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The role of GATA2 in lethal prostate cancer aggressiveness

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Abstract

Advanced prostate cancer is a classic example of the intractability and consequent lethality that characterizes metastatic carcinomas. Novel treatments have improved the survival of men with prostate cancer; however, advanced prostate cancer invariably becomes resistant to these therapies and ultimately progresses to a lethal metastatic stage. Consequently, detailed knowledge of the molecular mechanisms that control prostate cancer cell survival and progression towards this lethal stage of disease will benefit the development of new therapeutics. The transcription factor endothelial transcription factor GATA-2 (GATA2) has been reported to have a key role in driving prostate cancer aggressiveness. In addition to being a pioneer transcription factor that increases androgen receptor (AR) binding and activity, GATA2 regulates a core subset of clinically relevant genes in an AR-independent manner. Functionally, GATA2 overexpression in prostate cancer increases cellular motility and invasiveness, proliferation, tumorigenicity, and resistance to standard therapies. Thus, GATA2 has a multifaceted function in prostate cancer aggressiveness and is a highly attractive target in the development of novel treatments against lethal prostate cancer.

Prostate cancer is the second most frequently diagnosed type of tumour and the fifth leading cause of cancer-related deaths in men worldwide[1]. A subset of patients experience disease relapse and the development of metastases in distant organs despite undergoing watchful waiting or local therapy[2,3]. Treatment modalities that improve the survival of patients with advanced disease include androgen withdrawal[4,5], taxane chemotherapy[6–10], and second-generation androgen signalling inhibitors[11–14], among other therapeutic approaches including radium-223-mediated α emission[15] or active cellular immunotherapy using

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Competing interests statement

There authors declare no competing interests.

Review criteria

Literature for this review was selected by searching PubMed for the following key words: “prostate cancer”, “GATA2”, “pioneer transcription factor”, and “androgen receptor” alone or in combination. All papers selected were full text and in English. No limitations were used for year or period of publication. A number of references were identified from the reference lists of included papers.

sipuleucel-T[16]. However, advanced prostate cancer inevitably progresses to a therapy-resistant state that ultimately precedes lethality. Dissecting the molecular determinants of tumour progression to lethal prostate cancer will, therefore, stimulate new therapeutic strategies that will serve to improve the clinical outcome of patients with this disease.

The seminal studies by Huggins and Hodges in the 1940s[17,18] showed that tumour growth and survival depends on androgen receptor (AR) signalling throughout almost all stages of disease, despite its heterogeneous nature and without a full understanding of the natural course of this disease[19,20]. The mechanisms of disease progression to requirement for AR ablative therapy are classified as androgen-dependent (involving AR amplification or mutation) and androgen-independent (such as phosphatase and tensin homologue (PTEN) loss and RAC-α serine/threonine-protein kinase (AKT) activation). Mechanisms of progression to taxane therapy (docetaxel or cabazitaxel), the other mainstay of prostate cancer therapy, have also been extensively studied and include aberrant regulation of molecules involved in cell survival and death, overexpression of membrane efflux pumps, and modifications in the expression of the taxane target β-tubulin, among others[21].

In this Review we provide insight into the key function that endothelial transcription factor GATA-2 (GATA2), a transcription factor classically linked with haematopoiesis, exerts as a mediator of prostate cancer progression in both an AR-dependent and AR-independent manner and through the early to late stages of the disease. Whether it is via its role in regulating AR signalling as a pioneer factor, or owing to its function in driving a network of AR-independent signalling pathways, targeting of GATA2 might contribute to the development of new, much-needed therapeutic strategies for treating lethal prostate cancer.

The GATA2 transcription factor

The *GATA* family is a set of evolutionarily conserved genes that encode pioneer transcription factors, which regulate the development and differentiation of diverse tissues in eukaryotic organisms[22–24]. The family owes its name to their ability to bind specific DNA consensus sequences containing the GATA sequence (A/T-GATA-A/G) through two highly conserved zinc finger motifs[25,26]. In mammals, the GATA family contains six proteins, GATA1–GATA6, which in turn can be grouped into two subfamilies: GATA1–GATA3 are mostly involved in haematopoietic cell fate, and GATA4–GATA6 are implicated in endodermal and cardiovascular development[23,27,28] (FIG. 1a).

Most nuclear DNA is inaccessible owing to nucleosomal chromatin organization, leaving the vast majority of DNA-binding sites for transcription factors unoccupied, as has been shown using genome-wide location analyses[29]. In this context, pioneer transcription factors are characterized by their special ability to bind directly to these DNA regions of ‘closed’ chromatin, which is inaccessible to other factors, at some point before transcriptional activation. They either gain access passively or actively by opening and reorganizing chromatin, facilitating subsequent hierarchical binding of other regulators (transcription factors, cofactors, chromatin-modifying, and remodelling proteins) and activating gene expression[30] (FIG. 1b).

This nucleosome-binding ability is deemed essential for triggering transcription activation at otherwise silent chromatin sites that act as master regulators of signalling networks[31]. Interestingly, pioneer transcription factors might cooperate to achieve gene expression regulation, as shown by the close association between GATA and forkhead box protein A1 (FOXA) families in their role establishing temporal gene expression patterns in the undifferentiated gut endoderm[32,33].

GATA2 function in development

GATA2 was first demonstrated to be a critical component in the proliferation and differentiation of early haematopoietic progenitors and it has long been known to be an indispensable haematopoietic transcription factor[34,35]. Early loss-of-function studies showed that mice bearing null mutations at the *GATA2* locus developed severe anaemia early in embryogenesis, and, therefore, provided the first strong evidence that GATA2 involvement is fundamental to primitive haematopoiesis[34,35]. This evidence correlates with the observation that the emergence of definitive haematopoietic stem cells (HSCs) is dependent on the activation of GATA2 through neurogenic locus notch homologue protein 1 (NOTCH1)[36,37], which is fundamental for cell fate establishment and classically linked with haematopoietic development, among several other upstream regulators such as stem cell leukaemia protein (SCL), LIM domain only 2 protein (LMO2) and runt related transcription factor 1 protein (RNX1)[36]. Consequently, an interruption of the GATA2 signalling cascade results in an impairment of primary haematopoiesis, causing anaemia, decreased thrombopoiesis, and embryonic death owing to haematopoietic failure[24]. Moreover, GATA2 is mainly expressed in HSCs, early haematopoietic precursor cells (HPCs) and erythroid precursors, but its levels gradually decline as cells undergo differentiation and proliferation, and other factors such as GATA1 are eventually required for terminal maturation[38]. However, this decline in expression does not mean that GATA2 is absent in haematopoietic tissues of adult organisms[39,40], as GATA2 continues to have an important role in the maintenance of HSCs in the bone marrow of adults[41].

