayo.edu

Objective: High GATA2 expression has been associated with an increased risk of poor clinical outcomes after radical prostatectomy; however, this has not been studied in relation to risk of biochemical recurrence (BCR) after salvage radiation therapy (SRT) for recurrent prostate cancer after radical prostatectomy. Our aim was to evaluate the association between protein expression levels of GATA2 in primary prostate cancer tumour samples and the risk of BCR after SRT.

Methods: 109 males who were treated with SRT were included. The percentage of cells with nuclear staining and GATA2 staining intensity were both measured. These two measures were multiplied together to obtain a GATA2 H-score (range 0–12) which was our primary GATA2 staining measure.

Results: In unadjusted analysis, the risk of BCR was higher for patients with a GATA2 H-score >4 (hazard

ratio = 2.04, $p = 0.033$). In multivariable analysis adjusting for SRT dose, pre-SRT PSA, pathological tumour stage and Gleason score, this association weakened substantially (hazard ratio = 1.45, $p = 0.31$). This lack of an independent association with BCR appears to be the result of correlations between GATA2 H-score >4 and higher pre-SRT PSA ($p = 0.021$), higher Gleason score ($p = 0.044$) and more severe pathological tumour stage ($p = 0.068$).

Conclusion: Higher levels of GATA2 expression appear to be a marker of prostate cancer severity; however, these do not provide independent prognostic information regarding BCR beyond that of validated clinicopathological risk factors.

Advances in knowledge: A higher GATA2 expression level appears to be correlated with known measures of prostate cancer severity and therefore is likely not an independent marker of outcome after SRT.

INTRODUCTION

Prostate cancer is the most common newly diagnosed non-cutaneous cancer in American males each year in the USA and is the second leading cause of cancer death.¹ Radical prostatectomy (RP) provides curative treatment for the majority of patients; however, between 15% and 25% of males experience disease relapse following RP as evidenced by an elevated serum prostate-specific antigen level (PSA).^{2,3} Treatment options for patients with biochemical recurrence (BCR) of disease include androgen-deprivation therapy (ADT), which is typically reserved for metastatic cancer, and salvage radiation therapy (SRT), a potentially curative treatment option for locally recurrent disease.⁴ Reported success rates of SRT vary considerably, ranging from approximately 10–65%.⁴

Determining which patients are most likely to respond to SRT poses a significant clinical problem. A number of clinical and pathological factors have been shown to be reproducible independent prognostic markers of BCR after SRT, including the PSA level before SRT, PSA doubling time after RP, pathological tumour stage, Gleason score and surgical margin status.⁴ These factors have been combined into prediction models by several groups; however, there is a notable proportion of patients in the lower risk groups who still experience relapse and *vice versa*.^{5,6} Interestingly, the results of a recent relatively small study suggest that the Decipher® genomic classifier has the potential to be a strong predictor of SRT outcome; however, validation in larger patient cohorts is needed.⁷ Another possibility that has

potential to improve treatment decision making is to identify tissue biomarkers present in the initial tumour that are independent prognostic factors for BCR and to incorporate this information into the aforementioned predictive algorithms. This approach may also identify potential therapeutic targets or provide greater insight into the mechanisms of resistance to SRT.⁸

The GATA family of nuclear regulatory proteins plays an essential role in the development of urogenital and haematopoietic cells,⁹ and impaired GATA2 function has been linked to leukaemia; notably, mutations in GATA2 are found in 10% of chronic myeloid leukaemia patients and GATA2 is frequently overexpressed in acute myeloid leukaemia.¹⁰ In the prostate, GATA2 is expressed in normal epithelial tissue,¹¹ and laser capture microdissection gene expression has identified higher expression in neoplastic prostate tissue.¹² GATA2 expression is further upregulated in metastatic prostate cancer.^{13–15} Functionally, GATA2 interacts with the androgen receptor (AR), enhancing its binding to chromatin and mediating androgen-induced gene expression.^{11,16} Clinically, high expression of GATA2 has been associated with an increased risk of BCR and distant metastases after RP.^{14,17} Furthermore, upregulation of GATA2 is associated with development of hormone-refractory tumours after androgen deprivation therapy as well as chemotherapy resistance and progression to lethal disease.¹⁸ However, the degree to which GATA2 expression predicts BCR in males who have undergone RP and also have been subsequently treated with SRT due to a rising PSA following RP remains unknown. In this study, we evaluated the association of protein expression levels of GATA2 in primary prostate cancer tumour samples with the risk of BCR after SRT.

