The Genetics and Biology of *FOXL2*

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Keywords

Blepharophimosis, ptosis, and epicanthus inversus syndrome · *FOXL2* · Ovarian development · Premature ovarian insufficiency

Abstract

FOXL2 encodes a transcription factor that regulates a wide array of target genes including those involved in sex development, eyelid development, ovarian function and maintenance, genomic integrity as well as cellular pathways such as cell-cycle progression, proliferation, and apoptosis. The role of *FOXL2* has been widely studied in humans and animals. Consistent with its role in ovarian and eyelid development, over 100 germline variants in *FOXL2* are associated with blepharophimosis, ptosis, and epicanthus inversus syndrome in humans, an autosomal dominant condition characterised by ovarian dysgenesis/premature ovarian insufficiency, as well as defective eyelid development. Reflecting its role in apoptosis and proliferation, a somatic variant in *FOXL2* causes adult granulosa cell tumours in humans. Despite being widely studied and having clear relevance to human disease, much remains unknown about the genes *FOXL2* regulates and how it exerts its wide-reaching effect on multiple organs. This review focuses on *FOXL2* and its varied roles as a transcription factor in sex determination, ovar-

ian maintenance and function, eyelid development, genome integrity, and cell regulation, followed by discussion of the in vivo disruption of *FOXL2* in humans and other species.

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Introduction

Sex determination, the process by which a gonad is committed to differentiate into either a testis or ovary, is a complex finely-tuned process that differs substantially between species. In mammals, including humans, sex is governed by the sex chromosomes, with males being the heterogametic sex, harbouring an X and a Y chromosome, and females being homogametic, with 2 X chromosomes. The chromosomal complement determines which genetic cascade is activated, with the interacting pathways being delicately balanced to promote one fate while inhibiting the alternative. The molecular components of these pathways and how they interact, is not fully understood. Although the male sex-determining gene, *SRY*, was discovered in 1990 [Sinclair et al., 1990], genes directing female fate have remained far more elusive. One key gene in the female determination pathway, that is not only responsible for directing female sex development but also for maintaining ovarian function, is *FOXL2*.

FOXL2 as a Transcription Factor

FOXL2 is a single-exon gene that encodes a 376 amino acid transcription factor with a forkhead domain. This domain, also called a winged helix domain, is a conserved ~100 amino acid DNA-binding motif that is present in a large number of proteins involved in embryogenesis and/or cell differentiation [Golson and Kaestner, 2016]. The canonical forkhead domain consists of 3 α -helices, 3 β -sheets, and 2 “wing” regions, a structure that enables monomeric DNA binding. This family of transcription factors originated in unicellular eukaryotes but has expanded through evolution to over 40 members in mammals [Hannenhalli and Kaestner, 2009]. Mammalian forkhead proteins have evolved to be tissue-specific and have specialized roles in the development of specific organs, such as *FOXO1*, which has a role in thyroid development [Trueba et al., 2005] or *FOXN1* with a role in the development of hair, skin, and thyroid [Frank et al., 1999]. The *FOXL2* (*forkhead box L2*) gene is an important member of this extensive family and is primarily responsible for ovarian development and function [Uhlenhaut and Treier, 2006].

Evolution of FOXL2

FOXL2 homologues are present in all metazoans and have a complex evolutionary history, involving whole genome duplication followed by gains and losses [Bertho et al., 2016]. The *FOXL2* gene resulted from duplication of an ancestral gene prior to the explosion of vertebrate speciation [Crespo et al., 2013; Geraldo et al., 2013]. This duplication event led to 2 paralogs, namely *Foxl2* (also referred to as *Foxl2a*) and *Foxl3* (also referred to as *Foxl2b*). The *Foxl3* paralog has experienced independent losses through evolution, being absent in placental mammals and amphibians, for example, but present in some fish, birds, and marsupials [Crespo et al., 2013; Geraldo et al., 2013]. In contrast, *Foxl2* has been retained in all vertebrate species and repeatedly exhibits sexually dimorphic expression consistent with a role in sex determination.