Given that GATA2 expression is especially observed in early progenitor cells, its implication in vascular development (especially through regulation of the commitment of haemangioblasts[42] — multipotent cell precursors that can differentiate into both endothelial cells and HPCs during normal vertebrate development) is not surprising[42,43]. The common origin of endothelial cells and HPCs indicates that several transcription factors involved in the differentiation of haemangioblasts to the blood lineage can also regulate their differentiation in to endothelial cells[44]. Indeed, GATA2 has been shown to bind and regulate the promoter activity of several endothelium-specific genes, including *PECAM1* (REF. 45) and *EDN1* (REF. 46), in addition to regulating the expression of *VEGFR2* (also known as *KDR*) during both vascular development and angiogenesis[47,48].

The central role that GATA2 has in early development hindered the investigation of its potential functions in later stages of development, owing to embryonic mortality in *GATA2*-null mice (as a result of an impairment in primitive haematopoiesis), and, therefore, masked the sequelae of *GATA2* ablation in these mice[49]. The critical role of GATA2 in urogenital development was revealed when *GATA2*-null mice were rescued using a yeast artificial

chromosome (YAC) transgene[49]. YAC-rescued *GATA2*-null mice survived embryonic development but succumbed to lethal hydronephrosis and displayed a complex array of genitourinary abnormalities that resulted in postnatal mortality[49]. Further analysis revealed that GATA2 is indeed expressed in embryonic structures that give rise to the urogenital system, explaining the numerous postnatal abnormalities observed in rescued *GATA2*-null mice, including defects in the ureters, seminal vesicles, and vas deferens[49]. These findings were later confirmed by the discovery of at least three separate urogenital-specific *GATA2* enhancer elements in rescued *GATA2*-null mice[50]. Moreover, GATA2 gene and protein expression have been further confirmed in the developing mouse prostate as well as the adult mouse and human prostate[51]. Altogether, these studies suggest that GATA2 is a lineage marker with important master regulatory functions in the development of both haematopoietic and genitourinary systems.

Role of GATA2 in prostate cancer

The role of GATA2 in the pathogenesis of prostate cancer is of increasing interest, owing to the observation that it is a major contributor to the development of the urogenital and haematopoietic systems. GATA2 is involved in the pathogenesis of malignancies originating in organs in these systems. For example, heterozygous mutations in *GATA2* are associated with a broad spectrum of haematological disorders[52–56] leading to the hypothesis that GATA2 expression might also contribute to the development and progression of prostate cancer (FIG. 2). GATA2 is a critical component in the complex regulatory network of transcription factors that sustains prostate cancer growth in both AR-dependent prostate cancer and castration-resistant prostate cancer (CRPC), in which it acts as a supporter and mediator of AR signalling[57,58]. Aside from this well-characterized role, data demonstrate that GATA2 further contributes to the lethal progression of CRPC in an AR-independent manner[59].

Computational analysis of the gene expression profiles of paired metastatic and nonmetastatic prostate cancer xenografts identified *GATA2* as a potential regulator of metatasis[60]. Moreover, *GATA2* silencing using small interfering RNA in LNCaP cells showed a decrease in cell migration and tissue invasion. *GATA2* silencing resulted in persistence of focal adhesion complexes, indicating that it has a crucial role in focal adhesion disassembly. Genes associated with prostate cancer progression and metastasis, including *FOXM1, c-MYC, UHRF1, EZH2, BMP6, AURKA*, and *BIRC5* were also downregulated in *GATA2*-silenced LNCaP cells[61]. Notably, increased GATA2 mRNA and protein expression in tissue samples from patients with clinically localized prostate cancer was significantly associated with increased Gleason score and tumour stage ($P = 0.008$ and $P = 0.001$, respectively)[62] and an increased probability of disease relapse and developing distant metastasis[61–63]. GATA2 mRNA and protein levels were higher in samples from metastatic sites than primary tumour specimens[59,61,62]. This evidence suggests that GATA2 is a novel target for the treatment of prostate cancer.

GATA2 and androgen-dependent prostate cancer

The androgen-dependent stage of prostate cancer is arguably one of the earliest stages of disease progression[19,20] and is defined by a marked dependence on androgens for cell survival and tumour growth. In this disease stage, the AR and its co-activators bind to canonical AR regulatory elements in genes such as *PSA* (also known as *KLK3*), which is upregulated in prostate cancer[64], and *TMPRSS2*, which can fuse with the oncogenes *ERG* or *ETV1* and can mediate their androgen-responsive overexpression in prostate tumours[65].

GATA2 regulates the metastatic potential of prostate cancer cells in the early stages of disease through various regulatory mechanisms. GATA2 has been shown to increase the metastatic potential of androgen-responsive LNCaP cells. *In vitro*, *AZGP1* expression was decreased in *GATA2*-overexpressing LNCaP cells on treatment with 5α-dihydroxytestosterone[63]. Decreased *AZGP1* has been reported as a strong predictor of poor clinical outcomes in men with prostate cancer[66]. GATA2-mediated and AR-mediated changes in gene expression in prostate cancer suggest that crosstalk between both of these transcription factors has an essential role in androgen-dependent prostate cancer[57,58,63,67]. Discerning the role of GATA2 in early-stage prostate cancer could have major implications for understanding disease progression and is a new avenue for research in this area. Wu and colleagues[58] observed that GATA2 acts as a pioneer factor that drives androgen-responsive gene expression through a three-tiered role acting at a genome-wide level. Firstly, it binds to an upstream promoter region of *AR* regulatory elements and enhances basal and androgen-stimulated *AR* expression. Secondly, GATA2 mimics FOXA1 function in creating an accessible chromatin environment and establishes an accessible local chromatin environment in *AR* enhancers by recruiting p300, a histone acetylase transferase (HAT) that activates chromatin via acetylation of H3K27 (REFS 58,68). Finally, it forms and maintains regulatory chromatin loops between *AR*-bound distal enhancers and AR-target-gene promoters via recruitment of the mediator co-regulatory complex subunit MED1 (REF. 58).