METHODS AND MATERIALS

Patient selection and outcome definition

Included in this study were all 109 patients who were treated with SRT for a rising PSA following RP between July 1987 and February 2003 at our institution and who had archived tumour tissue available. Information was collected retrospectively regarding baseline characteristics (pre-RP PSA, pre-SRT PSA, pre-SRT ADT and age), pathological information (Gleason score, pathological tumour stage and surgical margin status), SRT information (length of time between RP and SRT, SRT dose) and post-SRT PSA measurements. The primary end point of this study was BCR after SRT, which was defined as a PSA of 0.2 ng ml^{-1} or greater and rising following the post-SRT nadir. The date of BCR was considered as the date of the defining PSA value without backdating. This study was approved by our institutional review board.

Salvage radiation therapy information

Patients were simulated and immobilized supine with contrast in the bladder and rectum. The majority of patients underwent retrograde urethrography. CT-based treatment planning was used in most cases with custom blocking and 4–8 stationary conformal or rotational fields. Intensity modulation, inverse planning and image-guidance techniques were not used during the study period. The prostatic fossa was targeted with 6–20 MV photons to a median dose of 64.8 Gy (range: 58.4–70.2 Gy) in daily 1.8–2.0 Gy fractions; the pelvic lymph nodes were not treated. After treatment was complete, patients were typically seen in follow up every 3–4 months for 2 years and every 6–12 months thereafter.

Figure 1. Representative GATA2 immunostaining showing examples of prostate tumours with (a) negative staining intensity, (b) weak staining intensity, (c) moderately staining intensity and (d) strong staining intensity. Images are shown at $\times 40$.

Figure 2. Representative GATA2 immunostaining showing examples of prostate tumours with (a) 0–25% staining, (b) 26–50% staining, (c) 51–75% staining and (d) 76–100% staining. Images are shown at $\times 10$.

Immunohistochemistry

5- μ m-thick slides were cut from the original prostatectomy specimen with the highest-grade tumour. Immunohistochemical staining was performed on the DAKO Autostainer Plus (Agilent Technologies, Glostrup, Denmark) using rabbit polyclonal anti-GATA2 antibody (Cell Signaling, Cat # 4595, Danvers, MA). Initially, 25-min heat-induced epitope retrieval was performed at pH 6.0 followed by a 5-min protein block. Primary antibody was applied for 60 min at titer of 1:150, followed by anti-rabbit polymer-horseradish peroxidase for 30 min and finally diaminobenzidine for 5 min. Rabbit anti-GATA2 was validated previously on human prostate tissue, and all experiments were performed using the same lot number of antibody (Lot #2).

After immunohistochemical staining, the percentage of cells with nuclear staining (*i.e.* GATA2 staining percentage, 1 = 0–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%) and GATA2 staining intensity (0 = negative, 1 = weak, 2 = moderate, 3 = strong) were measured manually by an experienced uropathologist who was blinded to all other patient data. GATA2 H-score was calculated by multiplying GATA2 staining percentage and GATA2 staining intensity together, which results in an H-score that ranges from 0 to 12. We considered GATA2 H-score as our primary staining measure, as this measure takes into account both overall tumour burden and total protein levels. The individual GATA2 staining percentage and GATA2 staining intensity variables were considered as secondary measures.