Sexually dimorphic *Foxl2* expression has been demonstrated in numerous fish species, with higher expression in ovaries compared to testes [Baron et al., 2004; Wang et al., 2007; Caulier et al., 2015; Hu et al., 2016]. In reptiles, such as the common snapping turtle or the American alligator, which experience temperature-dependent sex determination, *Foxl2* expression becomes sexually dimor-

phic in response to the temperature-sensitive period with increased expression at female-permissive temperature [Yatsu et al., 2016; Diaz-Hernandez et al., 2019]. In amphibians, such as the frog *Rana rugosa*, *Foxl2* is expressed before the sex determination period but becomes sexually dimorphic in female tadpoles during sex differentiation. Expression is maintained in both sexes of adult frogs but at a much higher level in females [Oshima et al., 2008]. *FOXL2* is expressed in the gonads of both male and female chickens, although at a higher level in female gonads, particularly the right ovary, reflecting the gonadal asymmetry of the species [Govoroun et al., 2004]. In mammals such as mice and humans, *FOXL2* is expressed in the somatic cells of the developing ovary and granulosa cells of developing ovarian follicles and is minimally expressed or absent in testes [Duffin et al., 2009].

The ubiquitous retention of *FOXL2* through evolution and its conserved tendency for dimorphic expression is the result of its ability to promote female fate while repressing male gonadal fate, as explored in the following section.

FOXL2 in Sex Development

The expression pattern of *FOXL2* in species across evolution clearly reflects its potential role in sex determination. The role of *FOXL2* in ovarian development is multifaceted (Fig. 1). *FOXL2* can be considered a key factor involved in the “battle of the sexes”, promoting female sex determination and repressing male sex determination [Veitia, 2010]. In 46,XY individuals, the sex-determining region on the Y (*SRY*) gene activates the cascade of gene expression that culminates in testes development. The first, and arguably most important, gene target of *SRY* is *SOX9*, which is able to maintain its own expression via 2 feedback loops, one involving fibroblast growth factor 9 (*FGF9*) [Colvin et al., 2001; Kim et al., 2007] and one involving Prostaglandin D2 (*PGD2*) [Malki et al., 2005; Moniot et al., 2009]. *Sox9* is essential for testis development and differentiation [Houston et al., 1983; Chaboissier et al., 2004]. In vitro experiments have shown that *Foxl2* can bind to an upstream enhancer of *Sox9* in mouse, *TESCO*, suppressing its activity and consequently preventing high levels of *Sox9* expression [Uhlenhaut et al., 2009]. Via this mechanism, *Foxl2* promotes the female pathway and the development of ovaries. As an additional means of repressing the male developmental pathway, *Foxl2* is believed to reduce *Nr5a1* expression in the developing ovary via antagonism with the WT1-KTS tran-

Fig. 1. Diagram summarising the role of *FOXL2* in ovarian development and function.

scription factor [Takasawa et al., 2014]. Although *Nr5a1* is required for early gonadal development of both sexes, its expression is upregulated in testes and downregulated in ovaries, likely governed by *Foxl2* [Takasawa et al., 2014]. Not only does *FOXL2* antagonise the male pathway, but it also promotes the female pathway. For example, it is believed to upregulate ovarian-specific genes such as *aromatase* (*CYP19*) and *follicle-stimulating hormone* (*FST*) [Pannetier et al., 2006; Kashimada et al., 2011]. The role of *FOXL2* in sex determination is best demonstrated by the study of sex reversal or ovarian dysfunction in animal models and humans with *FOXL2* disruption, as discussed in the final sections of this review.

***FOXL2* in Ovarian Maintenance and Function**

Many studies of humans and animals have revealed that the role of *FOXL2* in the ovary is not limited to its development (Fig. 1). *FOXL2* is instead required for multiple stages of ovarian physiology including folliculogen-

esis, maintenance of granulosa cell fate, as well as regulation of the hypothalamic pituitary axis.

A woman's oocyte pool is established by birth, with oocytes remaining arrested in prophase of meiosis I until sexual maturity, after which a subset of oocytes is recruited each month via folliculogenesis. Many growth factors, hormones, receptors, and enzymes are required for the coordinated signalling involved in this process, and *FOXL2* plays a role at multiple levels.

Primary recruitment refers to the initial activation of a group of oocytes and is mediated by growth factors and neuropeptides such as bone morphogenic proteins (BMPs), growth differentiation factor 9 (GDF9), nerve growth factor (NGF), and Newborn oogenesis homeobox (NOBOX). *FOXL2* plays a role in primary recruitment via its cooperation with, and regulation of, some such factors. AMH is expressed by primary follicles until the large pre-antral follicular stages, playing a role in regulating the number of follicles for initial recruitment, thereby preventing premature oocyte depletion via folliculogenesis [Durlinger et al., 1999; Broekmans et al., 2008]. *FOXL2*

has been shown to be the critical transcriptional activator of *AMH* [Park et al., 2014; Jin et al., 2016]. Consistent with this role in folliculogenesis, in the absence of *Foxl2*, granulosa cells fail to transition from the squamous to cuboidal morphology, as required for primary follicle formation [Schmidt et al., 2004].