GATA2, as a pioneer transcription factor, is required for AR binding to *PSA*, *TMPRSS2*, and *PDE9A* enhancers and has been suggested to be a critical factor in a theoretical hierarchical regulation network that could drive androgen-dependent, AR-target-gene expression[57]. It participates in a complex interaction between transcription factors, notably POU domain, class 2, transcription factor 1 (POU2F1, also known as OCT1) and FOXA1 (REFS 57,58). POU2F1 exerts its function subsequent to GATA2 and acts with AR and is, therefore, not considered a pioneer factor necessary for AR-mediated expression of PSA or TMPRSS2 (REF. 67). However, FOXA1 co-occupies AR-target-gene enhancers with GATA2 — GATA motifs and GATA2 binding events are considerably enriched in parallel with FOXA motifs and FOXA1 binding events within these regulatory regions[57,58]. Sequence analyses of AR, GATA2, and FOXA1 using chromatin immunoprecipitation showed that GATA2 and AR strongly enhance each other's transcriptional programmes and that FOXA1 regulates the cistromes of not only AR but also GATA2, acting, therefore, as a pioneer factor for both[69].

Collectively, these studies demonstrate that GATA2 is part of a complex hierarchical network of transcription factors that regulate AR signalling in the setting of androgen-dependant prostate cancer, and highlight its importance and contribution in these early stages of this disease.

GATA2 and castration-resistant prostate cancer

Androgen-deprivation therapy (ADT) can be achieved using surgical (bilateral orchiectomy) or chemical castration, and has been considered the frontline therapeutic strategy for locally advanced and metastatic androgen-dependent prostate cancer for over 40 years[70]. Chemical castration is generally achieved using gonadotropin-releasing hormone (GnRH) agonists, GnRH antagonists, or antiandrogens[70]. ADT has proven to be effective in achieving disease remission and PSA concentration decline in 90% of patients[71]. However, after a mean time of 24–36 months, the disease inexorably evolves to a castratation-resistant state associated with poor prognosis and a short survival time[72].

Increased tumour growth despite only castrate levels of androgens in circulation being present is a hallmark of CRPC[73]. Much experimental evidence demonstrates that AR signalling is enhanced during the development of CRPC[72,74]; however, mechanisms of CRPC development that bypass the AR altogether have also been described[74]. Mechanisms of progression towards CRPC include AR hypersensitivity (such as AR amplification), the outlaw AR pathway (including events such as AR activation by receptor tyrosine-protein kinase erbB-2), AR promiscuity (that can be caused by *AR* mutations that increase binding to nonandrogen steroids) and activation of bypass pathways (such as myc proto-oncogene protein activation)[75]. Notably, altered AR signalling in CRPC is also proposed to occur through several mechanisms that include changes in the expression of co-activators and co-repressors, including increased GATA2 gene and protein expression[67]. The contribution of GATA2 to progression to CRPC is supported by data from a prostate cancer xenograft mouse model of androgen-dependent disease that suggest GATA2 expression levels increase throughout the shift from an androgen-dependent disease status to a castration-resistant one[76]. Moreover, GATA2 levels are higher in hormone-refractory tumour samples than hormone-dependent specimens[59].

Nevertheless, the mechanisms through which GATA2 results in increased AR signalling and translates into cell proliferation and survival in the androgen-depleted context are far from being clearly understood. Mechanistically, resistance to androgen deprivation might act in a similar manner to that of androgen-dependent growth — that is, through the ability of pioneer factors FOXA1 and GATA2 to interact with AR and increase its transcriptional activity[67]. In this context, marked H3K4 histone methylation would recruit these transcription factors and induce AR binding to a set of M-phase cell-cycle, AR-target genes involved in prostate growth[67,77,78]. He and colleagues[62] showed that GATA2 regulates AR signalling in CRPC; silencing *GATA2* resulted in decreased AR gene and protein expression in LNCaP cells. Notably, exogenous restoration of full-length AR or truncated AR variants failed to restore the activity of AR-regulated genes including *PSA*, *TMPRSS2*, and *FKBP5* when *GATA2* was silenced. Taken together, these results suggest that GATA2 has a key role in maintaining AR signalling activity in CRPC.

Alternative treatment modalities such as taxane therapy (including docetaxel and cabazitaxel) and second-generation androgen inhibitors (for example, enzalutamide) have emerged as therapeutic strategies capable of improving survival of patients with CRPC[21,79]. However, despite all current therapeutic efforts, the disease can eventually progress to an AR-independent state in which the tumour does not inherently rely on AR signalling. One of

the most important pathways that bypasses AR involves the loss of the tumour-suppressor gene *PTEN*, resulting in the activation of RAC-α serine–threonine-protein kinase (AKT), phosphorylation of Bcl2-associated agonist of cell death (BAD) and, finally, in the release of apoptosis regulator Bcl-2 (REF. 80), which triggers cell survival and proliferation. Overexpression of ubiquitin-conjugating enzyme E2 C (UBE2C) has also been observed to drive AR-negative CRPC cell growth and survival *in vitro*, although the mechanisms that regulate this process have not been fully elucidated[78]. GATA2 seems to be a newly discovered mediator of aggressiveness in AR-negative CRPC cells through the regulation of a signature of cancer-progression-associated genes which include *IGF2, PAK4, FOXM1, GLNT7* and *ARRDC3* (REF. 59). Upregulation of *GATA2* is associated with chemotherapy resistance and tumorigenicity in docetaxel-resistant cell lines and progression to lethal disease in men treated with chemotherapy, and *GATA2* knockdown resensitizes docetaxel-resistant cell lines to docetaxel and cabazitaxel and increases apoptosis on treatment. *In vivo*, knockdown of *GATA2* in patient-derived, castration-resistant, taxane-resistant xenografts generated from circulating tumour cells results in reduced tumour growth[59]. RNA sequencing revealed a signature of 28 GATA2-regulated genes that are associated with cancer development and progression, cell death and survival, and cell growth and proliferation. The genes in this signature were enriched in a population of men who had lethal prostate cancer and men with prostate cancer who had received chemotherapy. Analysis of a subset of these genes showed their expression was unaffected by AR knockdown *in vitro* suggesting that GATA2 regulates clinically relevant AR-independent genes that contribute to resistance to chemotherapy[59]. Mechanistically, results from GATA2 silencing and overexpression studies demonstrated that it regulates insulin-like growth factor II (IGF2) expression. GATA2 achieves this regulation by directly binding to the *IGF2* promoter region and IGF2 then activates a downstream survival polykinase network that renders cells resistant to taxane therapy. The contribution of GATA2 and its effector gene *IGF2* to the aggressiveness of late lethal disease stages has been validated in tumour samples from patients; immunohistochemistry showed IGF2 expression increases during the progression of prostate cancer from primary disease to CRPC, and that expression levels are highest in men who have received taxane therapy. Furthermore, increased levels of both GATA2 and IGF2 were associated with poor response to docetaxel treatment in chemotherapy-naive men with CRPC[59].