Statistical analysis

Continuous variables were summarized using the median and range. Categorical variables were summarized using number and percentage of patients. Patient baseline, pathological and

SRT characteristics were compared according to GATA2 H-score when categorizing this measure by the sample median (≤ 1 vs > 1) using a Wilcoxon rank-sum test or Fisher's exact test. We estimated the cumulative incidence of BCR using the Kaplan–Meier method, censoring on the date of the last PSA measurement for patients who did not experience this outcome. Associations of each of the three different GATA2 staining measures [H-score (primary measure), staining percentage and staining intensity] with BCR were examined using single variable (*i.e.* unadjusted) and multivariable Cox proportional hazards regression models. Multivariable models were adjusted for pre-SRT PSA, pathological tumour stage and Gleason score as these three variables have previously been significantly associated with BCR in this same patient cohort¹⁹ and also any variable that differed between ≤ 1 and > 1 H-score groups with a *p*-value of 0.05 or lower. Hazard ratios (HRs) and 95% confidence intervals were estimated. Several different cut-points were considered for GATA2 H-score (0 vs > 0 , ≤ 1 vs > 1 , ≤ 2 vs > 2 , ≤ 4 vs > 4), GATA2 staining percentage ($\leq 25\%$ vs $> 25\%$, $\leq 50\%$ vs $> 50\%$, $\leq 75\%$ vs $> 75\%$) and GATA2 staining intensity (negative vs weak/moderate/strong, negative/weak vs moderate/strong) in Cox regression analysis.

In order to adjust for multiple testing in our primary analysis that involved evaluating the association between GATA2 H-score and BCR, we utilized a Bonferroni correction, after which *p*-values of 0.0125 or lower were considered as statistically significant due to the aforementioned four different cut-points that were examined for GATA2 H-score. For all remaining analysis which is considered as secondary, *p*-values of 0.05 or lower were considered as statistically significant. All statistical analysis was

performed using R Statistical Software v. 2.14.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

As shown in [Supplementary Table A](#), the majority of the tumours showed positive staining for GATA2 (82%), which was most commonly weak staining intensity (60%) or moderate staining intensity (21%). For GATA2 staining percentage, this was 25% or less for the majority of patients (61% of patients) and was fairly evenly distributed across the higher staining percentage categories. Representative images of GATA2 staining intensity and staining percentage are shown in [Figures 1 and 2](#), respectively. GATA2 H-score is the primary staining measure for our study, combining staining intensity and percentage to provide a more complete understanding of total expression levels. In our patient cohort, GATA2 H-score was equal to 0 in 20 patients (18%), 1 in 39 patients (36%), 2 in 22 patients (20%), 3 in 4 patients (4%), 4 in 11 patients (10%) and >4 in 13 patients (12%).

A comparison of patient clinical, pathological and SRT characteristics according to GATA-2 H-score is shown in [Table 1](#), where GATA2 H-score was dichotomized by the sample median (≤ 1 vs > 1) to allow for balanced sample sizes between the two groups. A higher GATA2 H-score was associated with a significantly higher pathological tumour stage (T3b: 42% vs 19%,

$p = 0.023$) and a lower SRT dose (median: 64.4 vs 66.6 Gy, $p = 0.002$). No other significant differences were observed between ≤ 1 and > 1 H-score groups (all $p \geq 0.13$).

In our cohort of 109 patients, 66 (60.6%) experienced BCR with a median follow-up duration of 8.8 years after SRT. Cumulative incidences of BCR at 3, 5 and 10 years after SRT were 44%, 52% and 64%, respectively. The association between GATA2 H-score and risk of BCR after SRT is shown in [Table 2](#). There was a nominally significant ($p \leq 0.05$) increased risk of BCR for patients with a GATA2 H-score > 4 (HR: 2.04, $p = 0.033$) in single variable analysis without adjustment for other variables, although this finding did not quite remain significant after adjustment for multiple testing ($p \leq 0.0125$ considered significant in this portion of the analysis). More specifically, the 5-year cumulative incidence of BCR was 49% for patients with a low GATA2 staining (H-score ≤ 4) compared with 76% for those patients with high levels of GATA2 (H-score > 4 , [Figure 3](#)). However, in multivariable analysis adjusting for SRT dose, pre-SRT PSA, pathological tumour stage and Gleason score, this association weakened noticeably and no longer approached statistical significance (HR: 1.45, $p = 0.31$, [Table 2](#)). Importantly, when adjusting only for a SRT dose in multivariable analysis, the association between GATA2 levels and increased risk of BCR remained consistent (HR: 2.03, $p = 0.040$); it was only after the