After primary recruitment, secondary recruitment occurs and is largely controlled by the hypothalamus-pituitary-ovarian axis. This involves the pulsatile secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus. GnRH stimulates the cyclical secretion of LH and FSH by the pituitary. FSH activates the development of antral follicles. A developing follicle increases its oestrogen output, which then acts in a feedback loop to downregulate further FSH release, encouraging a single dominant follicle in each ovulatory cycle [Hillier, 1994]. Again, *FOXL2* has been shown to have a role in this process. *FOXL2* binds a regulatory region of GnRH and may contribute to the onset of its expression [Ellsworth et al., 2003]. Furthermore, *FOXL2*, in partnership with *SMAD4*, activates expression of the β subunit of FSH, *FSHB* [Ongaro et al., 2020]. *FOXL2* is also implicated in oestrogen signalling via its transcriptional activation of the gene encoding the beta subunit of the oestrogen receptor, *ErbB* [Hirano et al., 2017].

Steroidogenesis is another process critical for ovarian functioning that also relies on *FOXL2*. *Foxl2* has been implicated in multiple steps of the steroidogenesis pathway. For example, *Foxl2* transcriptionally represses the steroidogenic acute regulatory protein (*StAR*) [Pisarska et al., 2004]. *FOXL2* in human granulosa cells negatively regulates the transcriptional activation of the key steroidogenic enzyme responsible for androgen production, *CYP17* [Park et al., 2010]. *FOXL2* also acts as a direct transcriptional activator of the gene encoding aromatase, *CYP19* [Pannetier et al., 2006; Fleming et al., 2010], which is responsible for the conversion of androgen to oestrogen.

Ovarian function, including folliculogenesis, hormonal signalling, and steroidogenesis, clearly relies on *FOXL2*, but so too does ovarian maintenance. Just as there are antagonistic factors and pathways that establish sex determination, there are also molecular mechanisms in the post-natal gonads that ensure their continued differentiated fate and prevent activation of the opposing fate. In males, *Dmrt1* is required for the maintenance of Sertoli and Leydig cells via inhibition of the ovarian retinoic acid and *Foxl2* pathways [Matson et al., 2011; Minkina et al., 2014]. Conversely, *Foxl2* suppresses *Dmrt1* expression and prevents transdifferentiation of granulosa and theca cells into testicular Sertoli and Leydig cells [Uhlenhaut et al., 2009].

***FOXL2* in Genomic Maintenance**

Genomic stability and integrity rely on efficient repair of DNA damage. DNA double strand breaks (DSB) can be the result of environmental insult, such as ionising radiation, or can be orchestrated for functional purpose, such as breaks initiated by *SPO11*, which allow genetic recombination in meiosis. The repair of DSB can occur via multiple mechanisms including homologous recombination (where a homologous chromosome or sister chromatid is used as template for repair) or non-homologous end joining (NHEJ). The balance between these processes is partly controlled by the Ku complex. The Ku complex, a *XRCC5/6* heterodimer, is involved in this process, promoting NHEJ while preventing HR. Recently, *FOXL2* has been shown to bind and sequester the Ku complex in an acetylation-dependent manner [Jin et al., 2020]. DSB induction leads to deacetylation of *FOXL2* at Lys142, which frees the Ku complex to bind DSB and promote repair via NHEJ. Without *FOXL2* regulation of the Ku complex, the balance between HR and NHEJ is disrupted, having catastrophic consequences for genomic integrity.

Oocytes are unique mammalian cells in that they are suspended in meiosis for decades, from development until after sexual maturation. During this time, oocytes must maintain their genetic integrity, and must be protected from DNA damage. During meiosis, oocytes must undergo careful replication and crossover of genetic material between maternal and paternal chromosomes. It has been suggested that the balance of HR and NHEJ for successful meiosis is mediated by competition between *MRE11A* and the Ku complex for access to the DSB site [Lee et al., 1998; Zhou and Paull, 2021]. Whether regulation of Ku by *FOXL2* is also necessary for efficient meiosis and the genomic stability of oocytes is avenue for further investigation.

