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FOXA1: a transcription factor with parallel functions in development and cancer

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Synopsis

When aberrant, factors critical for organ morphogenesis are also commonly involved in disease progression. FOXA1 (forkhead box A1), also known as HNF3α (hepatocyte nuclear factor 3α), is required for postnatal survival due to its essential role in controlling pancreatic and renal function. In addition to regulating a variety of tissues during embryogenesis and early life, rescue experiments have revealed a specific role for FOXA1 in the postnatal development of the mammary gland and prostate. Activity of the nuclear hormone receptors ERα (oestrogen receptor α) and AR (androgen receptor) is also required for proper development of the mammary gland and prostate respectively. FOXA1 modulates ER and AR function in breast and prostate cancer cells, supporting the postulate that FOXA1 is involved in ER and AR signalling under normal conditions, and that some carcinogenic processes in these tissues stem from hormonally regulated developmental pathways gone awry. In addition to broadly reviewing the function of FOXA1 in various aspects of development and cancer, this review focuses on the interplay of FOXA1/ER and FOXA1/AR, in normal and cancerous mammary and prostate epithelial cells. Given the hormone dependency of both breast and prostate cancer, a thorough understanding of FOXA1's role in both cancer types is critical for battling hormone receptor-positive disease and acquired anti-hormone resistance.

Keywords

androgen receptor (AR); breast; foxhead box A1 (FOXA1); foxhead box A2 (FOXA2); oestrogen receptor (ER); prostate

INTRODUCTION

FOXA1 (forkhead box A1) is the founding member of the FOX family of transcription factors that is comprised of at least 40 members (reviewed in [1]). FOXA1/HNF3α

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(hepatocyte nuclear factor 3α), FOXA2/HNF3β and FOXA3/HNF3γ constitute the FOXA subfamily, which was originally identified for its transcriptional regulation of the genes liver-specific (*Ttr*) transthyretin and α1-antitrypsin (*Serpinal*) [2]. Since this seminal study, FOXAs have been found to regulate many genes involved in developmental specification of not just hepatic, but several other tissues (reviewed in [3]). FOXAs contain an ~ 100 amino acid DNA-binding domain or FOX/winged helix domain that is highly conserved (at least 92%) within the FOXA family [4], and shares extremely high homology (90%) with that of its namesake, the *Drosophila* homologue *fkh* (forkhead) [4,5]. FOXAs also contain conserved nuclear localization sequences and homology in the N- and C-terminal transactivation domains [4,6,7]. FOXAs bind as monomers [8] to the consensus element A(A/T)TRTT(G/T)RYTY [9] and crystallization of the DNA-binding domain of FOXA3 revealed a 'winged helix' structure bound to DNA in a manner similar to that of linker histones [8]. Importantly, unlike linker histones, FOXAs lack the basic amino acids required for chromatin compaction [10]. Thus, FOXA binding to nucleosomes induces an open chromatin configuration enabling the recruitment of other transcriptional regulators[10–12]. This function has led to FOXAs being coined as 'pioneering' or 'licensing' factors. Recently, the participation of FOXA1 in chromatin remodelling has been further described, where FOXA1 binding to DNA precedes the loss of cytosine methylation and the dimethylation of histone H3 lysine K4 (H3K4) during the differentiation of pluripotent P19 cells [13].

The ability of FOXAs to remodel heterochromatin provides a mechanistic basis for how these factors initiate transcriptional cascades involved in both development and disease. Specifically, FOXA1 is required for normal development of the mammary gland and prostate, and is necessary for both ER (oestrogen receptor)- and AR (androgen receptor)-regulated transcription in hormone receptor-positive breast and prostate cancers respectively. An understanding of FOXA1 during development has generated great insight into its function in cancer progression and *vice versa*. This review focuses on the similarities and differences between the normal developmental role of FOXA1 and that involved in cancer.

FOXA1 IN DEVELOPMENT

Identifying FOXAs as transcriptional regulators of hepatic specification [2] led multiple groups to perform expression pattern analyses for these factors from early development throughout adulthood. *Foxa2* mRNA is the first to be expressed during embryogenesis, and is observed during gastrulation in the anterior primitive streak and node with subsequent expression in the notochord, floor plate and gut [14–16]. *Foxa1* becomes detectable at the late primitive streak stage in the midline endoderm of mouse embryos, followed by expression in the ventral floorplate, notochord and gut [14–16]. *Foxa3* is the last to be activated, being expressed during hindgut differentiation [15]. In the adult mouse, expression of FOXA1 and FOXA2 is observed within endoderm-, mesoderm- and ectoderm-derived tissues [17,18]. *Foxa3* mRNA is less restricted in adult tissues, being present within the heart, adipose tissue, ovary and testis, in addition to endoderm-derived liver and gastrointestinal tissues [17]. These results, in combination with the chromatin-remodelling function observed for FOXA, provided evidence that FOXAs may function in developmental specification. Through both germ-line and conditional knockout approaches, the functions of

FOXA1, FOXA2 and FOXA3 have been investigated both independently and, in the case of FOXA1 and FOXA2, in combination, and each has been proven to be required during various aspects of development (reviewed in [1,3,19]). Briefly, the loss of *Foxa2* is embryonic lethal due to failed node and notochord development [20]. *Foxa1* null mice survive through embryogenesis, but are postnatally lethal due to severe hypoglycaemia and dehydration as described in detail below [21,22]. In contrast, *Foxa3* knockout mice are normal, developing without morphological defects through adulthood [23], but are hypoglycaemic in response to fasting [24] and the males are subfertile [25]. The severity of these respective phenotypes parallels the onset of expression of *Foxa2*, then *Foxa1* and then *Foxa3* during embryogenesis, and suggests compensatory roles for FOXA2 in the *Foxa1* knockout mice, and for both FOXA2 and FOXA1 in *Foxa3* knockout mice. The involvement of FOXA1, specifically, will be discussed herein and is overviewed in Table 1.

Pancreas and kidney

Mice that are homozygous null for *Foxa1* lack any overt morphological abnormalities [21,22]. However, they are postnatally lethal due to a combined phenotype of severe hypoglycaemia and dehydration. Interestingly, loss of *Foxa1* also dramatically decreases circulating glucagon levels. This is paradoxical to the observed hypoglycaemic state because the pancreas normally responds to hypoglycaemia by releasing glucagon, which then induces conversion of glycogen stores into glucose in the liver. Further investigation revealed that FOXA1 is normally expressed in glucagon-expressing pancreatic α-islet cells, where it is necessary to transcriptionally activate the proglucagon promoter (*Gcg*) [21]. Loss of *Foxa1* reduces glucagon transcription, subsequently decreasing glucagon secretion into the circulation. These results confirm a role for FOXA1 in regulating glucose homoeostasis through directing α-islet cell function. In addition to the loss of proglucagon gene expression, *Foxa1* null mice also fail to secrete insulin in response to glucose administration [22]. This defect is attributed to the up-regulation of mitochondrial *Ucp2* (uncoupling protein 2) that occurs in the pancreatic β-cells of *Foxal* null mice [26]. Increased UCP2 expression uncouples oxidative phosphorylation, which decreases glucose-mediated ATP synthesis and insulin secretion from β-islet cells. Together, these studies revealed that FOXA1 modulates glucose homoeostasis through multiple mechanisms: transcriptional activation of the proglucagon gene and repression of *Ucp2* expression.

To investigate the role of FOXA1 during pancreatic development without the physiological complexity of a global knockout, mice were generated with *loxP* sites flanking exon 2 of *Foxa1*. These mice were then crossed with *Pdx1-Cre*^{Early} transgenics mice [27]. PDX-1 (pancreas and duodenal homeobox protein-1) expressing cells generate each cell type (exocrine, endocrine and duct) of the pancreas allowing for pancreas-selective expression of *Cre* DNA recombinase and disruption of floxed transgenes [28]. Surprisingly, *Pdx1-Cre*^{Early}; *Foxa1*$^{loxP/loxP}$ mice are viable and fertile indicating that the postnatal lethality observed in the germ-line knockouts [21] was not solely due to an isolated defect in the pancreas, but likely also involved severe dehydration [27]. Compound conditional knockout mouse studies subsequently revealed that FOXA1 and FOXA2 co-operatively control pancreatic acinar and islet morphogenesis [27]. The presence of at least one wild-type allele of *Foxa2* can compensate for complete loss of *Foxa1* in the pancreas, resulting in viable mice.

Interestingly, the absence of one or two wild-type alleles of *Foxa1* dictates the level of specification of exocrine and endocrine cell lineages in the pancreas of mice completely lacking *Foxa2* [27,29]. In other words, mice that are wild-type, heterozygous null and homozygous null for *Foxa1* in the pancreas have the most normal, intermediary and severe disruption respectively of pancreatic lineage specification when combined with the homozygous null *Foxa2* allele. For a detailed description of FOXA1/2 compound mutants that have been generated to investigate their functions in the pancreas and other tissues, see [3,19]. Notably, mice null for *Foxa3* are hypoglycaemic when fasted due to decreased expression of the GLUT2 (glucose transporter) [24], and thus each FOXA family member is necessary for maintaining glucose homoeostasis.