Table 1. Comparison of patient characteristics according to GATA2 H-score

Variable	GATA2 H-score ≤1 (<i>n</i> = 59)	GATA2 H-score >1 (<i>n</i> = 50)	<i>p</i> -value
Pre-RP PSA (ng ml ^{−1})	9.7 (2.3, 155.0)	13.8 (2.0, 219.0)	0.13
Pre-SRT PSA (ng ml ^{−1})	0.6 (0.1, 4.9)	0.7 (0.1, 15.3)	0.76
SRT dose (Gy)	66.6 (60.0, 70.2)	64.4 (58.4, 70.2)	0.002
Age (years)	66.6 (44.0, 80.7)	67.4 (52.6, 76.6)	0.15
Length of time from RP to SRT initiation (months)	12.7 (2.1, 75.5)	11.7 (1.5, 99.3)	0.69
Pathological tumour stage			
T2	7 (11.9%)	5 (10.0%)	0.023
T3a	41 (69.5%)	24 (48.0%)	
T3b	11 (18.6%)	21 (42.0%)	
Surgical margin			
Positive	32 (54.2%)	34 (68.0%)	0.17
Negative	27 (45.8%)	16 (32.0%)	
Gleason score			
3–6	24 (40.7%)	19 (39.6%)	0.45
7	26 (44.1%)	16 (33.3%)	
8–10	9 (15.3%)	13 (27.1%)	
Pre-SRT ADT			
Yes	9 (15.3%)	13 (26.0%)	0.23
No	50 (84.7%)	37 (74.0%)	

ADT, androgen deprivation therapy; PSA, prostate-specific antigen; RP, radical prostatectomy; SRT, salvage radiation therapy. The sample median (minimum, maximum) is given for continuous variables. p -values result from a Wilcoxon rank-sum test (continuous variables) or Fisher's exact test (categorical variables). Information was unavailable regarding pre-RP PSA ($n = 5$) and Gleason score ($n = 2$).

additional adjustment for the known clinicopathological risk factors that the association weakened markedly. There was not a statistically significant association with BCR for any other cut-points in GATA2 H-score (Table 2), GATA2 staining percentage or staining intensity (Supplementary Table B).

Given the association between high GATA2 and increased risk of BCR that was observed in single-variable (*i.e.* unadjusted) analysis, we examined key clinicopathological characteristics of the 13 patients with an H-score >4 and compared them with those of patients with low GATA2 staining levels (Supplementary Table C). Patients with high GATA2 levels had a significantly higher pre-SRT PSA ($p = 0.021$), a significantly higher Gleason score ($p = 0.044$) and a borderline significantly higher pathological tumour stage ($p = 0.068$) (Supplementary Table C). All of three of these markers were also significantly associated with the risk of BCR (Supplementary Table D). In our data set, GATA2 levels correlate with these known risk factors of disease aggressiveness.

DISCUSSION

There is an unmet need for an accurate method to identify which patients will benefit from SRT for BCR after RP.²⁰ Clinical makers of disease aggressiveness such as pathological Gleason score, tumour stage and pre-SRT PSA correlate with outcome but are insufficient to identify all patients who will benefit from SRT.^{5,6,19} The ability to identify males who are more likely to develop metastases after SRT is critically important, as these patients may benefit from alternative treatment strategies, perhaps focused on systemic therapies. Recently, the Decipher genomic classifier has shown very strong ability to predict outcome after SRT; however, further study is needed to validate that finding.⁷ There is also potential for tissue biomarkers of prostate cancer aggressiveness to be used as a tool to identify patients who are more likely to relapse after SRT. For example, previous immunohistochemical studies have identified Ki67 and B7-H3 as independent prognostic markers of BCR after SRT.^{21,22}

In this study, we observed for the first time evidence suggesting that GATA2 staining level is associated with an increased risk of

BCR after SRT. Specifically, we observed a two-fold increased risk of BCR in males who had a GATA2 H-score >4. However, multivariable analysis revealed that this association was not independent of known prognostic factors (pre-SRT PSA, Gleason score and pathological tumour stage). High GATA2 staining levels have previously been associated with a shorter time to BCR in the setting of RP; however, as noted in our investigation, this finding was not independent of key clinicopathological parameters.^{14,17} The associations of greater GATA2 expression with higher Gleason score and pathological tumour stage that we noted were also observed in the study by He et al.¹⁴ As such, it appears that GATA2 is a marker of prostate cancer severity. In agreement, higher GATA2 staining levels have been previously associated with a higher risk of distant metastatic progression after RP,¹⁷ and GATA2 expression is increased in metastatic prostate cancer.^{14,15}

