Molecular and Cellular Endocrinology 356 (2012) 55–64

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Review

The transcription factor FOXL2: At the crossroads of ovarian physiology and pathology

Sandrine Caburet [a,b,1], Adrien Georges [a,b,c,1], David L'Hôte [a,b,1], Anne-Laure Todeschini [a,b,1], Bérénice A. Benayoun [a,b,c], Reiner A. Veitia [a,b,*]

[a] CNRS UMR 7592, Institut Jacques Monod, Equipe Génétique et Génomique du Développement Gonadique, 75205 Paris Cedex 13, France
[b] Université Paris Diderot-Paris VII, 75205 Paris Cedex 13, France
[c] Ecole Normale Supérieure de Paris, 75005 Paris, France

ARTICLE INFO

Article history:
Available online 8 July 2011

Keywords:
FOXL2
Premature ovarian failure
Stress response
BPES
Apoptosis
Cell cycle

ABSTRACT

FOXL2 is a gene encoding a forkhead transcription factor. Its mutations or misregulation have been shown to cause the blepharophimosis–ptosis–epicanthus inversus (BPES) syndrome and more recently have been associated with the development of Ovarian Granulosa Cell Tumors (OGCT). BPES is a genetic disorder involving mild craniofacial abnormalities often associated with premature ovarian failure. OGCTs are endocrine malignancies, accounting for 2–5% of ovarian cancers, the treatment of which is still challenging. In this review we summarize recent data concerning FOXL2 transcriptional targets and molecular partners, its post-translational modifications, its mutations and its involvement in newly discovered pathophysiological processes. In the ovary, FOXL2 is involved in the regulation of cholesterol and steroid metabolism, apoptosis, reactive oxygen species detoxification and cell proliferation. Interestingly, one of the main roles of FOXL2 is also to preserve the identity of ovarian granulosa cells even at the adult stage and to prevent their transdifferentiation into Sertoli-like cells. All these recent advances indicate that FOXL2 is central to ovarian development and maintenance. The elucidation of the impact of FOXL2 germinal and somatic mutations will allow a better understanding of the pathogenesis of BPES and of OGCTs.

Contents

1. Introduction

The blepharophimosis–ptosis–epicanthus-inversus syndrome (BPES; MIM 110100) is a genetic disease characterized by eyelid malformations and minor craniofacial abnormalities. Two forms of BPES have been defined: in type II BPES craniofacial stigmata appear isolated while in type I BPES there is also a premature ovarian failure (POF) (Zlotogora et al., 1983). BPES is basically an autosomal dominant disease caused by mutations in the *FOXL2* gene, which encodes a forkhead transcription factor (Crisponi et al., 2001). FOXL2 contains a typical forkhead DNA-binding domain. The other salient feature of this transcription factor is the presence of a polyalanine tract of 14 residues, strictly conserved in Eutherian mammals (Cocquet et al., 2002). FOXL2 is expressed in the developing

* Corresponding author at: CNRS UMR 7592, Institut Jacques Monod, Equipe Génétique et Génomique du Développement Gonadique, 75205 Paris Cedex 13, France.
E-mail address: veitia.reiner@ijm.univ-paris-diderot.fr (R.A. Veitia).
[1] These authors have contributed equally to this review and are listed alphabetically.

0303-7207/$ - see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2011.06.019

eyelids and in fetal and adult ovaries. This expression pattern correlates with the BPES phenotype (Cocquet et al., 2002; Crisponi et al., 2001; Pailhoux et al., 2001). Expression of FOXL2 in the mammalian ovary is detectable at the moment of gonadal determination and is maintained throughout fetal life and adulthood. FOXL2 expression at the protein level seems restricted to the somatic compartment of the ovary and is especially important in granulosa cells (Cocquet et al., 2002; Crisponi et al., 2001; Pannetier et al., 2003). *Foxl2* is also expressed in the pituitary gland (Kioussi et al., 1999; Treier et al., 1998) and might be involved in its organogenesis. In the adult pituitary, Foxl2 is mainly expressed in gonadotrope and thyrotrope cells (Ellsworth et al., 2006). Online data suggest that FoxL2 is also expressed in other cells or tissues, such as macrophages, blood reticulocytes, hepatocytes, colon, heart (Moumne et al., 2008a,b). However, the role of FOXL2 in the development and/or function of these tissues remains to be studied.

Two constitutive *Foxl2* knock-out (KO) mouse models have been produced thus far (Schmidt et al., 2004; Uda et al., 2004). Homozygous KO animals undergo strong perinatal lethality (Uda et al., 2004). The surviving animals have a small size (Uda et al., 2004), which might be linked to a reduction of the plasmatic level of insulin-like growth factor 1 (Uda et al., 2004). However, the molecular and physiological bases of this phenomenon are unexplained. These animals have also severe craniofacial abnormalities, severe eyelid hypoplasia and are born with open eyes (Uda et al., 2004).

As expected, female *Foxl2*$^{-/-}$ mice are sterile (Schmidt et al., 2004; Uda et al., 2004). They display small and disorganized ovaries where primary follicles are not formed (Schmidt et al., 2004; Uda et al., 2004). Schmidt et al. (2004) described the formation of primordial follicles whose granulosa cells did not undergo the normal 'squamous-to-cuboidal' morphological transition (which represents the maturation from primordial to primary follicles) (Schmidt et al., 2004). In this model, granulosa cells stay 'flat' and fail to initiate proliferation (Schmidt et al., 2004). On the contrary, Uda et al. (2004) described a seemingly earlier defect leading to a perturbation of the fragmentation of ovigerous cords, which fail to produce primordial follicles (Uda et al., 2004). Interestingly, germ cells do not seem to be affected during the first stages of folliculogenesis (Schmidt et al., 2004; Uda et al., 2004). Around birth, the number of oocytes is similar between mutant and wild-type mice but a massive atresia appears early, leading to a premature depletion of the follicular stock and infertility (Schmidt et al., 2004; Uda et al., 2004).