In addition to the deregulation of pancreatic proglucagon and *Ucp2* expression, pups lacking FOXA1 are severely dehydrated [21], prompting studies to investigate FOXA1 function in the kidney. *Foxa1* null mice develop nephrogenic diabetes insipidus as shown by the inability to respond to arginine-vasopressin [ADH (anti-diuretic hormone)] [30]. Interestingly, the development of this disease could not be explained by a decrease in the expression of genes encoding the vasopressin 2 receptor, aquaporins or other proteins involved in water reabsorption. Although the precise mechanism by which FOXA1 modulates kidney function remains to be determined, it has been postulated that the lethality of the *Foxa1* null animals occurs as a result of the combined impact of pancreatic insufficiency and kidney defects. This is supported by the viability of mice with a pancreatic specific deletion of *Foxa1* (*Pdx1-Cre*^{Early}; *Foxa1*$^{loxP/oxP}$) as discussed above. It is unclear whether the kidney defect in *Foxa1* null mice is due to a direct effect on this organ or changes that occur in response to an abnormal developmental programme. Use of a kidney-specific inducible knockout would facilitate addressing this question, as well as provide a resource for identifying the specific genes which are controlled by FOXA1 that ultimately establish arginine-vasopressin responsiveness. In addition, such animals would generate a potential model for nephrogenic diabetes insipidus in humans. Identifying the specific role FOXA1 plays in water reuptake should ultimately provide important insights for clinical management of this disease.

Liver

Given the role for FOXA proteins in regulating liver-specific gene transcription [2], it was hypothesized that FOXA1 would be necessary for normal liver development. However, while *Foxa1* null mice die postnatally [21], they have a morphologically normal liver. Similar to the maintenance of normal pancreatic morphology in *Foxa1* knockouts due to FOXA2 compensation, FOXA2 also offsets loss of *Foxa1* in the liver. When investigated combinatorially, genomic loss of *Foxa1*, within the context of an endoderm-specific conditional deletion of *Foxa2* (*Foxa3-Cre*; *Foxa2*$^{loxP/loxP}$), completely blocked the onset of liver specification whereas conditional loss of *Foxa2* alone had no effect [31]. The endodermal disruption of *Foxa2* occurs at E8.5 (embryonic day 8.5) when using *Foxa3-Cre* to induce recombination, and this precedes liver specification. To investigate liver development after the onset of specification, *Alf-pCre*; *Foxa1*$^{loxp/loxp}$; *Foxa2*$^{loxp/loxp}$ mice were generated [32]. In these mice, *Cre* expression occurs at E10.5 in the liver primordium, but complete loss of *Foxa1/2* was not observed until P2 (postnatal day 2). These mice

undergo normal hepatocyte differentiation, but during adulthood develop bile duct hyperplasia and fibrosis as a result of increased IL-6 (interleukin-6) expression. Under normal conditions, FOXA1/2 and the GR (glucocorticoid receptor) co-operate to repress *Il6* transcription. In the absence of FOXA1/2, this repression is ablated, allowing NF-κB (nuclear factor κB)-induced up-regulation of *Il6*. The excessive IL-6 then induces proliferation of bile duct epithelial cells (cholangiocytes) [32]. These results demonstrate the combined importance of FOXA1 and FOXA2 in liver cell specification and differentiation, and suggest a potential role for FOXA1/2 in human diseases of the liver. Along these lines, FOXA2 expression is decreased in the livers of humans with cholestatic disease [33], but a role for FOXA1 in biliary diseases remains to be established.

Lung

FOXA1 is observed in the lung bud at E10.5, and is maintained in mature conducting airway epithelial and secretory alveolar type II cells [18]. Mice null for *Foxa1* have delayed lung development [34]. Normally, the fetal lung develops through three distinct morphological phases: pseudoglandular, canalicular and saccular. While lungs of wild-type mice reach the saccular stage by E16.5, those lacking FOXA1 are delayed in the canalicular stage until E17.5. Similarly, lung septation in postnatal *Foxa1* knockout mice (P5) was significantly delayed. Loss of *Foxa1* also decreased the expression of lung differentiation markers [e.g. CCSP (clara cell secretory protein), SP-B (pro-surfactant protein-B) and SP-C (pro-surfactant protein-C)] during embryogenesis. This is at least partly due to the dependency of both CCSP (*Scbglal*) [35] and SP-B (*Sftpb*) [36,37] promoters on FOXA1 for activation in lung epithelium. How FOXA1 modulates SP-C expression is not known. Although FOXA1 deficiency causes a developmental delay during embryogenesis, histology of the knockout lungs was indistinguishable from controls by P13. That said, cuboidal type II cells had higher glycogen levels, a decreased number of lamellar bodies and less surfactant than those in wild-type lungs. As in other tissues (e.g. pancreas and liver), FOXA2 eventually compensates for loss of *Foxa1* in the lung. Complete loss of *Foxa1*, combined with doxycycline-induced deletion of *Foxa2* driven by *Cre* expression under control of the SP-C lung-specific promoter [$Foxa1^{-/-}$ / $SPC\text{-}rtTA^{-ltg}/(tetO)_7Cre^{-ltg}/Foxa2^{loxP/loxP}$] during embryogenesis, drastically impairs lung branching morphogenesis and epithelial differentiation [38]. Similar to *Foxa1* null lungs, CCSP expression is absent in the compound mutants. In addition, loss of *Foxa1* and *Foxa2* decreases mRNA expression of sonic hedgehog (*Shh*), a necessary activator of lung epithelial specification [39,40]. FOXA1 expression is increased in a rat model of ALI (acute lung injury), in which it is suggested to function in alveolar type II epithelial cell apoptosis [41], and is similarly necessary for H_2O_2-induced apoptosis in A549 cells [42]. Together, these results imply FOXA1 may participate in the onset or progression of lung diseases not limited to cancer as discussed below. It will be important to generate mice with lung specific conditional deletion or transgenic overexpression of FOXA1 to gain mechanistic insight into the precise role FOXA1 may play in these diseases.

Brain

FOXA1 and FOXA2 are expressed in the notochord and floor-plate of the fetal brain [14–16], and *Foxa2* null mice are embryonic lethal as a result of failed node and notochord

development [20,43]. Although *Foxa1* null pups do not exhibit overt CNS (central nervous system) architectural defects [21,22], they have delayed maturation of mature mDA (midbrain dopaminergic) neurons [44]. Conditional loss of *Foxa2* using a *Nestin-Cre* transgene dose-dependently exacerbates the *Foxa1* null phenotype, indicating that even one copy of *Foxa2* compensates for *Foxa1* loss and *vice versa*. The phenotypic response to FOXA loss has been attributed to the ability of these proteins to control expression of neurogenin 2, Nurr1 and EN1 (engrailed 1), three factors essential for neuronal differentiation [45–47]. Complimenting these studies, conditional loss of both *Foxal* and *Foxa2*, through breeding with *En1-klCre* transgenic mice, led to disruption of each FOXA protein earlier in development (E9.75) [48] compared with *Nestin-Cre* (E10.5)-mediated disruption [44]. Through these two approaches, Lin et al. [48] showed that FOXA1/2 positively regulate expression of the LIM homeodomain transcription factors Lmx1a/1b and negatively regulate the morphogenically associated homeodomain protein Nkx2.2 earlier in development. Intriguingly, FOXA1 and FOXA2 are redundantly necessary for the initiation of SHH expression in the midbrain [48,49], paralleling the regulation of *Shh* by FOXA1 observed in the lung that was discussed previously. Further demonstrating the importance of FOXA in SHH signalling, at least in this context, FOXA1/2 are also responsible for restricting the expression of Gli1 and Gli2, transcriptional mediators of SHH signalling, and patched-1, the receptor for SHH, in the ventral midbrain [49].

Gastrointestinal tract

FOXA1/2 are also expressed in the epithelia of the gastrointestinal tract [18]. To investigate how the combined loss of *Foxa1* and *Foxa2* specifically affects intestinal development, floxed alleles were conditionally disrupted using the villin promoter to direct expression of Cre (*Vil-Cre*) to the intestinal epithelium [50]. The compound-conditional mutants are viable, but grow slower, and have decreased body weight due to a reduction in lean muscle mass and less body fat. Although overall intestinal morphology is normal, the number, secretory capacity and differentiation of goblet cells are reduced in the combined mutants. FOXA1, but not FOXA2, was found to bind to the promoter of the *Muc2* gene that encodes mucin 2. These results compliment previous *in vitro* studies in which FOXA1 and FOXA2 can both transactivate the *Muc2* promoter, with FOXA1 being more efficient [51]. In addition to diminished goblet cell formation, loss of *Foxa1/2* leads to decreased differentiation of L- and D-cell enteroendocrine lineages [51], which produce hormones involved in intestinal function. Paralleling *in vitro* regulation of the proglucagon promoter by FOXA1/2 [21,52], intestines of the combined conditional mutants lack D-cells, as shown by loss of cells expressing GLP (glucagon-like peptides)-1 and -2. In addition, cell populations that express somatostatin and PYY (peptide YY) (D- and L-cells respectively) were decreased. Furthermore, expression of Islet-1 and Pax6, two transcription factors involved in enteroendocrine cell differentiation, are decreased, providing evidence that FOXA1/2 precede Islet-1 and Pax6 expression in the enteroendocrine transcriptional hierarchy. These results indicate that FOXA proteins are essential for development of both mucin-producing goblet and enteroendocrine cellular populations. The loss of these cell types probably results in insufficient nutrient absorption required for normal growth, thus providing an explanation for the decreased growth rate of these mice during early life.