In summary, study results have unveiled a multifaceted and critical role for GATA2 in driving the aggressiveness of prostate cancer through all the stages of disease. First by enhancing AR signalling through its pioneer transcription factor function in early stage disease, and then by acting as a master regulator that controls a signalling network of effector genes associated with cancer aggressiveness independently of the AR.

Targeting GATA2 in prostate cancer

Targeting GATA2 is a highly attractive therapeutic strategy that might improve the clinical outcome of patients with prostate cancer, owing to its contribution to disease progression and cancer aggressiveness. In particular, inhibition of GATA2 in the context of both CRPC and taxane-resistant prostate cancer would be a valuable and novel alternative strategy to targeting AR signalling independently of androgen ligand binding and also to disrupting the

survival signalling network that contributes to the acquisition of taxane resistance. GATA2 blockade could be applicable and beneficial for a broad spectrum of men with prostate cancer in different clinical contexts, ranging from early-stage disease to aggressive CRPC.

To date, attempts to directly target GATA2 have not been pursued and GATA2 continues to be considered an undruggable transcription factor[81]. Indeed, critical information that is required in order to attempt direct targeting of GATA2 has not been gathered. For example, the 3D protein conformational structure of GATA2 (REFS 82,83), which would help identify compounds that bind specific domains and inhibit GATA2 activity, remains to be defined. Despite these limitations, studies investigating the mechanisms that underlie GATA2 expression and its signalling network have been undertaken. Novel therapeutic strategies that inhibit GATA2 activity have been discovered in these studies, which can be divided into the targeting of upstream *GATA2* regulators, post-translational and/or epigenetic GATA2 activating mechanisms, and downstream GATA2 effector signalling pathways (FIG. 3).

Targeting upstream regulatory signalling pathways of GATA2

The NOTCH signalling pathway has been extensively reported to be an upstream regulator of GATA2 activity in the haematopoietic system and to also regulate GATA2 expression in prostate cancer cells[84–89]. NOTCH signalling controls haematopoietic progenitor cell expansion and differentiation via upregulation of *GATA2* expression[84–89] by directly binding the promoter region of *GATA2* and consequently activating its transcription[37]. In prostate cancer cells, NOTCH signalling regulates proliferation, metastasis, and progression[90–93]. Functionally, the biological effects of NOTCH are mediated directly by activation of the canonical NOTCH pathway and through crosstalk between NOTCH and other pathways such as the PTEN–phosphoinositide 3-kinase (PI3K)–AKT pathway[94]. Indeed, prostate cancer cell aggressiveness properties are considerably increased through activation of the PI3K–AKT pathway by NOTCH[93]. Results from a 2015 study demonstrated that knockdown of *NOTCH2* substantially reduced GATA2 protein levels in chemotherapy-resistant CRPC cells, thereby establishing that GATA2 is a target of NOTCH signalling in prostate cancer cells[59]. Considered together, these studies suggest that GATA2 activity can be targeted through inhibition of NOTCH activity. Currently, strategies that target NOTCH signalling, either through anti-NOTCH antibodies or by inhibiting γ secretase activity, are being investigated[95,96]. Testing the capacity of these therapeutics to inhibit GATA2 activity as a surrogate marker of efficient NOTCH pathway inhibition in prostate cancer could be of particular interest in the future.

Several other upstream pathways have also been reported to regulate GATA2 activity and these pathways also have a key role in the pathogenesis and aggressiveness of prostate cancer[97–104]. However, the definitive role of these pathways in regulating GATA2 activity in the prostate cancer setting is yet to be investigated. For example, the protein C-ets-1 (ETS) transcription factor potassium voltage-gated channel subfamily H member 2 (ERG), which is known to have a critical role in prostate cancer tumorigenesis[105], directly regulates the transcription of *GATA2* by binding to its promoter region in embryonic haematopoietic stem cells[97]. Similarly, the ETS1 transcription factor regulates *GATA2* expression in haematopoietic progenitor cells[98]. Furthermore, the Hedgehog signalling pathway, which is

critical in the pathogenesis of prostate cancer[106], controls *GATA2* expression and considerably effects in the cell fate of neurons[99], adipocytes[100] and the development of the pituitary gland[101], therefore, suggesting that Hedgehog signalling could increase prostate cancer aggressiveness through GATA2. Moreover, members of the transforming growth factor β (TGFβ) family activin A, bone morphogenetic protein 2 (BMP2), and BMP4 that have been implicated in the regulation of haematopoiesis have been shown to regulate *GATA2* by repressing its transcription[102–104]. In a mouse model, loss-of-function mutations in TGFβ pathway genes resulted in decreased plasminogen activator inhibitor-1 levels, a downstream target of TGFβ, and was associated with increased prostate tumour grade and metastasis[107], therefore, suggesting that TGFβ could be regulating the invasiveness properties of prostate cancer cells through GATA2. These data indicate a potential link between the regulation of GATA2 by the TGFβ pathway in prostate cancer development and progression.

In conclusion, many molecules and signalling pathways that are involved in the pathogenesis of prostate cancer also serve as regulators of GATA2 expression. Future studies are warranted to elucidate their specific role in regulating GATA2 expression in the context of prostate cancer and to discover novel targeting approaches that can be implemented as therapeutic measures against this disease.