Mature granulosa cells devoid of *Foxl2* acquire Sertoli cell-specific characteristics, including expression of *Sox9*, *Amh*, as well as other genes involved in testicular determination/differentiation (Ottolenghi et al., 2005). Interestingly, overexpression of *Foxl2* in XY transgenic mice induces seminiferous tubule disorganization and the development of ovotestis-like gonads (Ottolenghi et al., 2007). In a recent paper, FOXL2 has been shown to be required to prevent transdifferentiation of follicle cells in the adult ovary into 'testis-like' cells (Uhlenhaut et al., 2009). Indeed, inducible deletion of *Foxl2* in adult ovarian follicles leads to histological changes affecting most of the gonad (Uhlenhaut et al., 2009). For instance, the typical follicular structure of the ovary turned into structures resembling seminiferous tubules 3 weeks after triggering *Foxl2* deletion. Most tubule-like structures lost their oocytes and granulosa cells acquired morphological characteristics of Sertoli cells (Uhlenhaut et al., 2009). Transcriptomic analyses of sex-reversed gonads have shown the up-regulation of typical Sertoli (*Gata1*, *Tif2*, *Dax1*, *Dhh*, *Dmrt1*, and notably *Sox9*) and Leydig cell (*Hsd17b3*) markers. This and other experiments have led the authors to propose that granulosa cells become reprogrammed into Sertoli-like cells in XX *Foxl2*$^{-/-}$ gonads (Uhlenhaut et al., 2009).

2. An overview of *FOXL2* germinal and somatic mutations and variants

More than 260 *FOXL2* mutations and variants have been identified since its discovery in 2001 (details in the FOXL2 mutation database at http://medgen.ugent.be/LOVD2/home.php). Intragenic *FOXL2* mutations, which can alter protein function and structure, are the most abundant in BPES. Indeed, more than 100 unique mutations have been identified within the coding sequence (for review, see (Benayoun et al., 2009a,b,c; Beysen et al., 2008)). Reduced dosage of *FOXL2* also causes BPES (as pointed out by complete allele deletions), but dominant negative effects in the case of missense mutations cannot be ruled out. Over 40% of intragenic mutations affect the portion coding for the forkhead domain, which represents about 25% of the protein length. This domain is very well conserved among Eucaryotes excluding plants (*i.e.* the Opistokhonts) (Carlsson and Mahlapuu, 2002). It has multiple roles (*i.e.* protein–protein and DNA–protein interactions, nuclear localization, etc.) and it is thus easy to understand that its alterations can perturb protein function (Carlsson and Mahlapuu, 2002).

The coding region of *FOXL2* displays an outstanding mutational hotspot, which encodes the polyalanine domain. Indeed, the expansion of the polyalanine run from 14 to 24 residues accounts for about one third of all intragenic mutations (De Baere et al., 2003). This polyalanine expansion leads to intranuclear aggregation and cytoplasmic mislocalization of the protein (whereas the normal FOXL2 protein is exclusively nuclear) (Caburet et al., 2004). Aggregation and mislocalization should interfere with the availability of normal FOXL2 (Caburet et al., 2004). Indeed, a promoter-specific dominant-negative effect has been described (Benayoun et al., 2008). However, the polyalanine-expanded protein retains a partial transactivation capacity on high-affinity target promoters, which might explain why it is often associated with type II BPES (*i.e.* without POF; Benayoun et al., 2008; Moumne et al., 2008a,b).

More than 70% of BPES patients bear intragenic mutations in *FOXL2*, yet no clear correlation has been drawn between such mutations and the type of BPES developed (type I or II). Dipietromaria et al. dissected the molecular and functional effects of *FOXL2* missense mutations, known to induce BPES associated with POF (type I) or not (type II) (Dipietromaria et al., 2009). We found a clear correlation between the transcriptional activity of FOXL2 variants on two different reporter promoters and the type of BPES. In short, a mutant completely lacking activity on such reporter promoters is likely to lead to BPES with POF.

Thus far, the vast majority of FOXL2 mutations have been detected in BPES cases. Interestingly, two missense mutations in the ORF, leading to the amino-acid substitutions p.Y258N (Harris et al., 2002) and p.G187D (Laissue et al., 2009), were identified in non-syndromic POF patients. A deletion of 10 residues of the polyalanine tract has also been described associated with isolated POF (Gersak et al., 2004; Harris et al., 2002). Moreover, very recently, the recurring somatic mutation c.402C>G, which leads to the amino acid substitution p.C134W was identified in adult-type Ovarian Granulosa Cell Tumors (OGCTs) (Shah et al., 2009). The presence of this mutation has been confirmed in over 95% of adult-type OGCTs by different laboratories (Schrader et al., 2009; Benayoun et al., 2010; Jamieson et al., 2010; Kim et al., 2010a,b; Hes et al., 2011; Al-Agha et al., 2011; D'Angelo et al., 2011; Gershon et al., 2011). No mislocalization of the mutated protein was observed (Benayoun et al., 2010; Shah et al., 2009). Very recently, two laboratories have detected significant differences between FOXL2 WT and the p.C134W mutant. Specifically, Fleming et al. found that p.C134W increased induction of aromatase, a known target of FOXL2 and Kim et al. found a lowered capacity of the p.C134W variant to induce apoptosis (Fleming et al., 2010; Kim et al., 2010a,b). Thus,

the mutant protein might no longer be able to induce cell death. We have recently reported that this mutation might also compromise the capacity of FOXL2 to modulate cell cycle (Benayoun et al., 2011). In order to better understand the long-term physiological and molecular impact of p.C134W homologous mutation in the mouse, it would be interesting to create an inducible tissue specific knock-in mouse model, much like the one that has been described for the oncogenic mutation p.G12D of the K-Ras gene (Chan et al., 2004). This system relies on a translational inhibition of the mutated allele that is specifically removed using an inducible CRE recombinase/Lox technology.