Prostate

Morphogenesis of the prostate is dependent on stromal AR expression, whereas prostatic secretory function relies on epithelial AR expression [53,54]. FOXA1 is an established regulator of AR transcriptional activity in prostate cancer cells (reviewed below) leading to an investigation of FOXA1 during prostate development. Expression analyses revealed that FOXA1 is present in prostate tissue from the onset of development at E18 in the urogenital sinus epithelium throughout adulthood, with no detectable expression in the stroma [55–57]. Considering the postnatal lethality of the *Foxa1* null mice, Matusik and co-workers [58] investigated prostate morphogenesis in the absence of FOXA1 through utilization of two distinct rescue strategies: renal capsule grafting and tissue recombination. While loss of *Foxa1* does not alter rudiment formation, luminal epithelial lineage differentiation is blocked as shown by luminal CK8 (cytokeratin 8) staining being present only in concert with the basal epithelial lineage marker p63. Furthermore, CK5-positive basal cell and α-SMA (α-smooth muscle actin)-positive smooth muscle cell populations were aberrantly expanded. The epithelium within the *Foxa1* null prostate also fails to polarize and undergo proper lumen formation, and, as expected, results in a secretion defect. While prostatic tissue lacking epithelial AR has a similar secretory phenotype [54], *Foxa1* null prostates maintain epithelial AR expression. Since FOXA1 facilitates AR transcriptional activity as discussed below, it is possible that while FOXA1 is not required for AR expression, it is necessary for AR function in the developing prostate, thus resulting in the observed secretory defect. This is supported by the loss of *Pbsn* (probasin) and *Sbp* (spermine-binding protein) mRNA, which are normally stimulated by AR activation, in the *Foxa1* null prostate glands [58].

Interestingly, FOXA2 is aberrantly up-regulated in *Foxa1* knockout prostate epithelium [58]. Normally, FOXA2 is only found in prostatic epithelia during embryogenesis, and in a small population of mature basal epithelial cells that co-express syn-aptophysin, an NE (neuroendocrine) marker [57,59]. Thus, unlike in other tissues (e.g. lung, liver and pancreas), FOXA2 does not compensate for FOXA1 loss in the prostate. As discussed below with regard to prostate cancer, the up-regulation of FOXA2 in the *Foxa1* null prostates may enable AR function in an androgen-independent manner, resulting in the observed hyper-proliferative phenotype. Of note, loss of *Foxa1* also decreases expression of the homeobox gene *Nkx3.1*, a putative tumour suppressor whose deficiency also leads to prostatic hyperplasia [60]. Prostates lacking FOXA1 also have increased SHH, an established contributor to prostate carcinogenesis [61–63]. Interestingly, the negative regulation of SHH by FOXA1 in the prostate differs from the positive regulation observed in lung and brain. This may be due to the absence of FOXA1/FOXA2 collaboration in the prostate, but this possibility has not yet been tested.

Mammary gland

In breast cancer, FOXA1 expression positively correlates with that of ER and another transcription factor, GATA3 [64–66], both of which are necessary mediators of normal mammary gland development [67–70]. This strong correlation, the known up-regulation of *FOXA1* as a result of GATA3 overexpression in HEK-293T cells [HEK-293 cells expressing the large T-antigen of SV40 (simian virus 40)] [71], and the co-expression of FOXA1 and GATA3 in mouse mammary epithelia, prompted Kouros-Mehr et al. [70] to investigate

whether GATA3 could regulate FOXA1 expression in the normal mammary gland. Using ChIP (chromatin immunoprecipitation), the authors showed that GATA3 binds to the *Foxal* promoter in mouse primary mammary epithelial cells. Further investigation into a mammary-specific role for the GATA3-interacting protein, FOG2 (friend of GATA2), revealed that conditional disruption of *Fog2* by *WAP* (whey acidic protein)-*Cre*-mediated excision resulted in decreased *Foxal* mRNA expression during involution [72]. However, limitations in the technical design of this study, specifically the lack of epithelial-specific normalization approaches, leave open the possibility that FOG2 does not directly regulate *Foxal* expression. A more recent study investigating FOXA1 protein expression in virgin adult mammary glands lacking *Gata3* following *MMTV* (murine mammary tumour virus)-*Cre*-induced conditional deletion failed to detect a change in the percentage of FOXA1-positive cells in response to *Gata3* disruption compared with wild-type controls [73]. These results indicate that GATA3 is not required for FOXA1 expression in the normal mammary gland. However, they do not negate GATA3 binding to the *Foxal* promoter in primary mammary cells [70] and leave open the possibility that GATA3 may regulate FOXA1 expression in other tissue types [71].

The observed correlation between FOXA1 and ER in human breast cancer also led to *in vitro* functional studies that revealed an essential role for FOXA1 in mediating transcriptional regulation by ER, as reviewed below. These results suggested that FOXA1 may be necessary during mammary morphogenesis simply due to its requirement for full ER activity. Supporting this hypothesis, FOXA1 and ER are co-expressed in mammary luminal epithelium during development [73]. To directly evaluate the role for FOXA1 in this process, we rescued *Foxa1* null mammary tissue via renal capsule grafting and orthotopic transplantation [73]. Loss of *Foxa1* completely blocks epithelial outgrowth in the orthotopic transplant model, and inhibits ductal invasion in renally transplanted glands. Unlike in the prostate, *Foxal* knockout mammary epithelium exhibits no observable alterations in the lineage markers α-SMA, CK8 and E-cadherin. Another difference between mammary and prostate responses to FOXA1 loss involves expression of their cognate steroid hormone receptors. While AR expression is maintained in the *Foxal* null prostate, loss of *Foxal* in the mammary gland is accompanied by a lack of epithelial ER expression. In support of these *in vivo* findings, FOXA1 binds to the *ESRl* promoter and is required for ER mRNA and protein expression in breast cancer cells [73]. Combined, these results revealed that FOXA1 is not only required for ER activity but also for its expression. The transcriptional hierarchy of FOXA1, ER and GATA3 has also been further refined. Mammary glands deficient in ER maintain expression of FOXA1 and *Gata3* [73]. In contrast, mammary glands deficient in GATA3 maintain FOXA1 [73], but lose ER [69,70]. Together, these results place both GATA-3 and FOXA1 upstream of ER, and also reveal that the expression of GATA-3 and FOXA1 are not interdependent. Interestingly, unlike the secretary defects seen in the pancreas, lung, intestine and prostate discussed above, loss of *Foxal* in the mammary gland does not affect lobulo-alveolar maturation and milk production [73]. This result was unexpected because epithelial ER is required for lobulo-alveologenesis [67,74,75]. One explanation is the possibility that FOXA1 actively represses maturation of the alveolar lineage, and its loss in virgin epithelia leads to premature alveologenesis independently of ER. To test this possibility, a novel mouse model must be developed wherein FOXA1

expression is enforced during pregnancy. If alveologenesis fails to occur in the presence of pregnancy-associated hormones, this would suggest that FOXA1 plays two, somewhat opposing, roles in the mammary gland. Specifically, it may promote morphogenesis of ducts, but inhibit terminal differentiation of alveoli. Lastly, it is interesting to note that like in the prostate, FOXA2 is not expressed in the mature mammary gland [18]. Thus the mammary gland represents a second tissue where FOXA1 acts independently of its related FOXA member to control morphogenesis.

FOXA1 IN CANCER

Over the past decade, FOXA1 expression has been examined in several human cancers, with proposed oncogenic and tumour suppressive roles depending on the type, and, in some cases, cancer subtype (Table 2). Our knowledge of FOXA1's participation in four of the cancer types [AML (acute myeloid leukaemia), oesophageal, lung, pancreatic and thyroid] is very much in its infancy with only one or two reports evaluating a role for FOXA1 in each of these diseases. Briefly, *FOXA1* is increased in AML patient samples that are chromosomally normal, but harbour oncogenic mutations in FLT3 (fms-related tyrosine kinase 3) and NRAS (neuroblastoma rat sarcoma viral oncogene homologue) [76]. Similarly, the *FOXA1* gene is amplified in anaplastic thyroid carcinomas [77], and in a subset of oesophageal and lung cancers [78]. These results suggest that aberrant FOXA1 transcriptional activity may promote tumorigenesis or the level of tumour aggressiveness in these tissues. Further supporting this possibility, FOXA1 expression is correlated with lymph node metastases in ESCC (oesophageal squamous cell carcinoma) and loss of FOXA1 *in vitro* decreases cellular invasion and migration in an oesophageal cancer cell line [79]. In stark contrast, loss of either FOXA1 or FOXA2 in pancreatic cancer cells induces EMT (epithelial-to-mesenchymal transition), implying a metastasis suppressive role in this disease [80]. The combined knockdown of FOXA1/2 in pancreatic cancer cells leads to an even greater reduction in epithelial-specific gene expression and increased cell motility than silencing either factor alone, suggesting that these genes may co-operate to inhibit metastatic progression. Given the compensatory effect of FOXA2 observed in the pancreas-specific knockout of *Foxal* during development, it is not surprising that the two FOXA factors have similar functions in cancer cells derived from this tissue. Determining whether loss of either FOXA1 or FOXA2 actually increases metastasis formation in pancreatic cancer mouse models may reveal regulatory mechanisms that modulate this highly aggressive cancer, and lead to the identification of FOXA target genes that could serve as novel therapeutic targets.