GATA2 is a well characterized interacting partner of the AR, a hormone-dependent transcription factor that plays a pivotal role in the growth and development of prostate cancer.²³ By acting as a so-called pioneer factor, GATA2 recruits chromatin-modifying proteins to numerous sites in the genome and makes them more accessible to AR.²⁴ As such, GATA2 pre-determines sites in the genome where AR binds and dictates the androgen-responsive gene expression pattern.²⁴ A number of *in vitro* models and mRNA clinical databases have identified GATA2 as a possible key driver of prostate cancer progression.^{13,15,25} Knock-down of GATA2 gene expression decreases *in vitro* cell growth, migration and invasion,¹³ and in mice xenograft models, tumour growth is potentially slowed when treated with a small molecule inhibitor of GATA2.¹⁴

Our data supports the idea that increased expression of AR coactivators, such as GATA2, leads to a more aggressive prostate cancer phenotype that is resistant to second-line therapies such as SRT.²⁶ Previous studies suggest that GATA2 remains an important mediator of AR function in prostate cancer which becomes resistant to ADT.²⁷ Moreover, recent data suggests that GATA2 may also play a role in resistance to docetaxel, the first-

Table 2. Association between GATA2 H-score and biochemical recurrence following salvage radiation therapy

GATA2 H-score	<i>n</i>	Cumulative 5-year incidence of BCR, % (95% CI)	Single variable analysis		Multivariable analysis	
			HR (95% CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value
0	20	37.0 (10.6, 55.6)	1.00 (reference)	N/A	1.00 (reference)	N/A
>0	89	55.8 (43.9, 65.2)	1.54 (0.79, 2.03)	0.21	1.26 (0.62, 2.55)	0.52
≤1	59	48.5 (33.3, 60.3)	1.00 (reference)	N/A	1.00 (reference)	N/A
>1	50	56.9 (40.6, 68.8)	1.16 (0.71, 1.88)	0.56	1.02 (0.55, 1.89)	0.96
≤2	81	50.6 (37.9, 60.7)	1.00 (reference)	N/A	1.00 (reference)	N/A
>2	28	57.1 (34.3, 72.1)	1.22 (0.71, 2.07)	0.47	0.90 (0.48, 1.67)	0.74
≤4	96	48.7 (37.3, 58.0)	1.00 (reference)	N/A	1.00 (reference)	N/A
>4	13	76.1 (37.7, 91.4)	2.04 (1.06, 3.92)	0.033	1.45 (0.71, 2.98)	0.31

BCR, biochemical recurrence; CI, confidence interval; HR, hazard ratio; N/A, not applicable.

HRs, 95% CIs and *p*-values result from Cox proportional hazards regression models. Multivariable models were adjusted for pre-salvage radiation therapy (SRT) prostate-specific antigen, pathological tumour stage, Gleason score and SRT dose.

Figure 3. Cumulative incidence of biochemical recurrence (BCR) following salvage radiation therapy (SRT) according to GATA2 H-score (≤ 4 vs >4).

line chemotherapeutic agent used in the treatment of metastatic prostate cancer, although this is through androgen-independent mechanisms.¹⁵

There are several important limitations of this study that should be noted. First, patient clinical, pathological and SRT information were retrospectively collected at a single centre. Second, the sample size of 109 patients was relatively small; however, we were able to demonstrate a nominally significant association between GATA2 H-score and risk of BCR in unadjusted analysis despite the limited power that this sample size results in. Third, GATA2 staining was measured by a single uropathologist. A more optimal design would have utilized multiple uropathologists who independently measured GATA2 staining, after which interrater agreement could be assessed and consensus measures of GATA2 staining could have been utilized in the analysis. Finally, our tertiary care patient population is

mostly Caucasian ($>98\%$), and therefore our findings are not applicable to patients of other races.