Sequence variants, whose functional effects are still unknown, have been identified in the 3′ UTR of the *FOXL2* transcription unit (Li et al., 2005; Qian et al., 2004). It would be interesting to study the functional significance of such mutations in the 3′ UTR on transcript stability and translation as they might provide an entry point to better understand the regulation of FOXL2 at the post-transcriptional and translational levels.

Many other mutations involving the *FOXL2* locus have been reported. The most abundant defects are deletions involving the *FOXL2* gene itself in about 12% of BPES patients (Beysen et al., 2005; D'Haene et al., 2009, 2010). Deletions may also involve the 5′ or 3′ sides of the gene, and include partial to whole-gene deletions. Deletions can also encompass *FOXL2* and adjacent genes. Such larger genomic rearrangements represent 10% of defects found in BPES patients, but in these cases other associated pathologies can appear (*i.e.* contiguous gene syndrome). For example a patient with a 7.7 Mb deletion encompassing *FOXL2* and *ATR* presents a BPES and Seckel syndrome (de Ru et al., 2005). D'Haene et al. have reported a small deletion of 7.4 kb located at 283 kb 5′ to *FOXL2* (D'Haene et al., 2009). Interestingly, the deleted fragment contains conserved non-coding sequences. This mutation appears in a BPES patient bearing a wild-type *FOXL2* coding sequence on both alleles. The analysis of the spatial organization and interaction patterns of a normal and deleted region with the FOXL2 promoter has shown that the 7.4 kb fragment normally interacts with the latter to ensure a correct FOXL2 expression. Interestingly, this deleted sequence is homologous to a region encompassed by the PIS deletion in goat, which suggests a conserved molecular regulation of the locus activity (D'Haene et al., 2009; Pailhoux et al., 2001).

3. An overview of FOXL2 transcriptional targets and partners

In the ovary, FoxL2 is known to regulate aromatase expression in numerous vertebrate species, such as fishes, chicken, frog and goat (Baron et al., 2004; Nakamoto et al., 2006; Govoroun et al., 2004; Oshima et al., 2008; Pailhoux et al., 2002; Pannetier et al., 2006; Wang et al., 2007). Moreover, FOXL2 has been shown to repress the transcriptional activity of the promoter of the gene encoding the Steroidogenesis Acute Response protein (StAR) (Pisarska et al., 2004). StAR catalyzes the translocation of cholesterol from the outer to the inner mitochondrial membrane, where it is transformed into steroids (Stocco, 2001). *StAR* inhibition by FOXL2 might help maintain immature follicles in a quiescent state (Pisarska et al., 2004). A transcriptome study comparing granulosa-like KGN cells overexpressing FOXL2 or not revealed that it upregulates the expression of other actors of steroid metabolism, such as *PPARGC1A* and *NR5A2* (Batista et al., 2007). The levels of other genes involved in various cellular pathways were perturbed by FOXL2 overexpression in KGN cells. This was the case of genes encoding various inflammatory chemokine ligands and the *Prostaglandin Synthase 2* gene (*PTGS2*) (Batista et al., 2007).

FOXL2 overexpression in KGN cells also upregulates genes involved in the metabolism of reactive oxygen species (ROS) such as the *Manganese mitochondrial Superoxide Dismutase* (*MnSOD* or *SOD2*) (Batista et al., 2007). This suggests that FOXL2 is an actor of the stress response. Indeed, *FOXL2* expression is upregulated by oxidative stress (Benayoun et al., 2009a,b,c). The regulation of the oxidative stress in the ovary is a crucial issue because ovulation is accompanied by ROS generation (Agarwal et al., 2005). Very recently, using a functional genomics approach, we have found that FOXL2 modulates cell cycle regulators (Benayoun et al., 2011). Accordingly, FOXL2 upregulation promotes accumulation of cells in the G1 phase. This is accompanied by an increased protection from oxidative damage, notably by increasing oxidized DNA repair (Benayoun et al., 2011). We found that mutated *FOXL2* versions leading to type I BPES (with ovarian dysfunction) fail to transactivate cell-cycle and DNA-repair targets, whereas mutations leading to type II BPES activate them correctly (Benayoun et al., 2011). This is in agreement with clinical observations (for instance, no tendency to develop OGCTs in type II BPES patients) (Benayoun et al., 2009a,b,c). We also found a mild cell type- and promoter-dependent hypomorphy of the tumor-associated mutation p.C134W (Benayoun et al., 2011).

As FOXL2 is involved in various cellular pathways, the cell must have ways to address it to the right set of promoters in response to a particular signal. This might be achieved at least in part through physical interactions between FOXL2 and other factors. Indeed, FOXL2 has been shown to interact with DDX20 (also known under the aliases DP103 and Gemin-3), Steroidogenic Factor 1 (SF1, also known as NR5A1), estrogens receptors alpha and beta (ESR1 and 2, respectively) and Smad3 (Blount et al., 2009; Corpuz et al., 2010; Ellsworth et al., 2003; Kim et al., 2009; Lee et al., 2005a,b; Park et al., 2010; Uhlenhaut et al., 2009; Wang et al., 2007; Yang et al., 2010).