Targeting post-translational and epigenetic GATA2 activating mechanisms

Post-translational modifications have been extensively implicated in regulating GATA2 activity. For example, acetylation of lysine in the GATA2 protein controls protein structure and function[108]. Acetylation of histone and nonhistone proteins is mediated by histone acetyl transferases (HATs) and histone deacetylases (HDACs) and is paramount to the accessibility of transcription factors such as GATA2 to chromatin. Importantly, GATA2 contains multiple acetylation sites, which are predominantly located in its zinc finger regions[108]. Acetylation of GATA2 mediated by the co-activator histone acetyltransferase p300 (p300)–CREB-binding protein (CBP) enhances its DNA binding activity in haematopoietic precursors and other cell types, such as human kidney epithelial cells and fibroblast-like cells[108,109]. Furthermore, p300–CBP has a critical role in promoting AR-mediated transcription of prostate cancer associated genes, such as *PSA*, and prostate cancer progression[110,111]. Notably, p300 expression levels correlate with those of GATA2 and are highest in aggressive prostate tumours[111], suggesting that the relationship between GATA2 and AR could be regulated by p300 acetylase activity and providing a rational for modulating GATA2 acetylation status using targeted therapy to reduce its activity in prostate cancer.

Shah and colleagues[112] inhibited p300–CBP HAT activity using the isoflavone compound curcumin and observed decreases in pioneer factor function and AR signalling. Exposure of hormone-dependent and CRPC cells to curcumin did not affect AR protein expression, but did reduce AR signalling activity by decreasing GATA2 and FOXA1 occupancy of *PSA* and *TMPRSS2* enhancer regions. *In vivo*, treatment of xenografts with curcumin resulted in a considerable decrease in tumour growth in both hormone-dependent and CRPC tumours, an observation that might be explained by the ability of curcumin to interrupt AR signalling in

an AR-ligand-independent manner by binding to p300 and inhibiting its activity. Importantly, CRPC xenografts that responded to curcumin included those derived from 22Rv1 cells, which have constitutive AR activity and are unresponsive to second-generation antiandrogen therapy. This observation suggests that a disruption in GATA2 acetylation could be a useful therapeutic strategy for targeting prostate cancer tumour cells in which AR signalling continues to be active in a AR-ligand-independent manner.

In the clinical setting, encouraging results have been reported regarding the relevance of curcumin for treating prostate cancer. An initial study showed that curcumin decreased serum PSA concentrations in patients with chronic inflammation of the prostate[113], and another study showed that men with prostate cancer receiving a dietary supplement containing curcumin experienced a significantly lower rise in serum PSA concentration than patients receiving placebo ($P = 0.0008$)[114]. Curcumin has shown to be well tolerated[115] and patients have a good response rate to therapy[116] when it is combined with docetaxel therapy; a randomized study is now required to validate these results. Curcumin might synergize with second-generation AR antagonists such as enzalutamide, owing to inhibition of AR signalling through an AR-independent mechanism, and increase the therapeutic window for treating patients with CRPC. However, the clinical benefits of curcumin could also be attributed to its inherited pleotropic antitumour mechanisms of action (such as inhibition of nuclear factor κ-B (NF-κB) and signal transducer and activator of transcription 3 signalling) or to dietary curcumin supplements that contain other bioactive polyphenols with potential antitumour activity[117–119] that could disguise or alter the perceived effect of curcumin on tumour progression. Prospective clinical studies that clearly monitor GATA2 activity and subsequent tumour growth when treated with specific curcumin derivatives are needed to ensure that the reported antitumour effect curcumin exhibits are indeed mediated through inhibition of GATA2.

The regulation of GATA2 activity through acetylation has been well documented; however, the role of deacetylation of transcription factors in general and GATA2 in particular is not as well understood. Data suggest that deacetylation might be a general mechanism of GATA2 regulation in human cells, demonstrating that deacetylases HDAC3 and HDAC5 suppress GATA2 transactivation activity in haematopoietic and nontumour cells[109] and that the homopiperazine derivative K-7174 inhibits GATA2 transcriptional activity in hepatoma cells[120], and HDAC1, HDAC2, and HDAC3 in myeloma cells by inhibiting proteasome acitivity[121]. K-7174 disrupts GATA2 and AR signalling and inhibits hormone-dependent prostate cancer and CRPC growth *in vitro* and *in vivo*[62]. K-7174 acts differently in prostate cancer cells from myeloma cells[62], in which it strongly decreases GATA2 *in vitro*, suggesting that it acts by decreasing GATA2 protein stability. These data are counterintuitive as K-7174 inhibits HDAC3, which itself represses GATA2. Thus, one would expect K-7174 treatment to result in an increase in GATA2 expression in prostate cancer cells. Further investigation is needed to determine why the effects of K-7174 are different in these different contexts. Interestingly, GATA2 is turned over rapidly through the ubiquitin-proteasome pathway[122], and F-box and WD repeat domain-containing 7 is an E3 ubiquitin ligase for GATA2 (REF. 123) that destabilizes GATA2 and promotes its proteasome degradation, therefore, suggesting a potential mechanism through which K7174 could exert its effects on GATA2 in the context of prostate cancer. However, the mechanism of action of

K-7174 remains ill-defined and further studies are needed to identify the clear mechanism of action of this compound in prostate cancer cells.

Similar to acetylation, GATA2 activity can be modulated through phosphorylation. GATA2 phosphorylation occurs via mitogen-activated protein kinase 1 (MAPK) signalling in haematopoietic progenitor cells[124] and the PI3K–AKT signalling pathway in human embryonic kidney cells[125], different GATA2 phosphorylation sites have differing effects. For example, p38 MAPK phosphorylates GATA2 at serine 192 and activates GATA2 transcription in haematopoietic cells[126,127], whereas phosphorylation of serine 401 impairs GATA2 activity by decreasing nuclear translocation and DNA binding in adipocytes[125]. These studies indicate that phosphorylation of GATA2 can regulate its activity in a context-dependent manner. Experimental results from studies in prostate cancer cells suggest that a positive feedback loop exists, whereby activated AKT partially regulates GATA2 expression[59]. Overexpression of constitutively active AKT (myristoylated AKT) increased the mRNA and protein levels of GATA2 in prostate cancer cells, and treatment with the AKT inhibitor MK-2206 reduced GATA expression levels. Treatment with pharmacological inhibitors of AKT resensitized prostate cancer cells to taxane therapy and mimicked the results produced after knockdown of *GATA2* expression[59]. Collectively, these studies highlight the potential crosstalk between GATA2 and kinases that control prostate cancer aggressiveness. Additional work is required to characterize the kinases implicated in the phosphorylation of GATA2 and the sites upon which they exert their function, as well as the functional consequences that such post-translational modifications of GATA2 bear in such setting.