Studies investigating the development of the lung, brain and prostate in the absence of FOXA1 have revealed a role for FOXA1 in regulating SHH expression as discussed above. SHH is oncogenic in several cancers [81] including those of the brain, oesophagus, lung and pancreas, all organs for which FOXA1 is either necessary for proper development, or has been implicated in tumorigenesis (Tables 1 and 2). Thus it is feasible that FOXA1 functionally interplays with SHH in one or more of these cancer types to promote initiation and/or progression. Experimental manipulation of FOXA1 expression in established cancer cell lines and transgenic mouse modelling should reveal what role, if any, FOXA1 plays in these cancers. The impact on SHH signalling should also be investigated upon altering FOXA1 expression to determine if FOXA1 mediates tumorigenicity through transcriptional

regulation of this pathway. Although FOXA1 has yet to be implicated in CNS tumours, the aberrant up-regulation of Gli1, a transcriptional modulator of SHH, in the developing frog leads to neural tube hyperplasia [82]. Loss of *Foxal* reduces expression of Gli1/2 in the murine brain [49], thus FOXA1 has the potential to mediate oncogenic SHH signalling in brain cancer. Additionally, although it is clear that FOXA2 compensates for loss of *Foxal* during development of the brain, lung and pancreas, it will be critical to investigate whether this compensation exists in cancerous cells.

As indicated above, loss of both *Foxal* and *Foxa2* induces bile duct hyperplasia in the murine liver [32]. These results suggest that FOXA proteins may reduce tumour susceptibility, but, interestingly, expression of both FOXA1 and FOXA2 is maintained in chemically induced mouse liver cancer models [83,84]. Although counterintuitive, these results may indicate that FOXA1 is differentially expressed in a less aggressive subtype of HCC (hepatocellular carcinoma) possibly represented in these mouse models. This is supported by results suggesting both FOXA1 and FOXA2 positively regulate mir122, a microRNA expressed in a subset of HCC that is correlated with favourable prognosis [85], and would be similar to the differential expression of FOXA1 seen in the varying subtypes of breast cancer as discussed below. The bile duct hyperplasia observed in the *Foxal/2* null liver stems from diminished FOXA1/2 co-operation with GR in the repression of *Il6* [32]. Importantly, FOXAs are implicated in GR-mediated transactivation of numerous other target genes [86–94], and GR signalling is anti-apoptotic in hepatoma cells [95]. Thus, the potential for pro-tumorigenic FOXA/GR signalling in HCC requires further evaluation. It will be necessary to investigate the expression profile of FOXA1 and FOXA2 in a large cohort of HCC patient samples and compare the presence or absence of FOXA1/2 with aberrant GR/IL-6 signalling to determine whether there is a clinically relevant connection between these pathways.

It is critical to note that while the studies discussed above are suggestive that FOXA1 functions in a pro-tumorigenic or anti-tumorigenic capacity, the rigorous approaches necessary to define FOXA1 as an oncogene (overexpression causes cancer), tumour suppressor (loss causes cancer) or metastasis suppressor (overexpression blocks metastasis) have not been performed for any cancer type. That said, the role for FOXA1 in prostate and breast cancers has been studied to a much greater degree than in any other type of cancer. In these cancers, disease aggressiveness and associated patient prognosis are complicated by multiple cancer subtypes and states of hormone dependence or independence. The known participation of FOXA1 in prostate and breast cancer is detailed below.

FOXA1 in prostate cancer

As previously discussed, FOXA1 is critical for prostate development and loss of *Foxa1* causes epithelial hyper-proliferation, activation of oncogenic SHH and decreased expression of the tumour suppressor Nkx3.1 [58]. These results suggest a tumour suppressive role for FOXA1 in prostate epithelia, and pose several questions regarding if, and how, FOXA1 regulates prostatic tumour initiation, progression and/or metastasis. While *Foxa1* null prostates maintain epithelial AR expression, they lack AR-dependent secretory differentiation, implying that FOXA1 may modulate AR activation of this process [58].

Given the majority of prostate cancers are driven by aberrant AR signalling, much work has been done to define a role for FOXA1 in this process.

FOXA1 expression in prostate cancer

The LPB-Tag (T antigen) LADY mouse model of prostate cancer expresses SV40 large Tag under control of a large (12 kb) fragment of the prostate-specific probasin promoter (LPB), giving rise to prostate tumours that recapitulate human disease [96]. LADY tumours have increased nuclear expression of FOXA1 and AR that accompanies Tag [59]. Interestingly, FOXA1 is retained in both androgen-dependent (12T-7f) and androgen-independent (12T-10) models. Furthermore, 12T-10-associated liver metastases maintain FOXA1. Together, these results argue against a tumour suppressive role for FOXA1 and suggest that it may function independent of androgens. FOXA1 has also been examined in the TRAMP (transgenic adenocarcinoma of mouse prostate) mouse model that differs from LADY by expressing both SV40 large T- and small t-antigens downstream of a minimal probasin promoter [97,98]. Chiaverotti et al. [99] examined murine strain-specific differences on TRAMP-induced tumour formation and found that expression of the transgene in the FVB/N background decreases survival and increases the incidence of NE carcinomas compared with TRAMP-C57BL/6 animals. NE carcinoma, although rare in humans, is unresponsive to androgen ablation therapy [100]. Interestingly, TRAMP-induced NE carcinomas co-express FOXA1 and FOXA2. Normal prostate epithelia and atypical hyperplasia have high levels of FOXA1 and AR, whereas FOXA2 is only expressed within a small percentage of mature prostate basal epithelial cells [57,59]. This, along with co-expression of epithelial (E-cadherin) and NE (syn-aptophysin) lineage markers, support the possibility that NE carcinomas initiate within a bipotential epithelial population that is more susceptible to tumorigenic processes in FVB/N compared with C57BL/6 mice. The combined expression of FOXA1 and FOXA2 in NE tumours may provide a growth advantage in the absence of androgens. Indeed, *in vitro* analyses revealed that FOXA2 activates PSA (prostate-specific antigen) promoter activity in an androgen-independent fashion, and suggested that the conversion from FOXA2-negativity into FOXA2-positivity may be responsible for the transition from androgen-dependence to androgen-independence [59].

FOXA1 mRNA positively correlates with human prostate cancer cell line and xenograft models that have been defined as androgen dependent [101]. Examination of FOXA1 and FOXA2 expression in a small cohort of human prostate adenocarcinomas indicated that FOXA1 is present in all samples independent of Gleason score, with the level of expression being indistinguishable from benign tissue [59]. In contrast, FOXA2 is undetectable in low-grade prostate adenocarcinomas, but is found in NE small cell carcinomas similar to the TRAMP mouse model discussed above. The expression of FOXA1 was not reported for these human NE samples [59]. Recent studies investigating FOXA1 protein levels in primary and metastatic human prostate tumours, revealed high FOXA1 expression positively correlates with metastatic disease and poorer outcomes [102,103]. In addition, an amplicon at 14q21, which includes *FOXA1*, has been identified in metastatic prostate tumours [104]. Lastly, examination of FOXA1 and AR expression revealed poorer outcomes from prostate cancer when these two proteins are co-expressed compared with expression of AR alone [103]. Overall, these studies confirm that FOXA1 expression is maintained in mouse models

of prostate cancer as well as human tumours, but more robust expression analyses in human prostate tumours are necessary to delineate its association with patient outcomes and anti-androgen treatment response. In addition, the specific functions that FOXA1 may have in androgen-dependent compared with androgen-independent disease require further exploration. Determining if FOXA1 can be used as a predictor of anti-androgen therapeutic success could provide a valuable diagnostic in the management of prostate cancer.

FOXA1 and the androgen receptor

Most men diagnosed with prostate cancer have AR-positive, hormone-dependent disease that is initially responsive to androgen-ablation therapy. Unfortunately, the majority of these cases become resistant to this therapeutic approach due to acquisition of androgen-independence [105]. To better understand AR signalling within both androgen-dependent and -independent contexts, the co-operation of FOXA1 with AR transactivation has been investigated, albeit with some discrepancies in the resulting data [59,106–109]. Initial reports indicated that FOXA1 is necessary for AR activation of target gene expression [106]. These studies involved blockade of FOXA1 binding to the probasin and PSA promoters by mutating the FOXA1 consensus sequences within the promoters. Such disruption blocked androgen (R1881)-induced promoter activity [106], suggesting that FOXA1 is necessary for androgen activation of the expression of these genes (Figure 1). In contrast with these results, overexpression of FOXA1 in other studies inhibited DHT (dihydrotestosterone)-induced PSA promoter activity [59] and AR activation of transcription [109]. While the overexpression of FOXA1 *in vitro* could non-specifically squelch the transcriptional machinery, and explain these discrepant results, additional studies have shown that reducing FOXA1 expression does not alter DHT-induced expression of *PSA/KLK3* (kallikrein-related peptidase 3) [107,108], *TMPRSS2* (transmembrane protease serine 2) or *PDE9A* (phosphodiesterase 9A) [107], nor is FOXA1 necessary for androgen-induced cell cycle progression [107]. However, loss of FOXA1 increases expression of a subset of DHT-induced genes, including *PSA/KLK3*, and intensifies androgen-induced cell cycle progression [110]. Interestingly, Jia et al. [108] observed a reduction in DHT-induced expression of *TMPRSS2* following FOXA1 silencing in an LNCaP variant (C4-2B) that is AR-positive, but androgen-independent, suggesting that FOXA1 facilitates AR-stimulated transcription of at least some genes. Supporting this finding, FOXA1 is also necessary for expression of *UBE2C* (ubiquitin-conjugating enzyme E2C) in an AR-positive, androgen-independent LNCaP variant (abl), but not in parental cells [111]. FOXA1 also binds to, and may regulate the homeobox transcription factor *Hoxbl3* [112], which is a uniquely androgen-independent gene [113]. Overall, these results suggest that FOXA1 modulates AR target gene transcriptional activity, but through various mechanisms that depend on the level of responsiveness of the cell or gene to androgens. They also indicate that FOXA1 can modulate ligand-dependent and -independent actions of AR in prostate cancer cells.