In particular, DP103 is a DEAD-box RNA helicase, which may regulate transcription (Gillian and Svaren, 2004; Grundhoff et al., 1999; Ou et al., 2001; Yan et al., 2003). In the mouse ovary, DP103 protein is detected in granulosa and thecal cells and to a lower extent in the oocyte (Mouillet et al., 2008). Interestingly, heterozygous DP103 mouse mutants display ovarian abnormalities consisting in the presence of follicles without oocyte (Mouillet et al., 2008). DP103 was shown to interact directly with Foxl2 in a yeast two-hybrid screen (Lee et al., 2005a,b). Moreover, DP103, when co-transfected with Foxl2, potentiates the pro-apoptotic ability of the latter in ovarian CHO and primary granulosa cells (Lee et al., 2005a,b). Apoptosis is one of the mechanisms of follicle stock regulation, thus direct or indirect stimulation of apoptosis by FoxL2–DP103 was suggested to be implicated in the etiology of follicular depletion in BPES patients. More recently, it has been shown that the C134W mutation, which appears in granulosa cell tumors, is less effective at inducing apoptosis than wild-type Foxl2 (Kim et al., 2010a,b). However, the potential role of DP103 in this defect was not investigated, and it would be interesting to test whether the interaction between DP103 and mutated FOXL2 is disturbed.

DP103 is also known to be an inhibitor of the trans-activation activity of SF1, which is an orphan nuclear receptor (NR5A1) involved in pituitary, adrenal and gonadal development (Ou et al., 2001). DP103 physically interacts with SF1 and induces its SUMOylation mediated by PIAS1. This SUMOylation represses SF1 induction of aromatase (Cyp19), StAR and 3β-hydroxysteroid dehydrogenase genes (Lee et al., 2005a,b). Interestingly, SF1 also directly interacts with FoxL2 (Park et al., 2010; Wang et al., 2007; Yang et al., 2010). Mouse conditional mutants for Sf1 (in granulosa cells) display a sterile phenotype, associated with a lack of corpora lutea, indicating a potential implication of SF1 in ovulation (Jeyasuria et al., 2004). SF1 is also an important regulator of steroidogenesis as it is implicated in the regulation of the expression of several enzymes such as CYP11, CYP17, CYP19A1 and StAR (Parker, 2004). Interestingly, Foxl2 was shown to specifically

inhibit SF1 binding to the Cyp17 promoter and to induce a decrease of SF1-mediated trans-activation of Cyp17 expression (Park et al., 2010). As the Foxl2–Sf1 protein complex seems to enhance promoter activity of Cyp19a1/aromatase gene, it was proposed that this dual regulation could play a role in the pro-androgenic (Cyp17) and pro-estrogenic (Cyp19) balance, the perturbation of which would lead to folliculogenesis abnormalities (Park et al., 2010). Interestingly, both FOXL2 and SF1 have been implicated in ovarian/follicular development, but also in gonadal determination. Indeed, SF1 mutations can lead to male-to-female sex reversal in human (Lin et al., 2007) and Foxl2 misregulation has been implicated in female-to-male sex reversal in the goat (Pailhoux et al., 2001) and in the mouse (Uhlenhaut et al., 2009). These two players act directly on the expression of SOX9, a major testis-determining gene.

Foxl2 and SF1 also synergistically up-regulate the promoter of Mc2r (Yang et al., 2010), which encodes an ACTH receptor required for steroidogenesis and adrenal gland development (Chida et al., 2010). In addition, Mc2r is known to be expressed in *corpora lutea* and stroma of bovine ovaries, but its potential regulation by Foxl2 and SF1 in such a context remains to be explored.

Foxl2 also interacts with estrogen receptor alpha (Erα/Esr1) (Kim et al., 2009; Uhlenhaut et al., 2009). Indeed, Foxl2 inhibits the activation of *PTGS2* promoter by ERα. Interestingly, this inhibition seems to be DNA-independent, suggesting that Foxl2 can act as a trans-repressor of a transcription factor even if no Foxl2 binding element is present in the promoter sequence. ERα mouse mutants display multiple phenotypes including ovarian abnormalities. The ovarian defect consists in massive follicular atresia associated with hemorrhagic cysts and no *corpora lutea* formation (Dupont et al., 2000). ERα regulates expression of activin A and B, FSHβ, LHβ and αGSU genes in the pituitary (Glidewell-Kenney et al., 2008). Interestingly, Foxl2, in interaction with Smad3, is known to be involved in the pituitary–ovarian regulatory feedback through the regulation of α-GSU and FSH-β, which are also targets of ERα (Corpuz et al., 2010; Ellsworth et al., 2006). Unfortunately, little is known on the modulation of other ERα targets by Foxl2.

Smad3 is a transcription factor activated by phosphorylation by TGF-β and activin receptors. This activation leads to nuclear import of the Smad3/4 complex and its binding to target promoters. Smad3 directly interacts with Foxl2 and regulates the *GnRHR* (gonadotropin-releasing hormone receptor) promoter, one of its various pituitary targets. Its expression is regulated through the GnRHR Activating Sequence (GRAS), a composite regulatory element. Indeed, the GRAS sequence contains partially overlapping binding sites for Foxl2, Smad3 and AP-1 (Ellsworth et al., 2003). Foxl2 also modulates the expression of the glycoprotein hormone α-subunit (α-GSU) (Ellsworth et al., 2003, 2006). The potential role of Foxl2 in the regulation of gonadotropin secretion is further supported by its co-regulation with Smad3 of the *FSH-β* and *Follistatin* promoters (Blount et al., 2009; Lamba et al., 2009).

The conditional *Foxl2* KO model mouse model mentioned above displays a rapid upregulation of *Sox9* expression in *Foxl2*-ablated gonads. This suggests the existence of a transcriptional repression of *Sox9* by Foxl2 in the adult ovary. Indeed, the TESCO element, which regulates *Sox9* expression, is swiftly reactivated after *Foxl2* ablation. Not surprisingly, TESCO is directly recognized by Foxl2, as shown by chromatin immunoprecipitation experiments (Uhlenhaut et al., 2009). *In vitro* results show that Foxl2 is able to attenuate TESCO activation by Sf1, Sry/Sf1 and Sox9/Sf1. Further *in vitro* experiments have shown that Erα, which is not able to repress the TESCO alone, can synergize with Foxl2 to induce a significant repression (Uhlenhaut et al., 2009). Co-immunoprecipitation data show that Erα (and β) and Foxl2 physically interact (Uhlenhaut et al., 2009).