GATA2 is rarely mutated or amplified in prostate cancer, which is contrary to observations regarding *GATA2* in haematopoietic malignancies[128–130], leading to the hypothesis that *GATA2* expression can be epigenetically modulated. Methylation is known to have a crucial role in regulating gene expression in cancer cells, and DNA hypomethylation is generally associated with gene activation, whereas hypermethylation is associated to gene silencing. *GATA2* expression has been shown to be modulated epigenetically through hypermethylation and hypomethylation in haematopoietic stem cells[131] and haematopoietic malignancies[132]. A 2015 study reported that the methylation status of *GATA2* significantly ($P = 0.009$) varies between prostate adenoma and prostate cancer samples[133], suggesting that *GATA2* methylation could have a role in the development of prostate cancer. Targeting methylation has proved to be an efficacious clinical therapeutic strategy for a subset of patients diagnosed with myelodysplasia[134]. Thus, further studies are warranted to investigate whether methylation contributes to *GATA2* expression and whether *GATA2* can be pharmacologically targeted in prostate cancer through this mechanism.

Targeting GATA2 downstream effector pathways

Functional studies have identified and characterized the GATA2 gene and protein effectors in the haematopoietic system, lung cancer, and prostate cancer. Some of these effectors can be pharmacologically targeted, offering an opportunity to therapeutically inhibit GATA2 in prostate tumours and reduce their aggressiveness.

GATA2 regulates a well-organized signalling network that operates within the haematopoietic system and includes specific downstream effectors that drive haematopoietic cell fate and malignancy[24,135,136]. GATA2 downstream effectors have since been characterized in solid tumours, with Kumar and colleagues[81] describing how GATA2 contributes to the aggressiveness of *Kras*-mutated non-small cell lung cancer (NSCLC) by regulating the activity of the proteasome, IL-1–NF-κB and Rho signalling pathways. Moreover, combined suppression of these GATA2 -regulated pathways in lung cancer cells and in a *Kras*-mutated mouse model of NSCLC with clinically approved inhibitors bortezomib and/or fasudil resulted in a dramatic inhibition of tumour growth[81]. Collectively, these results demonstrate that joint inhibition of GATA2 effectors causes substantial regression of *Kras*-mutated NSCLC.

The majority of studies on GATA2 in prostate cancer have focused on its effects on AR effectors, owing to their observed interaction. Promising therapeutic strategies focused on inhibiting GATA2 acetylation are currently in preclinical and clinical development[62,112]. However, and most importantly within the context of advanced prostate cancer, novel GATA2 effectors independent of AR signalling have been identified[59]. Transcriptome profiling after *GATA2* knockdown in docetaxel-resistant cells yielded a signature of GATA2-regulated genes, which was enriched in clinical datasets of advanced prostate cancer and included genes with well-established oncogenic (*IGF2*, *FOXM1*) and tumour suppressor (*ARRDC3*) functions. Some of the cell lines used in this study do not express AR (DU145 and ArCaPM), therefore, this gene set did not include the large number of co-regulated GATA2–AR genes, meaning GATA2 effectors independent of AR could be identified. Mechanistic studies focused on elucidating the regulation of *IGF2* by GATA2 showed that GATA2 activates the transcription of *IGF2* by directly binding to its promoter, and activation of *IGF2* by GATA2 initiates a polykinase survival cascade that enabled prostate cancer cells to survive taxane therapy, among other stressful conditions.

IGF2 is a ligand for the insulin-like growth factor 1 receptor (IGF1R) and for the insulin receptor (INSR)[137], and dual pharmacological inhibition of IGFR1 and INSR using OSI-906 (REF. 138) increased taxane sensitivity and overall survival of mice bearing cell-line and patient-derived xenografts[59]. Thus, targeting downstream GATA2 effectors could offer a new therapeutic strategy for treating patients with prostate cancer. GATA2 downstream effectors are not limited to *IGF2* and for example include a multitude of metastogenic genes such as *ARRDC3*, *DPYSL3*, *FOXM1*, *GALNT7*, and *PAK4*. In addition, GATA2 might regulate the expression of other molecules with unknown implications for cancer, such as the transmembrane nucleoporin nuclear envelope pore membrane protein POM 121 and the postsynaptic protein neurogranin. Thus, mechanistic studies coupled with *in vivo* validation using preclinical models are strongly warranted to elucidate the function of the most clinically relevant GATA2 effectors that have the potential to be exploited by novel drugs as therapeutic targets to inhibit the lethal progression of advanced prostate cancer.

Conclusions

Therapeutic advances have improved the survival of patients with advanced prostate cancer, but the disease inexorably follows an eventually lethal course. A substantial and growing

body of evidence has been gathered suggesting that the pioneer transcription factor GATA2 contributes to the lethality of prostate cancer by enhancing metastatic dissemination of tumour cells and sustaining their survival and progression to standard therapies. The functional and molecular characterization of the mechanisms that activate GATA2 have resulted in the identification of novel potential therapeutic strategies that might delay the progression of this disease. However, from a clinical perspective, implementation of GATA2 targeting in prostate cancer still requires the development of robust and precise predictive assays that identify which patients are most likely to respond to therapy, as well as the rational design of clinical trials in which the combination therapy with the largest therapeutic index is identified.