***FOXL2* in Cellular Pathways (Cell Cycle Progression, Proliferation, Apoptosis)**

The critical requirement of *FOXL2* for ovarian function is also dependent on its role in fundamental processes such as cell cycle progression, proliferation, and apoptosis.

FOXL2 has been implicated in the regulation of the cell cycle. Its upregulation leads to accumulation of cells in the G1 phase [Benayoun et al., 2011]. Knockout of *FOXL2* in KGN cells leads to an excess of cells in the G0/G1 phase

and a reduction of cells in S phase [Tang et al., 2019]. The influence of FOXL2 on cell cycle progression may be due to its ability to regulate cell cycle mediators, such as cyclin D1 and cyclin-dependent kinase 4 [Benayoun et al., 2011; Tang et al., 2019].

Foxl2 interacts with Smad3 to stimulate follistatin expression, which in turn inhibits activin-induced proliferation [Cheng et al., 2014]. The anti-proliferative function of Foxl2 is reinforced by its ability to activate death receptors such as *Fas* and *Tnfr1* [Kim et al., 2011]. Foxl2 has also been shown to induce cell death in granulosa cells via activation of caspase 8, with a subsequent impact on BID and BAK [Kim et al., 2011].

FOXL2 in Eyelid Development

The expression and function of FOXL2 is not restricted to the ovaries alone. Its highest expression is in the granulosa cells of the ovary, but also the periocular mesenchymal cells during eyelid development [Crisponi et al., 2001], as well as other craniofacial structures [Heude et al., 2015]. FOXL2 has been shown to activate actin gene expression in the smooth muscle of the periocular mesenchyma, thereby controlling the development of the levator smooth muscle of the eyelid, which is responsible for raising the upper eyelid to its appropriate position [Liu, 2012].

FOXL2 Disruption in Animal Models

Cell-based and in vitro studies have demonstrated many of the aforementioned insights into FOXL2 function. The most conclusive insights, however, have been gleaned from in vivo studies of animal models, both naturally occurring and engineered.

In the farming industry, hornless animals have been selected for domestic breeding as they negate the need for surgical horn removal. As early as 1944, a genetic relationship between hornlessness and intersex was recognised [Asdell, 1944]. In 2005, a deleted region that is ~200 kb from the FOXL2 locus was identified as responsible for the polled intersex phenotype in goats and associated with a loss of FOXL2 expression in sex-reversed gonads [Pailhoux et al., 2001]. The naturally occurring deletion affected the expression of not only FOXL2 but also nearby long non-coding RNAs. The subsequent study of goats engineered to have biallelic loss of function FOXL2 provided definitive confirmation that the loss of FOXL2

alone was sufficient for the phenotype of XX female-to-male sex reversal [Boulanger et al., 2014]. These goats also had eyelid agenesis, consistent with the role of FOXL2 in eyelid development.

Two constitutive *Foxl2* null mouse models were described in 2004 [Schmidt et al., 2004; Uda et al., 2004], both resulting in neonatal lethality in most newborn mice. Surviving mice were small, had severe craniofacial abnormalities, and eyelid hypoplasia [Uda et al., 2004]. Homozygous female mice lacking *Foxl2* were sterile. Although the number of oocytes in Foxl2-deficient females was normal at birth, rapid and extensive follicular atresia led to premature depletion and subsequent infertility [Schmidt et al., 2004; Uda et al., 2004]. Foxl2 deficient mice have also been reported to have defects in embryonic bone and cartilage formation, as well as postnatal growth, suggesting also a role for Foxl2 in these processes [Marongiu et al., 2015].

The large degree of neonatal lethality in mice with ubiquitous loss of Foxl2 limits their investigation. Instead, many different conditional *Foxl2* knockout mice have been generated, giving further insight into the role of Foxl2 in specific tissues and organs.

The selective ablation of *Foxl2* in the anterior pituitary gonadotrope cells of mice led to normal development but subfertility in adulthood. Interestingly, this affected both sexes with impaired spermatogenesis in testis and the ovulation of fewer oocytes per ovarian oestrous cycle due to impaired *Fshb* transcription. This confirmed the essential role of *Foxl2* in the pituitary for FSH expression [Tran et al., 2013].