Foxl2 interacts with several transcription factors involved in the regulation of different pathways (apoptosis, steroidogenesis, steroid hormone and activin response). These interactions result often in a synergistic regulation of the pathway (*i.e.* DP103 alone does not affect apoptosis, while it enhances the pro-apoptotic action of FOXL2). Such non-linear behaviors might be a way to direct either FOXL2 or its partner(s) to specific subsets of target gene. These interactions also underline the ability of FOXL2 to regulate promoter activity either with direct DNA binding or through protein–protein interactions, suggesting that FOXL2 can act as classical transcription factor, but might also modulate the activity of other transcription factors without contacting DNA. This point is of practical importance when studying the FOXL2 direct transcriptional targets and in finding the DNA-binding sequence recognized by FOXL2.

4. A brief meta-analysis of FOXL2 targets

In order to gain further insights into the diversity of potential target genes directly regulated by FOXL2 and their role in the ovary, we performed a meta-analysis of microarray data. Specifically, we compiled ovarian transcriptomic data from the three FoxL2 homozygous knock-out experiments: during embryonic development (E16.5, GEO:GSE12989 (Garcia-Ortiz et al., 2009)), on the day of birth (P0, GEO:GSE12989 (Garcia-Ortiz et al., 2009)), at 3 days of age (P3, GEO:GSE16853 (Uhlenhaut et al., 2009)) and at 8 weeks of age (floxed conditional KO, W8, GEO:GSE16853 (Uhlenhaut et al., 2009)). On the basis of the KO/WT expression ratio, we extracted the transcripts deregulated in the absence of Foxl2, at least in one developmental time point, with a cut-off of 1.5 or 0.67. Then, we focused on the intersection of this set of transcripts with the list of the direct FoxL2 targets identified by ChIP-on-Chip in ovaries of 5 weeks-old mice (ArrayExpress: E-MTAB-400 (Benayoun et al., 2011)). We defined 12 biologically relevant regulation profiles, *i.e.* patterns of FoxL2 effect on its targets, such as "constantly upregulated when FoxL2 is present" (++++ profile). We extracted the list of deregulated direct targets that follow those patterns, focusing on genes with a False Discovery Rate equal or inferior to 0.05 in the ChIP-on-Chip analysis. Furthermore, we filtered this list on the basis of detection in a second ChIP-on-Chip analysis on human KGN cells overexpressing FOXL2 (ArrayExpress: E-MTAB-399, KF3 cells (Benayoun et al., 2011)). Next, we analyzed for each profile the role of the identified genes, by direct bibliographic search and DAVID annotation tool (Huang da et al., 2009). The FoxL2 direct target genes following those profiles were classified according to the broad known functions of FoxL2, as shown in Table 1 (a fully referenced version of this table is available as an on-line supplement) (Fig. 1).

It is obvious that we can miss known FoxL2 targets with this analysis, because of several reasons. Known targets can be present in the studied profiles, but below our FDR cut-off for mouse ChIP-on-Chip data, as evidenced by the aromatase gene (Cyp19a1). Moreover, the fact that the ChIP-on-Chip data involves 5-weeks old mice pinpoints more efficiently adult-stage FoxL2 targets while direct targets regulated during development and around birth might be overlooked. This can explain why targets implicated in sex determination and early gonadal development are not represented. In addition, a known direct target can be detected as regulated but indirectly (such as Sox9), because of the promoter array design for the ChIP-on-Chip, that was not build to encompass long-distance regulating sequences.

Despite these biases, this study identifies many direct targets that appear relevant to the described functions of FOXL2 in the ovary, namely in the regulation of apoptosis, cell cycle,

Table 1
Functional classification of direct FoxL2 targets identified by the transcriptomic and ChIP-on-chip meta-analysis. The regulation profiles are shown according to the action of FoxL2 on its targets (+: up-regulated by FoxL2, −: down-regulated by FoxL2). The (adult) direct target genes sharing the same regulation profile are shown under the relevant ovarian process (see text for details). One gene can be described by several transcripts, each showing a specific profile (*i.e.* FoxP1). Two genes sharing the same bibliographic reference(s) are joined by a & sign (*i.e.* Axin2 & Daxx). A question mark suggests a possible implication of the gene in the shown cellular process, although it could not be formally deduced from the literature. The bold font indicates a stronger level of confidence, as the homolog of the gene detected as a direct target in the murine ChIP-on-Chip was also found in the ChIP-on-Chip analysis of KGN cells overexpressing FOXL2, with an FDR ⩽ 0.05. Genes in normal font were also found in the ChIP-on-Chip analysis of KGN cells overexpressing FOXL2, but with an FDR > 0.05. A fully referenced version of this table is available as an on-line supplement.