The prognostic and predictive value of both gene and protein GATA2 expression in patients with prostate cancer indicate that GATA2 is a specific and targetable biomarker of prostate cancer aggressiveness that meets the criteria required for the design of specific detection assays that can be used to drive clinical decisions at the personalized level. Specific assessment of GATA2 activity in prostate cancer specimens from biopsy samples through GATA2 protein immunostaining and gene expression analyses have been developed, are easy to interpret, reproducible, and can be readily implemented into the clinic. In addition, the implications of targeting GATA2 have tremendous potential from a personalized medicine perspective, as it is a prognostic biomarker with predictive value for determining response to standard therapies, and is potentially targetable using novel therapeutic interventions such as GATA2 inhibitors. In this context, the therapeutic development of selective potent GATA2 inhibitory agents will have to meet the criteria for safety and will have to proceed with caution, especially in the context of preventing dose-limiting haematological toxicities, as GATA2 is known to be active in normal haematopoietic stem cells.

The anticipation of potential toxicities might favour the rational design of clinical trials testing combination strategies with standard therapies that do not increase the risk of severe haematologic toxicities, such as second-generation antiandrogen agents. In this context, notably, curcumin, which inhibits GATA2 activity in preclinical models of prostate cancer, was well tolerated in a phase II study when given in combination with the myeloablative taxane agent docetaxel in patients with prostate cancer, suggesting that taxane myelosuppressive effects are not enhanced when GATA2 inhibitors are used concomitantly. Despite these results, care and effort should be taken in the development of novel GATA2 inhibitors and these toxicity-related limitations acknowledged. The design of phase I clinical trials for GATA2 inhibitors should include close monitoring of this potential dose-limiting toxicity. Altogether, the translation of GATA2 inhibitors into the clinic will require the development and validation of new precise prognostic and predictive assays that measure GATA2 expression and the anticipation of toxicities specific to GATA2 inhibition in the design of clinical trials.

In summary, the molecular and functional characterization of GATA2 in prostate cancer has advanced the understanding of the development and progression of this disease and might result in improved stratification of patients with prostate cancer with regards to prognosis and response to standard therapy, as well as novel treatment approaches that might improve the survival of men with prostate cancer.

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Key points

- Endothelial transcription factor GATA-2 (GATA2) is a pioneer, master-regulator, transcription factor that binds DNA regions of closed chromatin, causing opening and facilitating subsequent hierarchical binding of other regulators that activate transcription
- GATA2 is crucial for the development of the genitourinary system and might be a lineage marker of mouse and human prostate tissue
- GATA2 drives androgen-responsive gene expression and contributes to prostate cancer metastasis by increasing tumour cell motility and invasiveness in early stages of the disease, through its pioneer transcription factor function
- GATA2 is important in prostate cancer progression to an androgen-refractory state and regulates an androgen-independent signalling network in late stages of the disease
- Preclinical experimental data have demonstrated the utility of inhibiting GATA2 through targeting its upstream regulators, post-translational modifications, and downstream effectors
- Integrating GATA2 inhibition into the therapeutic landscape of prostate cancer will require the development of precise predictive assays and identification of the most effective therapeutic combination

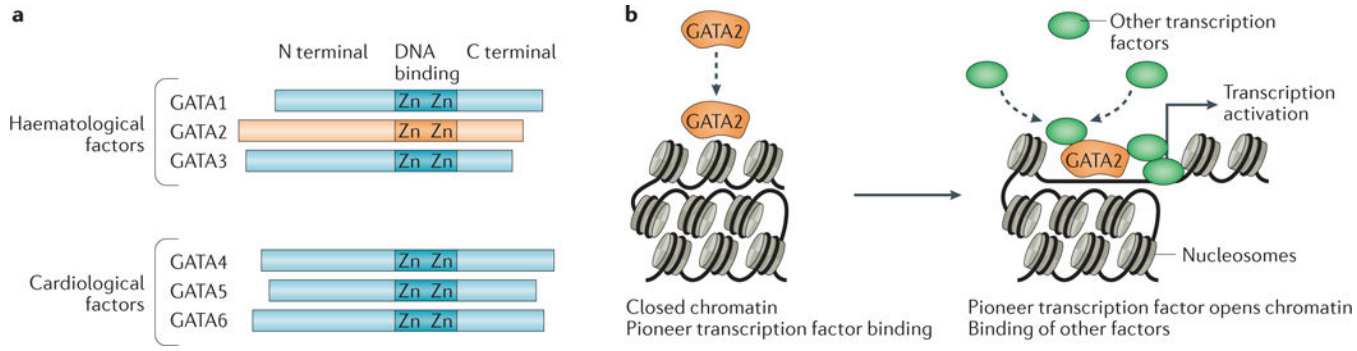

Figure 1. The pioneer endothelial transcription factor GATA-2 (GATA2)

a | Schematic representation of the six endothelial transcription factor GATA (GATA) family members containing two zinc DNA binding domains. GATA1–GATA3 are classified as haematological factors and GATA4–GATA6 are classified as cardiological factors. **b** | Schematic representation of the pioneer function of the GATA2 transcription factor. The GATA2 transcription factor is the first to bind to closed chromatin and enables the binding of other factors, activating gene transcription.

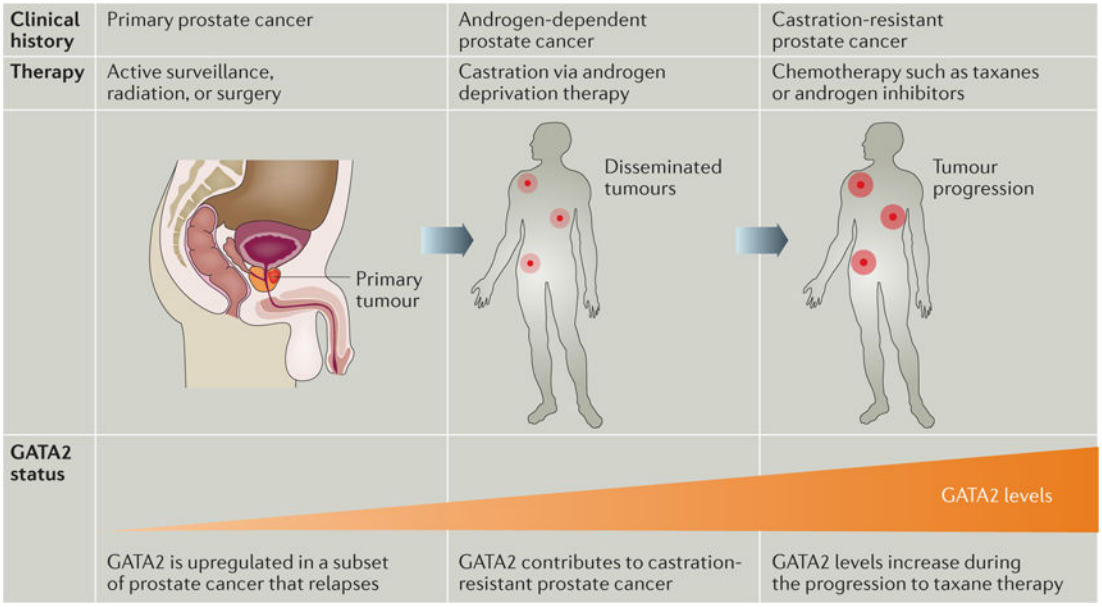

Figure 2. Endothelial transcription factor GATA-2 (GATA2) regulates lethal prostate cancer aggressiveness