The constitutive knockout models demonstrated the role of Foxl2 in ovarian development, however, more recently, the conditional deletion of *Foxl2* in mature granulosa cells of the postnatal ovary provided surprising new insights into the breadth of its role [Uhlenhaut et al., 2009]. This postnatal deletion led to upregulation of the testis-determining gene, *Sox9*, and subsequent transdifferentiation of granulosa and theca cell lineages into Sertoli-like and Leydig-like lineages, respectively. This study highlighted the role of *Foxl2* in ovarian maintenance in addition to its previously known role in ovarian development and function.

The role of *Foxl2* in female fertility is not only due to its ability to stimulate gonadotropin synthesis and direct ovarian development and maintenance, but also due to a role in uterine maturation and function. This was revealed by the conditional deletion of *Foxl2* in the mouse postnatal uterus, which led to infertility due to a severely reduced thickness of the stroma layer and a hypertrophic,

disorganized inner myometrial layer of the uterus. This defective uterine maturation likely results from disrupted *Wnt* gene expression in the absence of *Foxl2* [Bellessort et al., 2015].

Studies of *Foxl2* overexpression have similarly been informative. Ovaries overexpressing *FOXL2* exhibited disorganised ovarian structure, decreased steroidogenesis, and lack of ovulation [Nicol et al., 2020], demonstrating that the level of *FOXL2* expression must be finely tuned for proper ovarian development and function. The forced expression of *Foxl2* in Sertoli cells of XY mice leads to seminiferous tubule disorganization and in some cases ovotestis-like gonads [Ottolenghi et al., 2007]. These studies are consistent with the role of *Foxl2* in directing ovarian development and function.

In addition to goats and mice, a role of *FOXL2* in promoting ovarian differentiation has been proposed for an array of other species including, but not restricted to, fish [Baron et al., 2004; Fan et al., 2019], amphibians [Oshima et al., 2008; Okada et al., 2009], reptiles such as turtles and alligators [Moore et al., 2010; Shoemaker-Daly et al., 2010], birds [Govoroun et al., 2004; Major et al., 2019], crustaceans [Li et al., 2015], and molluscs [Wang et al., 2020]. This includes species for which sex is determined by the XX (female)/XY (male) system, the ZW (female)/ZZ (male) system, and even those for which sex is influenced by temperature. These animal studies demonstrate that *FOXL2* is clearly an evolutionary-conserved major player driving ovarian sex determination.

FOXL2 Variants in Human Disease

Given the fundamental and conserved role of *FOXL2* in ovarian development and function, it is not surprising that its disruption results in human disease.

The most commonly described association with *FOXL2* disruption in humans is blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES) (MIM#110100). This autosomal dominant condition is

Fig. 2. *FOXL2* with domains and variants highlighted (black = missense; red = loss of function; yellow = isolated premature ovarian insufficiency; boxed = somatic variant found in adult granulosa cell tumours). Pathogenic variants (ClinVar) are distributed along the length of the protein. The majority of variants are loss of function (red), but causative missense variants are also reported and cluster around the functional forkhead domain responsible for DNA binding. The 2 variants highlighted in yellow are reported in association with isolated premature ovarian insufficiency.

characterised by eye malformations, including narrowing of the eyelids (blepharophimosis) and drooping of the upper eyelid (ptosis). A skin fold running from the lower eyelid inwards and upwards (epicanthus inversus) is also observed, as are widely-set eyes (telecanthus). Type I BPES syndrome presents with premature ovarian insufficiency in females, whereas Type II BPES lacks ovarian involvement [Crisponi et al., 2001].

The genetic cause of BPES was discovered via positional cloning followed by candidate gene sequencing within the identified region in 7 families affected by BPES [Crisponi et al., 2001]. Every family, regardless of BPES subtype, harboured a *FOXL2* variant consistent with dominant inheritance of the condition. Since this initial discovery, a huge array of *FOXL2* variants have been identified in cases of BPES, including null-type variants such as nonsense or frameshift variants, missense variants, triplet repeat expansion, and microdeletions [De Baere et al., 2001]. Figure 2 summarises the nature and location of pathogenic and likely pathogenic variants reported in ClinVar. This demonstrates that the majority of reported variants are loss-of-function variants, but many causative missense variants have also been reported and tend to cluster in the forkhead domain (Fig. 2). This is consistent with the forkhead domain being critical for *FOXL2* function, and its disruption is therefore likely to abolish protein function. The most commonly identified causative variants are polyalanine expansions, accounting for ~30% of patients [De Baere et al., 2001]. Although this polyalanine tract is exclusive to mammalian species, it has been shown to have strong functional constraints [Cocquet et al., 2003], and its frequent involvement in BPES aetiology is consistent with it having a functional role. There is some degree of genotype:phenotype correlation, with a tendency for loss-of-function variants positioned before the forkhead and polyalanine tract being associated with premature ovarian insufficiency (POI) as part of BPES Type I [De Baere et al., 2001]. Microdeletions tend to associate with intellectual disability in addition to classical BPES syndrome. Genotype:phenotype correlations, however, are not concrete, with significant variation in clinical presentation of individuals with the same variant, even within single families [De Baere et al., 2001].