Analyses of the binding of FOXA1 to AR target genes and its role in AR transactivation have suggested varied functions for FOXA1 that are gene-specific. FOXA1 binds adjacent to ARE (androgen response elements) in the probasin, PSA [106,114] and Sbp [114] promoters similarly in the presence and absence of DHT. This is similarly seen on a genome-wide level [110]. Others have also reported that FOXA1 binds to AR bound regions, but recruitment of

FOXA1 to these sites is enhanced by DHT [107,108]. Importantly, while FOXA1 was necessary for androgen-induced transcriptional activity of the probasin and PSA promoters, blocking direct binding of FOXA1 to these promoters did not impact AR binding [106], suggesting that FOXA1 is not necessary for recruitment of AR. In contrast, AR binding to enhancer regions of several other genes [*UBE2C, CDKl* (cyclin-dependent kinase 1) and *CDC20* (cell division cycle 20)] was reduced in response to FOXA1 silencing in parental (LNCaP) and androgen-independent (LNCaP-abl) prostate cancer cell lines [111]. Hence, the dependency of AR binding on preoccupancy of FOXA1 at adjacent sites appears to be highly dependent on gene context [111]. Supporting the requirement for FOXA1 binding as an essential modulator of AR activity, a germ line SNP (single nucleotide polymorphism) identified in prostate cancer patients within the risk locus 8q24 increases its affinity for FOXA1 binding and potentiates the ability of this chromosomal region to mediate androgen responsiveness of a heterologous promoter compared with the wild-type sequence [115]. Although these results suggest a tight link between FOXA1 binding and androgen responsiveness, the authors did not directly investigate whether FOXA1 is necessary for either AR binding or enhancer activity in its native chromosomal context. As a mechanism for how FOXA1 dictates AR binding at specific genes, studies in both prostate and breast cancer cells revealed that H3K4 dimethylation occurs proximal to FOXA1-binding sites, and can precede FOXA1 binding to DNA [116]. The overexpression of the histone demethylase KDM1 (lysine-specific demethylase 1) reduced AR binding, probably due to loss of bound FOXA1 [111]. The presence of FOXA1 in LNCaP cells is also associated with DNA hypomethylation [13], and an open chromatin state as measured by FAIRE (formaldehyde-assisted isolation of regulatory elements), where high FAIRE FOXA1-binding sites are more likely to bind AR [117]. Overall, it is quite evident that FOXA1 participates in AR target-gene recognition, but it seems likely that this regulation is dependent on additional co-factors, androgen dependence and/or the distance from the transcriptional start site (i.e. promoter or enhancer).

As another mode of regulation, the winged helix/forkhead domain of FOXA1 has been found to complex directly with the DNA-binding domain/hinge region of AR [106,110,118]. This complex permits AR binding to promoter DNA even in the absence of AR consensus elements and vice versa for FOXA1 binding [118]. Formation of a FOXA1/AR multimeric transcriptional complex that requires only one of the two protein's consensus elements could explain why mutagenesis of FOXA1-binding sites does not alter AR binding [106], whereas decreasing FOXA1 expression does [111] as discussed above. The FOXA1/AR complex may also be necessary for AR nuclear localization. In this case, decreasing FOXA1 expression would greatly impede the access of AR to its target genes, whereas mutating the FOXA1 consensus element would only directly affect FOXA1 binding. Most recently, it has been demonstrated by two distinct groups that the FOXA1/AR interaction is required for maintaining a particular hormonal transcriptional programme [103,110]. Loss of this interaction, either through experimental knockdown of FOXA1, or via somatic mutation of the *AR* gene, results in an AR-induced transcriptional programme that is distinct from that induced by AR when co-expressed with FOXA1. This is due to the differential binding of AR to targets that are occupied by FOXA1 compared with those that lack a FOXA1-binding site. FOXA1 can also form complexes with USF2 (upstream stimulatory factor 2) in prostate

cancer cells, but USF2 does not bind to AR, nor is it required for androgen-induced *PSA/KLK3* expression [114]. Since USF2 binds to a similar DNA consensus element as FOXA1, it is also possible that a complex of FOXA1 and USF2 may modulate prostate-specific promoter activity, but this remains to be directly examined.

Additional roles of FOXA1 in prostate cancer

Although the majority of work investigating FOXA1 in prostate cancer has been aimed at understanding how it is involved in modulating AR transcriptional activity, FOXA1 has also been implicated in other signalling pathways. FOXA1/2 positively regulate transcription of the gene *AGR2* (anterior gradient protein 2), whose expression increases aggressiveness of prostate cancer cells. This regulation can be abrogated by the presence of EBP1 (ERBB3-binding protein 1) [119]. AGR2 is an established metastasis inducer [120], and its activation by FOXA1 was first discovered in goblet cells [121]. Placement of FOXA1 in the EBP1/FOXA1/AGR2 pathway indicates that it may play an important modulatory role in metastatic progression. Interestingly, EBP1 has also been shown to negatively regulate AR-mediated transcription [122]. While not specifically tested, it is possible that EBP1 represses AR transactivation by inhibiting FOXA1 function in a similar manner as seen for the transcriptional regulation of *AGR2*. In contrast to its pro-metastatic positive regulation of AGR2, expression of *FOXA1* is also down-regulated by SOX4 (sex determining region Y-box 4), a prostate cancer oncogene [123]. This contradiction exemplifies the broad spectrum of activity for FOXA1 in the cancerous prostate, and at least partially explains how loss of *Foxa1* in the normal prostate could lead to both tumorigenic (i.e. increased SHH) and anti-tumorigenic (i.e. loss of AR function) phenotypes. Lastly, it is intriguing to speculate a potential pro-carcinogenic functional interaction between FOXA1, Nkx3.1 and SHH in the prostate given the aberrant regulation of these three factors in the *Foxa1* null prostate. Future *in vitro* work should aim to delineate the connection between these pathways and their interdependent contributions to disease phenotypes.

FOXA1 in breast cancer

The molecular subtypes of breast cancer were identified via unsupervised hierarchical clustering of cDNA microarray data from a cohort of human breast tumours, in which each subtype was distinguished by the differential expression of numerous genes. In particular, *FOXA1* mRNA is expressed in luminal subtype tumours along with several other discriminatory genes, including *ESR1* and *GATA3* [124,125]. While the six distinct subtypes [luminal A, luminal B, luminal C, HER2/ERBB2, basal and normal-like] are classified by genome-wide expression profiling [125], they are often identified using the surrogate markers: ER, PR (progesterone receptor) and the epidermal growth factor receptor 2 (HER2/ERBB2), a proto-oncogene amplified in ~ 20–30% of breast cancer patients. Luminal A subtype tumours are ER^+ /PR^+ /$HER2^-$ and confer a favourable prognostic outcome that is at least partially due to the efficacy of anti-hormone therapies [126]. In contrast, basal subtype tumours tend to be triple negative (ER^- /PR^- /$HER2^-$) and confer a poor prognosis due to the inherent aggressiveness of these tumours and the absence of subtype-specific targeted therapies [127].

FOXA1 expression in breast cancer

Since being identified as a novel luminal subtype gene, the clinical significance of FOXA1 protein expression in breast tumours has been investigated by multiple groups. Wolf et al. [128] were the first to investigate a breast tumour TMA (tissue microarray) for FOXA1 expression, revealing that FOXA1 is associated with low tumour grade, and, not surprisingly, ER expression [129]. A number of publications soon followed with various TMA compositions, and, when combined, provide expression data for FOXA1 on over 5000 human breast cancers [64,65,129–132]. FOXA1 significantly associates with ER expression in each study. Moreover, FOXA1 positively correlates with the luminal subtype as defined by ER and/or PR positivity, HER2 negativity [64,129], and luminal CK expression [64,65,129]. Negative correlations have been observed for FOXA1 and the basal subtype when defined as ER-negative, HER2-negative with CK5/6 and/or EGFR positivity [129, 132] or by basal CK expression [65,129]. Importantly, several groups have proposed utilizing FOXA1 as a prognostic tool to stratify patients within the ER-positive luminal subtype [64,129,130]. Results from these studies suggest that FOXA1 can predict survival within this population, and thus aid in therapeutic decision-making. However, TMA data generated by one group argues that FOXA1 is unable to further stratify prognosis within the subgroup of patients with ER-positive disease [65]. To directly test the independent prognostic and predictive ability of FOXA1, Ademuyiwa et al. [131] investigated the correlation between FOXA1 expression and Oncotype DX recurrence scores in patients with ER-positive node-negative disease. The Oncotype DX is a molecular diagnostic used to predict recurrence and response to chemotherapy. FOXA1 negatively correlated with recurrence score leading the authors to suggest FOXA1 immunostaining could function as a more cost-effective pathological marker than the Oncotype DX. Of note, *FOXA1* is not a component of Oncotype DX. Lastly, increased copy number of chromosomal locus 14q13, where *FOXA1* is located, is associated with the ER- and PR-positive breast cancer subtype [133], providing further evidence that FOXA1 may promote tumour differentiation.