CONCLUSION

Our results suggest that a higher GATA2 staining level is associated with an increased risk of BCR in males undergoing SRT. However, due to its correlation with other well-established clinicopathological BCR risk factors, it does not appear to be an independent prognostic marker for BCR in the SRT patient population. Therefore, although an elevated GATA2 staining level appears to be a marker of prostate cancer severity, it does not appear that the measurement of GATA2 staining levels will provide beneficial prognostic information in SRT candidates beyond what is already known from standardly collected measures, and we cannot presently advocate its use in routine clinical care decision making. Nonetheless, our findings advance our understanding of the role of GATA2 in aggressive prostate cancer.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016; **66**: 7–30. doi: <https://doi.org/10.3322/caac.21332>
2. Stephenson AJ, Scardino PT, Eastham JA, Bianco FJ Jr, Dotan ZA, Fearn PA, et al. Preoperative nomogram predicting the 10-year probability of prostate cancer recurrence after radical prostatectomy. *J Natl Cancer Inst* 2006; **98**: 715–17. doi: <https://doi.org/10.1093/jnci/djj190>
3. Patel AR, Stephenson AJ. Radiation therapy for prostate cancer after prostatectomy: adjuvant or salvage? *Nat Rev Urol* 2011; **8**: 385–92. doi: <https://doi.org/10.1038/nrurol.2011.80>
4. Tzou K, Tan WW, Buskirk S. Treatment of men with rising prostate-specific antigen levels following radical prostatectomy. *Expert Rev Anticancer Ther* 2011; **11**: 125–36. doi: <https://doi.org/10.1586/era.10.210>
5. Tendulkar RD, Agrawal S, Gao T, Efstathiou JA, Pisansky TM, Michalski JM, et al. Contemporary update of a multi-institutional predictive nomogram for salvage radiotherapy after radical prostatectomy. *J Clin Oncol* 2016; **34**: 3648–54.
6. Lee RJ, Tzou KS, Heckman MG, Hobbs CJ, Rawal B, Diehl NN, et al. Proposed prognostic scoring system evaluating risk factors for biochemical recurrence of prostate cancer after salvage radiation therapy. *BJU Int* 2016; **118**: 236–42. doi: <https://doi.org/10.1111/bju.13229>
7. Freedland SJ, Choeurng V, Howard L, De Hoedt A, du Plessis M, Yousefi K, et al. Utilization of a genomic classifier for prediction of metastasis following salvage radiation therapy after radical prostatectomy. *Eur Urol* 2016; **70**: 588–96.
8. Tran PT, Hales RK, Zeng J, Aziz K, Salih T, Gajula RP, et al. Tissue biomarkers for prostate cancer radiation therapy. *Curr Mol Med* 2012; **12**: 772–87.
9. Zhou Y, Lim KC, Onodera K, Takahashi S, Ohta J, Minegishi N, et al. Rescue of the