Less commonly, germline variants in *FOXL2* have been described in cases of isolated POI [Harris et al., 2002; Laissue et al., 2009]. One such variant is a deletion within the polyalanine tract that is frequently expanded in cases of BPES and may therefore be considered likely to alter *FOXL2* function [Harris et al., 2002]. Whether deletions

within this mammalian-specific polyalanine tract have a deleterious impact, however, remains to be confirmed. A missense variant, p.(Y258N), was described in a patient with a milder POI phenotype diagnosed at 38 years, but the variant was present in her unaffected mother, indicating either it is a benign variant or is associated with reduced penetrance [Harris et al., 2002]. The third reported variant is a missense variant, p.(G187D), which led to reduced expression from an ovarian-specific *FOXL2* reporter construct [Laissue et al., 2009]. This same variant has been detected in an XY individual with BPES but was considered non-pathogenic due to its presence in unaffected family members [De Baere et al., 2002]. Although *FOXL2* has been associated with isolated POI, it is not a common cause as screens of large numbers of patients failed to identify other causative *FOXL2* variants [De Baere et al., 2002], and functional evidence confirming its involvement in isolated POI is scarce.

Given mouse studies suggested a role for *Foxl2* in pituitary function and postnatal growth [Tran et al., 2013; Marongiu et al., 2015], a recent study screened a small cohort of patients with congenital eyelid malformations in conjunction with hypopituitarism, and *FOXL2* variants were identified in 30% [Castets et al., 2020]. Again, functional studies are lacking, but the absence of another genetic cause and the known role of *FOXL2* in pituitary development indicates the potential involvement of these variants in pituitary disease pathology.

Somatic variants in *FOXL2* are also a known cause of human disease, specifically adult ovarian granulosa cell tumours (GCTs). These tumours are rare, accounting for only 5% of malignant ovarian tumours. Given the role of *FOXL2* in both proliferation and apoptosis, it can act as a tumour suppressor or oncogene, depending on the context [Rosario et al., 2014]. In contrast to the >100 germline variants resulting in BPES, only a single somatic variant in the forkhead domain is known to have a role in the development of adult ovarian GCTs, c.402C>G, p.(C134W). It is highly specific and sensitive for detecting these tumours, being present in 97% of adult GCTs [Shah et al., 2009] and absent in most other cancers, including those of ovarian origin [Schrader et al., 2009]. The exception to this, is occasional detection in related tumours, such as juvenile GCT, thecomas, and adult testicular GCTs in males [Shah et al., 2009; Lima et al., 2012]. Recent studies have made progress in understanding the mechanism by which this variant causes cancer. The variant enables the binding of *FOXL2* to the transcription factor, SMAD4 [Weis-Banke et al., 2020]. This results in altered DNA-binding specificity and the activation unique targets that

drive an oncogenic program [Carles et al., 2020]. Such insights pave the way for the development of therapeutic strategies.

Conclusion

FOXL2 is a transcription factor with many overlapping roles in development. It has a major role in ovarian development and function, which has been demonstrated in a broad range of animal models, from molluscs to humans. The role of FOXL2 is mediated by its transcriptional activation of genes involved in development, folliculogenesis, hormone signalling, steroidogenesis, cell regulation, proliferation, and apoptosis. It acts as a key gene required for female sex development, antagonising the male pathway via inhibition of SOX9. Its disruption in animal models causes female sex rever-

sal and/or infertility, and concordantly, germline *FOXL2* variants in humans cause BPES, a syndrome characterised by POI and abnormal eyelid development. The mechanism of FOXL2 action continues to be studied, and our understanding of the pathways it modulates is continually expanding.

Conflict of Interest Statement

The author declares no conflict of interest.

Funding Sources

This work was supported by an Australian National Health and Medical Research Council (NHMRC) fellowship (1054432 to E.J.T.) and the Victorian Government's Operational Infrastructure Support Program.

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