(1) Regulation Nb of genes (Nb of direct targets)	Regulation profile E16.5 P0 P3 W8	Folliculogenesis	Cholesterol and steroid biosynthesis	Ovulation and inflamation	Stress response and DNA repair	Apoptosis	Cell cycle	Cancer-related processes	Cell adhesion and matrix remodeling
Constant up 30 genes (8 direct targets)	+ + + +						Cdkn1b **FoxP1**	Cdkn1b	**Thbs1**
Constant down 37 genes (8 direct targets)	- - - -	Adora1		Prkg2				Adora1	
Switch up 9 genes (4 direct targets)	- + + +				**Eya2**			Acta2, Mfap5	
Switch down 5 genes (3 direct targets)	+ - - -					**Bnip2**		**Atp1a1**	
Rising 532 genes (113 direct targets)	- 0 + +	**Serpine2**	**Hmgcr**	**Ednra** Chrdl2?	**Txnl1** & **Txnrd2**	Itga2	**FoxP1, Ttk**	**Dock4**, Lum, Mras, **Socs2**	**Adamts10**, Itga2, Lum, **Serpine2**
Decreasing 264 genes (47 direct targets)	+ + 0 -	**App**		**Snap25**				Rab37	
Adult up-regulation 903 genes (205 direct targets)	0 0 0 +	**Fzd4 Gja1**	**Cyp51 Hmgcr**	**Ednra**	**Mlh1 Sesn3 Dnajb1**?	Chek2 **Mlh1 Steap3**	**Cdk4**, Chek2, E2f7, **FoxP1, Mlh1, Sesn3, Skil, Steap3, Timeless, Ube2c**	**Adamts6**, **Dock4**, Lum, Mras, **Ube2c, Wwox**	**Adamts6, Adamts10, Osbpl3**
Adult down-regulation 1199 genes (277 direct targets)	0 0 0 -	**Igf1**	**Igf1** Il6st	**Tnfaip3**		**Axin2** & **Daxx Bnip2, Fyn, Igf1**, Lass5	Cdkl3	**Fzd7, Fyn, S100pbp, Sos**	**Cdh23** Cldn7, **Fyn**, Vcl, **Jam2**, **Sos**
Post-natal up-regul. 81 genes (12 direct targets)	0 + + +	Hey2 **Serpine2**?					**FoxP1**	**Socs2 Serpine2**	**Serpine2**
Post natal down-regul. 57 genes (7 direct targets)	0 - - -		**Ppargc1a**			**Ppargc1a**			
Embryonic up-regul. 668 genes (102 direct targets)	+ 0 0 0	**Crem**	**Crem**				**Crem**	**Rab37 Cited2**	**Cited2**, Git2 **Col5a3**
Embryonic down-regul. 405 genes (73 direct targets)	- 0 0 0	**Btrc**?		**Runx1**			Cdca5	Cdca5	

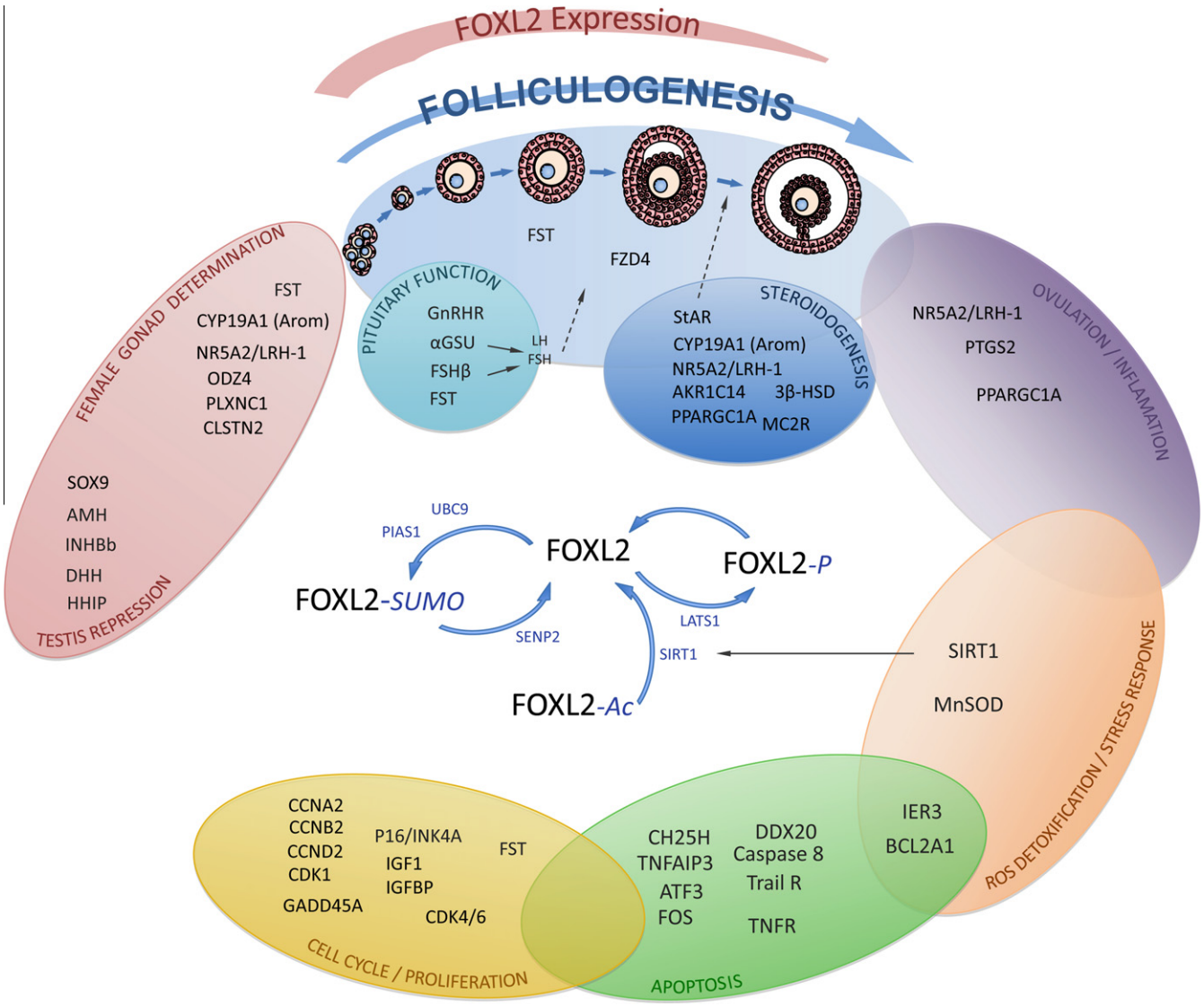

Fig. 1. Summary of FOXL2 targets.

cholesterol/steroid biosynthesis, ROS detoxification and stress response. In addition to these roles, other cellular processes are highlighted, such as cell-to-cell adhesion and matrix remodeling. These last two processes are strongly modulated throughout folliculogenesis and are crucial at particular stages, such as the squamous-to-cuboidal transition of granulosa cells, a hallmark of the formation of primary follicles and absent in FOXL2 KO mice (Schmidt et al., 2004). In the final stages of folliculogenesis, matrix remodeling is necessary to set up the hyaluronan-enriched matrix required for ovulation and for the subsequent luteinisation (Rodgers et al., 2003). Therefore, the potential implication of FoxL2 in regulating cellular adhesion and matrix remodeling reinforces its central role in ovarian function.