Although FOXA1 statistically correlates with ER, it has been noted that some ER-negative tumours also have high FOXA1 expression [65,128]. In fact, a subset of ER- and PR-negative tumours is molecularly more similar to ER- and PR-positive than to triple negative tumours. Most importantly, FOXA1 was identified as one of the differentially expressed genes within this ‘ER-positive-like’ subgroup [134]. These tumours are more likely to possess apocrine features, express AR and have an androgen responsive molecular signature, and a breast cancer cell line (MDA-MB-453) that recapitulates this phenotype is growth stimulated by androgens and inhibited by anti-androgens [135]. Given the role of FOXA1 in modulating AR transactivation in prostate cancer, it is possible that the presence of FOXA1 in these ER-negative tumours is required for androgen-dependent responsiveness mediated by AR. Supporting this hypothesis, testosterone increases *FOXA1* mRNA expression in another AR-positive breast cancer cell line (SUM190) [136]. Indeed, recent studies provide evidence that FOXA1 mediates AR binding and transcriptional regulation in the MDA-MB-453 cells [137,138]. FOXA1/AR signalling in these cells is similar to FOXA1/ER signalling in MCF7 cells, and is necessary for maintenance of the apocrine gene expression signature [137]. Further evidence revealed that AR transcriptionally regulates *WNT7B*, and subsequent induction of *HER3* gene expression via an AR/FOXA1/β-catenin complex [138].

Enhanced HER3/HER2 signalling likely drives tumorigenesis of apocrine tumors. Hence, FOXA1/AR induces a set of genes that convey the phenotype, and perhaps induces the formation, of apocrine of breast cancers.

FOXA1 and the oestrogen receptor

Functioning as a chromatin remodeller, FOXA1 was discovered to co-operate with ER in activating the liver-specific vitellogenin promoter [139], and had been shown to regulate the well-established ER target, *TFF1* (trefoil factor 1; pS2) [140]. In 2005, two groups independently extended these findings to breast cancer, demonstrating that FOXA1 was necessary for oestrogen-induced ER binding, and the up-regulation of several target genes, including *TFF1* [141,142]. Carrolletal. [141] identified forkhead consensus motifs in close proximity to approximately half of the ER-binding sites spanning chromosomes 21 and 22 of a breast cancer cell line. Likewise, Laganière et al. [142] analysed ER-binding sites via a human promoter array, and found 12% of ER-bound promoters also contained forkhead motifs [143]. Several genome-wide analyses have confirmed these data [116,143–146], although one group has reported much less (<10%) commonality in the location of forkhead and ER-binding motifs in the genome [147]. That said, considerable co-operativity between FOXA1 and ER has been repeatedly observed in comparisons of full genome ER and FOXA1 ChIP-chip [116] and ChIP-seq [146] data, showing that over half of ER-binding sites are also occupied by FOXA1. This co-operation is observed for both ER up-regulated and down-regulated genes [116,146] (Figure 2A). For example, FOXA1 is necessary for ER repression of Reprimo (*RPRM*) transcription [148]. FOXA1 and ER can also have opposing roles in regulating gene expression as demonstrated for *BASE* (breast cancer and salivary gland expression); FOXA1 is required for *BASE* expression, whereas ER represses transcription of this gene [149].

Functionally, FOXA1 is necessary for cell growth [150] and specifically for oestrogen-mediated cell-cycle progression of breast cancer cells [142,151]. These results are at least partly explained by the requirement for FOXA1 to mediate ER stimulation of *CCND1*, the gene encoding cyclin D1, through a downstream enhancer [151]. The presence of FOXA1 correlates with bound RNA polymerase II, active chromatin and histone H4 acetylation [151] at ER-activated genes, where each is a hallmark of proliferating cells. Similar to its role in prostate cancer cells, FOXA1 binding is associated with an open chromatin state as measured by FAIRE, where loss of FOXA1 decreases the level of open chromatin on a global scale [117,146]. Specifically, FOXA1 binding at high FAIRE regions is accompanied by increased histone H3, lysine 9 (H3K9) acetylation, decreased H3K9 mono- and dimethylation, and increased H3K4 dimethylation [117]. The presence of FOXA1 is also associated with DNA hypomethylation [13]. Together, these results support a pioneering function for FOXA1 in modulating chromatin structure for subsequent ER binding and activity (Figure 2A), similar to that predicted for AR, although the specific timing of events is not well established in cancer cell lines. In addition to modulating ER activity, we recently reported that FOXA1 also directly binds to the *ESR1* (oestrogen receptor 1) promoter and is required for expression of ER mRNA and protein in breast cancer cells [73]. Thus, unlike its relationship with AR in the prostate, FOXA1 controls both the expression and activity of ER in breast cancer (Figure 2B).

In response to oestrogen stimulation, FOXA1 is preferentially bound to intergenic enhancers and introns, mirroring that of ER [141]. It is recruited to DNA in the presence and absence of estradiol, but unlike with androgen stimulation, binding of FOXA1 generally decreases with oestrogen treatment [141,143]. A direct comparison of FOXA1 in ER and AR signalling using breast and prostate cells, respectively, revealed that while there are common binding sites, FOXA1 has a distinct binding profile depending on whether it is co-expressed with ER or AR [116]. This tissue specificity is dependent on differential recruitment of FOXA1 to ER compared with AR target genes due to lineage-specific H3K4 dimethylation [116]. Moreover, the presence of FOXA1 at the *TFF1* gene enhancer is also dependent on binding of the histone variant H2A.Z by p400 [152], providing further evidence that tissue-specific FOXA1 activity is dictated by histone modifications. Another factor has recently been identified that also delineates FOXA1-specific recruitment and subsequent ER transcriptional regulation. CTCF (CCCTC-binding factor) is an insulator binding protein [153,154] whose loss was shown to impede FOXA1 binding to known target genes [e.g. *TFF1* and *PGR* (progesterone receptor)], while loss of FOXA1 had no impact on CTCF binding [154]. In contrast, Hurtado et al. [146] found that loss of CTCF increases FOXA1-binding capacity. Despite the discrepancy between these two reports, it is apparent that CTCF can modulate FOXA1 binding. The context dependency of this interaction must be further explored to unravel the molecular mechanisms by which CTCF enhances or impedes binding of FOXA1 to DNA.

The interplay between FOXA1 and ER may extend beyond the ability of FOXA1 to control ER expression and activity. Using a different experimental approach than Carroll et al. [141] who identified FOXA1-binding sites in ER target genes through motif analysis, Laganière et al. [142] first identified FOXA1 as an oestrogen-induced ER target gene and then subsequently observed FOXA1-binding sites within ER responsive genes. Providing support for *FOXA1* being an ER target, a more recent analysis of the ER chromatin interaction network revealed that ER-binding sites flank the *FOXA1* gene [155]. Although these results suggest a positive regulatory loop between ER and FOXA1 (Figure 2B), others have described FOXA1 to be down-regulated with oestrogen treatment [128,156–158]. Moreover, mice-lacking *Esr1* maintain expression of FOXA1 in the mammary epithelium [73]. These disparate observations are further complicated by the varying roles of FOXA1 in mediating ER signalling in tissues other than the breast [146,159,160]. Osteosarcoma cells that have been engineered to express the ER (U2OS-ER) have undetectable levels of FOXA1 protein, suggesting that the ER is insufficient to induce expression of FOXA1. Given the absence of FOXA1 in U2OS-ER cells, it is not surprising that forkhead consensus motifs do not correlate with the ER cistrome, nor is FOXA1 required for ER-regulated transcription in this context [160]. These results suggest that the role of FOXA1 in modulating ER activity is context dependent, and are consistent with the observation that FOXA1 is necessary for ER expression only in the epithelium and not in the stroma of the murine mammary gland [73]. In support of an indispensable role for FOXA1 in ER activity in breast epithelial-specific gene regulation, exogenous expression of FOXA1 in U2OS-ER cells induces ER binding to otherwise breast-specific ER targets [e.g. *TFF1* and *XBP1* (X-box binding protein 1)] that is accompanied by increased expression of these genes [146]. Furthermore, FOXA1 overexpressing U2OS-ER cells also become sensitive to the growth inhibitory effects of

tamoxifen, a SERM (selective ER modulator). In contrast, FOXA1 is endogenously expressed in, and is necessary for, resveratrol-induced ER transactivation of BMP2 (bone morphogenetic protein-2) gene expression in the osteoblast cell line MC3T3-E1 [159]. It is possible that the distinct observations seen with FOXA1 and ER in these studies can be attributed to the differences between the U2OS cells that were derived from a human osteosarcoma compared with the non-transformed MC3T3-E1 cells that were isolated from newborn mouse calvaria [161]. FOXA1 has been proposed as a putative breast cancer therapeutic target based on the concept that ER dependency on FOXA1 will translate to an increase in the efficacy of tamoxifen in ER-dependent breast cancer [162,163]. Functioning as an SERM, tamoxifen has antagonistic properties in the breast, but is agonistic in the bone, where it maintains oestrogen signalling and is protective against osteoporosis. The differing roles of FOXA1 in cancerous and normal bone tissue should be further defined before considering targeting FOXA1 in breast cancer patients. If FOXA1 is expressed in normal bone, as seen in the MC3T3-E1 cells, but loss of FOXA1 correlates with tumorigenic progression, as shown by the lack of FOXA1 in transformed U2OS cells, then the systemic reduction of FOXA1 expression could inadvertently induce bone tumours.