During prostate cancer progression, GATA2 contributes to disease relapse in early stages of the disease and to the progression to androgen ablation and taxane therapies by promoting prostate cancer cell invasiveness, dissemination, and survival.

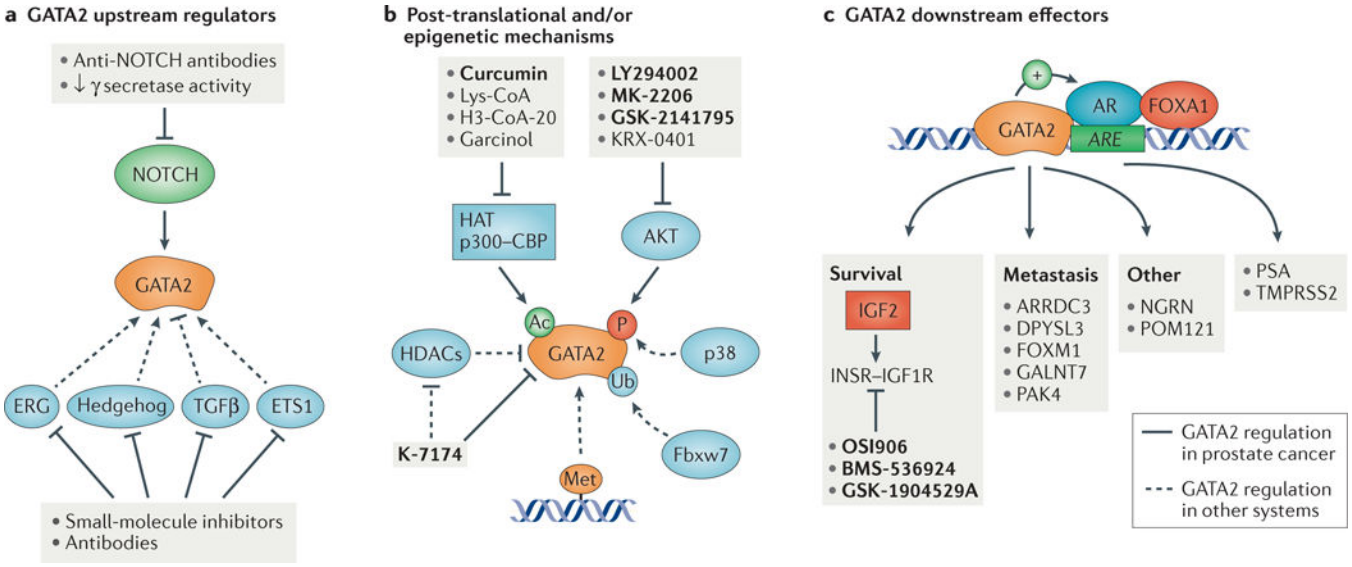

Figure 3. Different strategies for targeting endothelial transcription factor GATA-2 (GATA2) in prostate cancer

a | Inhibiting GATA2 upstream regulators such as notch homologue protein (NOTCH), which is known to affect GATA2 in prostate cancer, with anti-NOTCH antibodies or inhibiting γ secretase activity. Also targeting effectors involved in GATA2 regulation in other systems that might have a role in prostate cancer, such as Hedgehog, potassium voltage-gated channel subfamily H member 2 (ERG), protein C-ets-1 (ETS1) and transforming growth factor (TGFβ) using antibodies or small molecule inhibitors. **b** | Targeting post-translational and/or epigenetic mechanisms such as acetylation (Ac) by inhibiting molecules involved in signalling pathways such as histone acetylase transferase (HAT) p300–CREB-binding protein (p300–CBP) with curcumin, Lys-CoA, H3-CoA-20, or Garcinol; phosphorylation (P) by targeting RAC-alpha serine/threonine-protein kinase (AKT) with LY294002, MK-2206, GSK-2141795, or KRX-0401; targeting p38; targeting histone deacetylases (HDACs), or GATA2 itself with K-7174; targeting ubiquitination (Ub) by F-box and WD repeat domain-containing 7 (Fbxw7); or targeting methylation. **c** | Targeting downstream effectors involved in prostate cancer cell survival and metastasis by inhibiting specific effector pathways including survival through insulin-like growth factor 2 (IGF2) using dual insulin-like growth factor 1 receptor (IGF1R) and insulin receptor (INSR) inhibitors such as OSI906, BMS-536924, or GSK-1904529A; potentially, metastasis could be targeted through arrestin domain-containing protein 3 (ARRDC3), dihydropyrimidinase-related protein 3 (DPYSL3), forkhead box protein M1 (FOXM1), *N*-acetylgalactosaminyltransferase 7 (GALNT7), or serine/threonine-protein kinase PAK 4 (PAK4), or other pathways could be inhibited through targeting molecules such as neurogranin (NGRN) or nuclear envelope pore membrane protein POM 121 (POM121). Furthermore, androgen receptor (AR) activity could be affected through targeting androgen response elements (AREs) such as PSA and transmembrane protease serine 2 (TMPRSS2). Highlighted in bold are drugs that have been used experimentally to inhibit GATA2 in prostate cancer, NOTCH, acetylase, and dual IGF1R and insulin receptor INSR inhibitors that have shown antitumour activity. Continuous lines indicate pathways that have been

described in prostate and dashed lines described pathways that have been described in other systems.