Finally, many of its direct targets are members of well-known signaling pathways implicated in ovarian development and function. Indeed, FoxL2 deletion perturbs major members of the WNT signaling pathway (Wnt1, 2b, 3a, 4, 9a, 9b, 10a; Fzd1, 3, 4, 5, 7, 8; Lrp6; Axin2; Ras; Jnk; Plc, CaMkII; ...), of the MAPK pathways (FgfR, Sos, Ras, Nf1, Myc, NfkB, TgfBR, Daxx, Jun, p53, ...), of the insulin pathway (Insr; Irs2, 3, 4; Pik3; Sos; Mknk, ...) (Supplemental file 1).

5. Post-translational modifications of FOXL2 and their regulatory role

Covalent modifications of transcription factors can influence their stability, subcellular localization or their interaction with DNA targets or protein partners, as exemplified by the well-studied p53 and FOXO proteins (Calnan and Brunet, 2008; Kruse and Gu, 2008). During the last ten years, most studies on FOXL2 have focused on either its pathogenic mutations or its transcriptional targets. Little is known about the regulation of FOXL2 and even less about its post-translational modifications. One of the first studies on this topic concerning FOXL2 showed, using 2D-electrophoresis followed by Western blot, that this protein existed in KGN cells under several isoforms of different isoelectric points (pI) (Benayoun et al., 2009a,b). Such a variety of FOXL2 isoforms might account for the variety of biological processes it can regulate, including granulosa cells differentiation, proliferation, cell fate determination and apoptosis. A similar, though more complex, pattern of modified forms has been observed in murine ovary extracts, but it remains undecided whether they are all present in the same cells or in follicles at various maturation stages.

Protein phosphorylation is the most common post-translational modification in eukaryotic cells. The first study to bring mechanistic insights into FOXL2 phosphorylation showed that it is phosphorylated when transiently expressed in CHO cells (Pisarska et al., 2010). Moreover, Pisarska et al. identified the Large Tumor Suppressor 1 (LATS1) as a kinase of FOXL2, and showed that this interaction might take place *in vivo*, as LATS1 and FOXL2 are coexpressed in granulosa cells of early murine follicles (Pisarska et al., 2010). Co-overexpression of LATS1 and FOXL2 in CHO cells led to an increased transrepression of the *StAR* promoter by FOXL2 (Pisarska et al., 2010). Interestingly, mice lacking *Lats1* exhibit pituitary hyperplasia and dysfunction, associated with severe female infertility, mainly as a consequence of LH depletion (St. John et al., 1999). A reduction of the pool of follicles is also observed. Although the role of LATS1 in generating this phenotype is not clear, it would be interesting to identify the residue(s) modified by this particular kinase to assess the relevance of this modification *in vivo*.

High-throughput mass-spectrometry studies have also provided data on FOXL2 phosphorylation. Indeed, a study designed to identify phosphorylated tyrosine residues identified tyrosines 127 and 258 as modified in MO-91 acute myelogenous leukemia cells (http://www.freepatentsonline.com/y2010/0009463.html). Interestingly, a mutation of FOXL2 at residue 258 (p.Y258N) has been reported in an isolated POF case (Harris et al., 2002), suggesting that this residue and its modification are specifically required for a correct ovarian function of FOXL2. Other phosphorylated residues (S33 and S263), referenced in the Human Protein Reference Database (http://www.hprd.org/), seem to result from a wrong annotation misattributing data from FOXO3a to FOXL2. Therefore, although FOXL2 can be phosphorylated on serine residues, no formal identification has been reported so far.

SUMOylation is the best characterized post-translational modification of FOXL2. SUMOylated FOXL2 was first identified in extracts of KGN cells having undergone oxidative stress or heat shock. This was subsequently confirmed in αT3 cells (mouse pituitary immortalized cell line) and whole murine ovary extracts (Benayoun et al., 2009a,b,c). Later, two parallel two-hybrid screenings showed that the SUMO E2 ligase UBC9 directly interacted with FOXL2, bringing mechanistic details on its SUMOylation process (Kuo et al., 2009; Marongiu et al., 2010). Through transient transfection of FOXL2 in CHO or COS-7 cells, respectively, they observed SUMOylated forms with an apparent MW close to 110 kDa. Moreover, Marongiu and collaborators showed that the E3-ligase PIAS1 also interacted with FOXL2 and increased its degree of SUMOylation (Marongiu et al., 2010). Based on an alignment with a consensus site of SUMOylation in the mosquito homolog of FOXL2, Pisarska and collaborators predicted that lysine 25 might be a SUMOylation site of FOXL2 and they observed a decreased intensity of the 110 kDa band in a p.K25R mutant (Kuo et al., 2009). Marongiu and collaborators performed similar experiments, testing lysines 25, 87, 114 and 150 and concluded that lysines 114 and 150 were preferred to lysine 25 for SUMOylation (Marongiu et al., 2010). Despite this divergence, both teams agree that a loss of SUMOylation impairs the ability of FOXL2 to repress the *StAR* promoter. Marongiu et al. proposed that this may be due to a decreased protein stability independently of the ubiquitin–proteasome pathway.