- embryonic lethal hematopoietic defect reveals a critical role for GATA-2 in urogenital development. *EMBO J* 1998; **17**: 6689–700. doi: <https://doi.org/10.1093/emboj/17.22.6689>
10. Vicente C, Conchillo A, Garcia-Sanchez MA, Odero MD. The role of the GATA2 transcription factor in normal and malignant hematopoiesis. *Crit Rev Oncol Hematol* 2012; **82**: 1–17. doi: <https://doi.org/10.1016/j.critrevonc.2011.04.007>
 11. Perez-Stable CM, Pozas A, Roos BA. A role for GATA transcription factors in androgen regulation of the prostate-specific antigen gene. *Mol Cell Endocrinol* 2000; **167**: 43–53.
 12. Gregg JL, Brown KE, Mintz EM, Piontkivska H, Fraizer GC. Analysis of gene expression in prostate cancer epithelial and interstitial stromal cells using laser capture microdissection. *BMC Cancer* 2010; **10**: 165.
 13. Chiang YT, Wang K, Fazli L, Qi RZ, Gleave ME, Collins CC, et al. GATA2 as a potential metastasis-driving gene in prostate cancer. *Oncotarget* 2014; **5**: 451–61. doi: <https://doi.org/10.18632/oncotarget.1296>
 14. He B, Lanz RB, Fiskus W, Geng C, Yi P, Hartig SM, et al. GATA2 facilitates steroid receptor coactivator recruitment to the androgen receptor complex. *Proc Natl Acad Sci U S A* 2014; **111**: 18261–6. doi: <https://doi.org/10.1073/pnas.1421415111>
 15. Vidal SJ, Rodriguez-Bravo V, Quinn SA, Rodriguez-Barrueco R, Lujambio A, Williams E, et al. A targetable GATA2-IGF2 axis confers aggressiveness in lethal prostate cancer. *Cancer Cell* 2015; **27**: 223–39. doi: <https://doi.org/10.1016/j.ccell.2014.11.013>
 16. Wang Q, Li W, Liu S, Carroll JS, Janne OA, Keeton EK, et al. A hierarchical network of transcription factors governs androgen receptor-dependent prostate cancer growth. *Mol Cell* 2007; **27**: 380–92. doi: <https://doi.org/10.1016/j.molcel.2007.05.041>
 17. Böhm M, Locke WJ, Sutherland RL, Kench JG, Henshall SM. A role for GATA-2 in transition to an aggressive phenotype in prostate cancer through modulation of key androgen-regulated genes. *Oncogene* 2009; **28**: 3847–56. doi: <https://doi.org/10.1038/onc.2009.243>
 18. Rodriguez-Bravo V, Carceles-Cordon M, Hoshida Y, Cordon-Cardo C, Galsky MD, Domingo-Domenech J. The role of GATA2 in lethal prostate cancer aggressiveness. *Nat Rev Urol* 2017; **14**: 38–48.
 19. Buskirk SJ, Pisansky TM, Schild SE, MacDonald OK, Wehle MJ, Kozelsky TF, et al. Salvage radiotherapy for isolated prostate specific antigen increase after radical prostatectomy: evaluation of prognostic factors and creation of a prognostic scoring system. *J Urol* 2006; **176**: 985–90. doi: <https://doi.org/10.1016/j.juro.2006.04.083>
 20. Trock BJ, Han M, Freedland SJ, Humphreys EB, DeWeese TL, Partin AW, et al. Prostate cancer-specific survival following salvage radiotherapy vs observation in men with biochemical recurrence after radical prostatectomy. *JAMA* 2008; **299**: 2760–9.
 21. Parker AS, Heckman MG, Wu KJ, Crook JE, Hilton TW, Pisansky TM, et al. Evaluation of ki-67 staining level as an independent biomarker of biochemical recurrence after salvage radiation therapy for prostate cancer. *Int J Radiat Oncol Biol Phys* 2009; **75**: 1364–70. doi: <https://doi.org/10.1016/j.ijrobp.2008.12.061>
 22. Parker AS, Heckman MG, Sheinin Y, Wu KJ, Hilton TW, Diehl NN, et al. Evaluation of B7-H3 expression as a biomarker of biochemical recurrence after salvage radiation therapy for recurrent prostate cancer. *Int J Radiat Oncol Biol Phys* 2011; **79**: 1343–9.
 23. Heinlein CA, Chang C. Androgen receptor in prostate cancer. *Endocr Rev* 2004; **25**: 276–308. doi: <https://doi.org/10.1210/er.2002-0032>
 24. Wu D, Sunkel B, Chen Z, Liu X, Ye Z, Li Q, et al. Three-tiered role of the pioneer factor GATA2 in promoting androgen-dependent gene expression in prostate cancer. *Nucleic Acids Res* 2014; **42**: 3607–22. doi: <https://doi.org/10.1093/nar/gkt1382>
 25. Rosa-Ribeiro R, Nishan U, Vidal RO, Barbosa GO, Reis LO, Cesar CL, et al. Transcription factors involved in prostate gland adaptation to androgen deprivation. *PLoS One* 2014; **9**: e97080. doi: <https://doi.org/10.1371/journal.pone.0106797>
 26. Pienta KJ, Bradley D. Mechanisms underlying the development of androgen-independent prostate cancer. *Clin Cancer Res* 2006; **12**: 1665–71. doi: <https://doi.org/10.1158/1078-0432.ccr-06-0067>
 27. Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. *Cell* 2009; **138**: 245–56. doi: <https://doi.org/10.1016/j.cell.2009.04.056>