Additional roles of FOXA1 in breast cancer

In addition to the well-described ability of FOXA1 to control ER activity, there have been other facets of FOXA1 described in breast cancer. One of these is a possible role in HER2 signalling [136,150]. Treatment of the breast cancer cell line SUM190 with heregulin, an indirect activator of HER2, induces FOXA1 expression [136]. This suggests that FOXA1 may be downstream of HER2 signalling and mediate some of its actions. Indeed, FOXA1 silencing potentiates the cellular toxicity of herceptin, a humanized monoclonal antibody that inactivates HER2 in HER2-amplified breast cancer cell lines [150]. While the specific mechanisms remain unknown, these results support a role for FOXA1 in HER2-induced cell survival. However, it is important to note that the presence or absence of FOXA1 does not correlate with clinical outcome of patients with HER2-positive disease [164], indicating that FOXA1 may not play a critical role in HER2-induced signalling *in vivo*.

In addition to many other genes, FOXA1 binds to, and activates transcription of, the gene encoding HSP72 (heat-shock protein 72; *HSPA1A)*, the stress-inducible cytosolic form of HSP70 [165]. HSP70 independently associates with poorer outcome in breast cancer patients with node-negative disease [166]. Thus it is possible that FOXA1 and HSP72 co-operatively dictate the aggressive behaviours of these cancers, but this has not been tested. As mentioned earlier, FOXA1 also participates in ER-dependent transactivation of *CCND1* (cyclin D1) [151]. Intriguingly, FOXA1, ER and cyclin D1 are co-ordinately up-regulated in tumours arising in mice that are bi-transgenic for mammary gland-specific expression of ILK (integrin-linked kinase) and Wnt1 *(MMTV-Wnt1/ILK)* [167]. Forced co-expression of ILK and Wnt1 leads to increased epithelial proliferation, a greater percentage of luminal progenitor cells and increased tumour formation as opposed to the expression of either alone. The authors of this work proposed that the convergence of Wnt1 and ILK on β-catenin activation may result in up-regulation of FOXA1, which is downstream of SOX17/β-catenin during endodermal development in *Xenopus* [168]. Notably we have shown that FOXA1 is expressed in the mammary epithelial luminal progenitor population [73]. Whether

FOXA1 is necessary for expansion of luminal progenitors and the formation of mammary tumours, or is simply expressed as a bystander in this bi-transgenic or other mouse models of breast cancer, remains to be seen.

Given its correlation with the less aggressive luminal subtype of breast cancer, it is not surprising that FOXA1 activates the transcription of genes [e.g. *CDH1* (t-cadherin) and *CDKN1B* (cyclin-dependent kinase inhibitor 1B)] implicated in decreased breast cancer tumorigenicity independently of ER [169,170]. Exogenous overexpression of FOXA1 in a triple negative, basal breast cancer cell line (MDA-MB-231) induces expression of the cell-cell adhesion molecule, E-cadherin [171]. In this context, FOXA1 directly stimulates transcription of the E-cadherin gene *(CDH1)*, and the associated induction of E-cadherin expression decreases the migratory capacity of these cells, suggesting that FOXA1 may promote their differentiation. Activation of *CDH1* occurs in the absence of ER, supporting the notion that FOXA1 has ER-independent roles in dictating a more differentiated luminal cell phenotype. Another example of FOXA1 functioning in a tumour suppressive role is its regulation of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ [172]. FOXA1 binds to the promoter of $p27^{Kip1}$ (*CDKNlB*) in breast cancer cells and synergizes with BRCA1 (breast-cancer susceptibility gene 1) to induce its transactivation in a colon cancer cell line. In addition, breast cancer cells overexpressing FOXA1 have increased $p27^{Kip1}$ promoter activity and decreased cell number [128]. When combined, these studies paint a complicated picture of FOXA1 as a participant in multiple signalling pathways in breast cancer, which are both oncogenic and tumour suppressive. It is likely that the specific molecular functions of FOXA1 are dictated by many components, including, but not limited to, the epithelial origin of the tumour initiating cell and the co-expression of ER, HER2 and other regulatory factors that have not yet been identified.

CONCLUDING REMARKS

Since first described in liver-specific transcription in 1989, the FOXA subfamily has been linked to the development and differentiation of multiple tissues. FOXA1, in particular, has roles in the pancreas, kidney, liver, lung, gastro-intestinal tract, brain, prostate and mammary gland, and will likely prove to be expressed in, and modulate the function of, additional tissues. Commonly, FOXA1 is necessary for epithelial secretion in several organs, and is analogously critical in mediating lineage specification. FOXA2 can compensate for loss of *Foxa1* in the development of many tissues, but in others (e.g. mammary, prostate) the lack of redundancy can be, at least partly, explained by the lineage-specific expression of FOXA1 in the absence of FOXA2. Similarly, expression of FOXA3 is undetectable in the murine prostate [57] and in breast cancer cell lines [13]. Beyond having divergent expression profiles, the apparent lack of compensation by FOXA3 for either FOXA1 or FOXA2 is likely due to the dissimilarity of the amino acid sequence of this factor with FOXA1 and FOXA2 outside of the forkhead domain [4], and their distinct DNA-binding profiles [4,173]. To truly test whether FOXA3 is capable of compensating for loss of *Foxa1/2*, mice null for both *Foxa1/a2* and *Foxa3* will be necessary.

Developmental studies have revealed FOXA1 regulates certain genes indiscriminate of cell type (e.g. *Shh)*, while other FOXA1 targets are tissue-specific. The pattern of SHH

regulation by FOXA1 is also tissue specific, where loss of *Foxa1* decreases SHH in the lung and brain, but increases SHH in the prostate. The interplay between FOXA1 and SHH requires further evaluation given the presence of aberrant SHH signalling in multiple types of cancer. In addition, FOXA1 has a propensity to co-operate with nuclear hormone receptors (e.g. GR, ER and AR) in a somewhat similar manner in both development and cancer. The ability of FOXA1 to modulate AR and ER transcriptional regulation has been studied in the greatest detail in prostate and breast cancer respectively, but has been primarily limited to hormone-dependent disease. It will be critical to further investigate the potential role of FOXA1 in the transition to hormone independence and in hormone receptor negative disease, both features of more aggressive, less treatable cancers. The 'cell of origin' hypothesis predicts that a tumour maintains the molecular characteristics of the cell where the cancer originated. With regard to the breast, multiple subtypes of cancer exist representing the distinct lineages of the normal mammary epithelium, and these subtypes are predictive of patient prognosis. In this disease FOXA1 is expressed only in the luminal subtype, and is predictive of favourable patient prognosis. The differential expression and requirement for FOXA1 in the discrete lineages of tissue types other than breast will probably prove an equally valuable subtype-specific marker in cancers associated with these tissues, and may also be predictive of patient prognosis. In particular, the *Foxa1* null prostate exhibits a decrease in the luminal lineage, with a concomitant increase in the basal lineage.

In summary, the investigation of *Foxa1* knockout mice coupled with cell line studies has uncovered FOXA1-mediated pathways that, in addition to being necessary for normal development, are aberrant in cancer. The majority of our knowledge of FOXA1 in cancer relates to its function in hormone receptor driven breast and prostate cancers. While FOXA1 in hormone signalling should continue to be investigated, future research should also revolve around elucidating a role for FOXA1 in hormone independent breast and prostate cancers, and cancers of other tissues where FOXA1 has been implicated in normal development.

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Abbreviations used:

AGR2	anterior gradient protein 2
AML	acute myeloid leukaemia
AR	androgen receptor
ARE	androgen response elements
α-SMA	α-smooth muscle actin
BASE	breast cancer and salivary gland expression
CCSP	clara cell secretory protein
Chip	chromatin immunoprecipitation

CK	cytokeratin
CNS	central nervous system
CTCF	CCCTC-binding factor
DHT	dihydrotestosterone
E	embryonic day
EBP1	ERBB3-binding protein 1
EN1	engrailed 1
ER	oestrogen receptor
FAIRE	formaldehyde-assisted isolation of regulatory elements
FOG2	friend of GATA2
FOX	forkhead box
GR	glucocorticoid receptor
HCC	hepatocellular carcinoma
HNF	hepatocyte nuclear factor
HSP	heat shock protein
IL-6	interleukin-6
ILK	integrin-linked kinase
KLK3	kallikrein-related peptidase 3
MMTV	murine-mammary-tumour virus
NE	neuroendocrine
PDX-1	pancreas and duodenal homeobox protein-1
P	postnatal day
PR	progesterone receptor
PSA	prostate-specific antigen
Sbp	spermine-binding protein
SERM	selective ER modulator
SHH	sonic hedgehog
SP-B	pro-surfactant protein-B
SV40	simian virus 40

Tag	T antigen
TFF1	trefoil factor 1
TMA	tissue microarray
TMPRSS2	transmembrane protease serine 2
TRAMP	transgenic adenocarcinoma of mouse prostate
UBE2C	ubiquitin-conjugating enzyme E2C
Ucp2	uncoupling protein 2
USF2	upstream stimulatory factor 2

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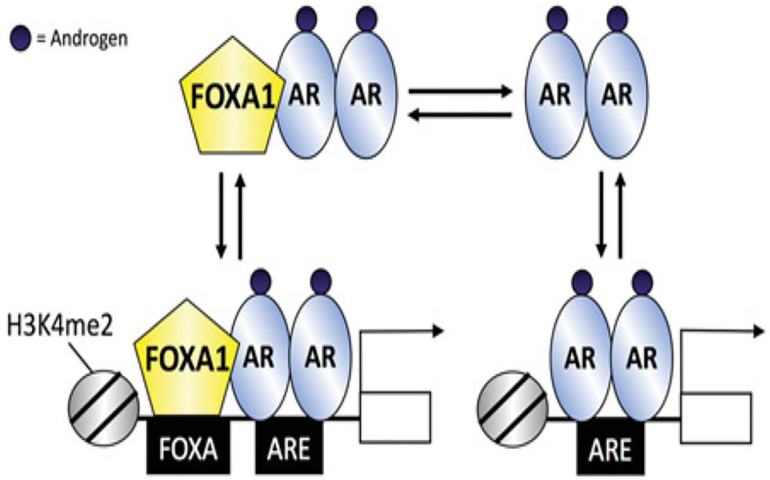

Figure 1. FOXA1/AR signalling in prostate cancer

Lineage-specific FOXA1-binding sites are marked by histone H3, lysine 4 dimethylation (H3K4me2). Under both androgen-dependent (shown) and androgen-independent (not shown) conditions, FOXA1 mediates AR transactivation of varied responsive genes. FOXA1 and AR also directly interact. Loss of this interaction leads to AR binding and transcriptional regulation at a different set of genes (*i.e.* hormonal reprogramming) compared with when co-expressed with FOXA1.

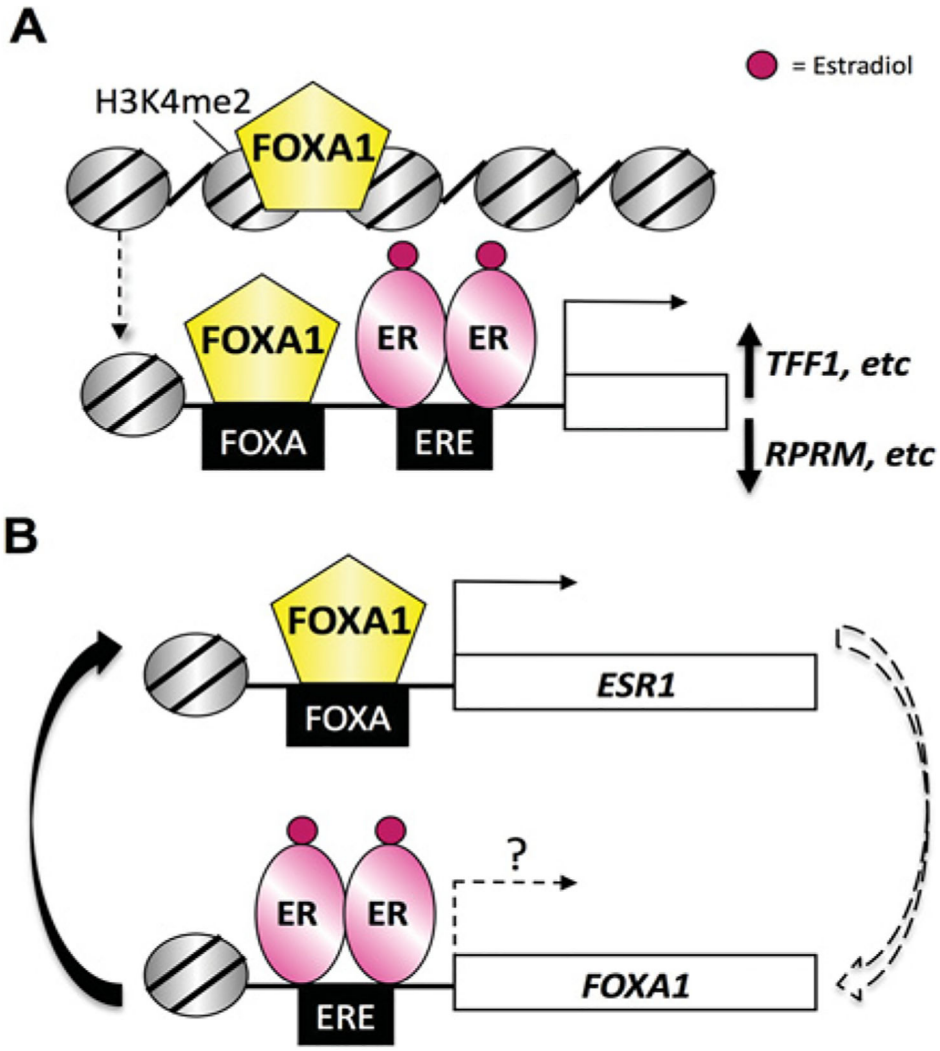

Figure 2. FOXA1/ER signalling in breast cancer

(**A**) Lineage-specific FOXA1-binding sites are marked by histone H3, lysine 4 dimethylation (H3K4me2). FOXA1 mediates both oestrogen-induced gene transactivation and repression. (**B**) FOXA1 is necessary for ER expression, and expression of ER has been proposed to modulate FOXA1 expression in an oestrogen-dependent manner.

Table 1

Overview of *Foxa1* mouse models of development

Tissue	Origin	Genotype	Viability	Phenotype	Foxa2 redundancy	Reference(s)
Pancreas	Endoderm	*Foxa1*$^{-/-}$	Lethal, P2–12/14	Insulin secretion defect; hypoglycaemia; hypotriglyceridaemia	No	[21,22,26]
		Pdx1-Cre^{E}*/Foxa*$^{loxP/loxP}$	Adulthood	No major defects	Yes	[27]
		Pdx1-Cre^{E}*/Foxa1*$^{loxP/loxP}$*/Foxa2*$^{loxP/+}$	Adulthood	No major defects	Yes	[27]
		Pdx1-Cre^{E}*/Foxa1*$^{loxP/+}$ */Foxa2*$^{loxP/loxP}$	Lethal, P5	Hypoplasia	n/a	[27]
		Pdx1-Cre^{E}*/Foxa1*$^{loxP/loxP}$*/Foxa2*$^{loxP/loxP}$	Lethal, P2	Failed endocrine and exocrine differentiation; hypoplasia	n/a	[27]
Kidney	Mesoderm	*Foxa1*$^{-/-}$	Lethal, P2–12	Dehydration; nephrogenic diabetes insipidus	No	[21,30]
Liver	Endoderm	*Foxa1*$^{-/-}$ */Foxa3Cre/Foxa2*$^{loxP/loxP}$	Lethal, E10	Failed fetal liver development	n/a	[31]
		Alf-pCre/Foxa1$^{loxP/loxP}$*/Foxa2*$^{loxP/loxP}$	Adulthood	Bile duct hyperplasia; fibrosis	n/a	[32]
Lung	Endoderm	*Foxa1*$^{-/-}$	Lethal, P2–13	Delayed alveolarization; surfactant secretion defect	Yes	[34]
		Foxa1$^{-/-}$ */SPC-rtTA*$^{-/tg}$*/(tetO)*$_{7}$*-Cre*$^{-/tg}$*/ Foxa2*$^{loxP/loxP}$	At least E18.5	Impaired branching morphogenesis		
Brain	Ectoderm	*Foxa1*$^{-/-}$	Lethal, P2–12	Delayed dopaminergic neuron maturation	Yes	[44]
		Foxa1$^{-/-}$ */Nestin-Cre/Foxa2*$^{loxP/loxP}$*	At least E18.5	Failed dopaminergic neuron maturation	n/a	[44]
		En1-klCre/Foxa1$^{loxP/loxP}$*/Foxa2*$^{loxP/loxP}$	At least E11.5	Failed dopaminergic neuron maturation	n/a	[48]
		Foxa1$^{-/-}$ */Wnt1-Cre/Foxa2*$^{loxP/loxP}$	At least E18.5	Failed ventral midbrain progenitor specification	n/a	[49]
GI tract	Endoderm	*Villin-Cre/Foxa1*$^{loxP/loxP}$*/Foxa2*$^{loxP/loxP}$	Adulthood	Impaired goblet and enterendocrine cell maturation; mucin secretion defect	n/a	[50]
Prostate	Endoderm	*Foxa1*$^{-/-}$	Rescued	Failed luminal lineage differentiation; epithelial hyperproliferation; secretion defect	No	[58]
Mammary	Ectoderm	*Foxa1*$^{-/-}$	Rescued	Impaired ductal invasion	No	[73]

*Investigated the dose-dependent effect of each allele of FOXA1/A2. For simplicity, these combinations were not reported here. n/a, not applicable; in these models *Foxa2* was deleted in combination with *Foxa1*. GI, gastrointestinal.

Table 2

Overview of FOXA1 in human cancer

Cancer type	FOXA1 expression relative to normal	Predicted activity	Reference(s)
AML	Increased	Oncogenic	[76]
Breast	No difference	Unclear	[64,65,128–132]
	Amplified in ER+/PR+ tumours	Oncogenic	[133]
Oesophageal	Amplified/overexpressed	Oncogenic	[78,79]
HCC	Expressed in favourable prognosis tumours	Tumour suppressive	[85]
Lung	Amplified/overexpressed	Oncogenic	[78]
Pancreatic	Decreased in poorly differentiated disease	Tumour suppressive	[80]
Prostate	No difference	Unclear	[59]
	Decreased in associated metastases/favourable outcome	Tumour suppressive	[110]
	Increased in associated metastases/poor outcome	Oncogenic	[102,103]
	Amplified in associated metastases	Oncogenic	[104]
Thyroid	Amplified/overexpressed	Oncogenic	[77]