SUMOylation and phosphorylation are unlikely to represent the whole post-translational modifications of FOXL2. For instance, hyperacetylating conditions (*i.e.* overexpression of p300) increase the transactivation ability of FOXL2 on most target promoters tested (Benayoun et al., 2009a,b,c). However, it is not known yet whether acetylation affects the intrinsic transactivation properties of FOXL2 or less specific properties, such as its stability. On the contrary, the action of the SIRT1 deacetylase decreases the transactivation capacity of FOXL2 on a series of stress-response promoters (Benayoun et al., 2009a,b,c). This effect is also visible on targets linked to cell cycle, growth factor signaling and DNA-repair (Benayoun et al., 2011). Interestingly, "deacetylated" FOXL2 displays an enhanced ability to directly upregulate the *SIRT1* promoter (Benayoun et al., 2009a,b,c).

6. Conclusions and perspectives

FOXL2 is a well-conserved transcription factor found in a wide range of species, and appears to have conserved crucial functions in reproduction. For historical reasons, the involvement of FOXL2 in pituitary and ovarian function has been the focus of most of the current research. However, FoxL2 expression outside these tissues suggests that it might have other functions that remain largely unexplored.

Despite current data on the post-translational modifications of FOXL2, little is known about the modified residues and their significance *in vivo* and nothing is known about the interplay between these modifications and their effects. Notably, the way they are set up on FOXL2 in response to intra- or extra-cellular signals, and their effect on FOXL2 partner and target selection, remain to be elucidated. The study of FOXL2 post-translational modifications is only at its beginning and its advance could help better understand FOXL2 function. Furthermore, other modifications known to be important for the regulation of transcription factor activity and signal transduction, such as ubiquitinylation or lysine/arginine methylation, should also be explored in the case of FOXL2.

Even though it is known that various kinds of FOXL2 mutations lead to protein aggregation, the precise molecular pathogenesis mechanisms are still poorly understood. The potential interference between aggregation of mutated FOXL2 and its post-translational modifications remains to be studied. These two items need to be addressed with suitable animal models. Such models might also prove valuable to test anti-aggregation molecules that would resolubilize, at least partially, the mutated FOXL2 protein. This kind of approach would be an interesting therapeutic possibility for BPES patients and deserves further exploration, notably in order to restore or improve FOXL2 function in the ovary.

Recent evidence has incriminated FOXL2 in processes such as the ovarian response to oxidative stress (Benayoun et al., 2009a,b,c, 2011). The premature ovarian failure observed in type I BPES female patients is compatible with an involvement of FOXL2 in the control of the rate of ovarian ageing in mammals and potentially in other animals. Cellular ageing can be considered as counterbalancing tumorigenesis, and six key events are believed to be crucial to allow or promote malignant transformation, namely, evasion of apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, increased invasivity, ability to sustain angiogenesis, and an unbound replicative potential (Hanahan and Weinberg, 2000). The involvement of FOXL2 in many of these processes can explain its contribution to cancer progression when mutated or misregulated. Indeed, FOXL2 in normal conditions may counter cancer (i) by promoting apoptosis of severely damaged cells, (ii) by promoting DNA-repair and genomic stability, (iii) by slowing the cell cycle and (iv) by interfering with growth-factor signaling. A synthetic model of the potential action of FOXL2 in the regulation of reproductive longevity and tumor suppression is displayed in Fig. 2. We hypothesize that, in the event of a severe stress, FOXL2 would logically promote granulosa cell apoptosis, leading to the elimination of follicles damaged beyond repair. In the cases of milder stress, we propose that FOXL2 would rather promote ROS detoxification, repair of genotoxic lesions and favor their return to pre-stress cellular homeostasis. By protecting cells against ROS damage and by regulating cell proliferation (and thus

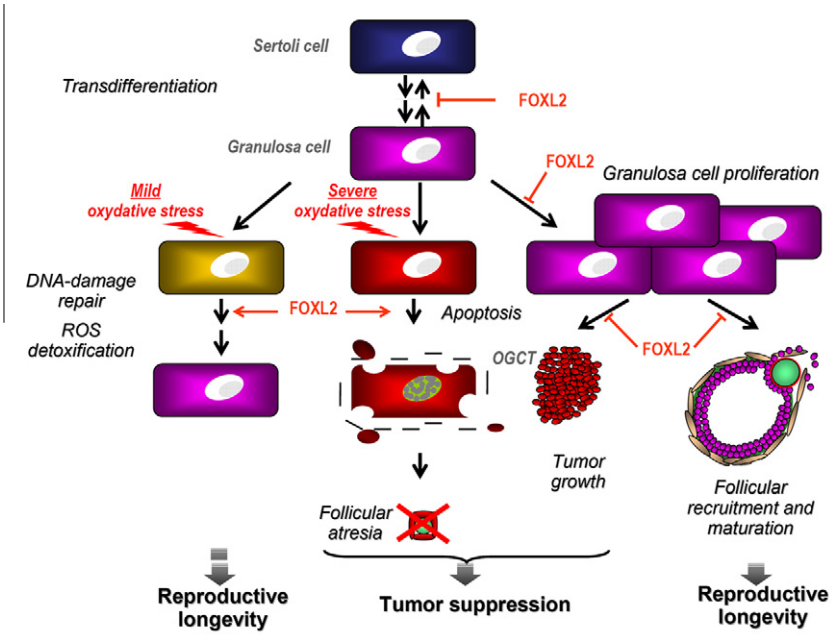

Fig. 2. Synthetic model of FOXL2 action and potential implications in ovarian ageing and granulosa cell tumor suppression. We used the data reviewed here, concerning the role of FOXL2 in the regulation of cell survival/apoptosis, ROS homeostasis and cell proliferation, to compile a synthetic model of the action of FOXL2 in ovarian physiology and pathology. Concerning apoptosis, FOXL2 seems to have a dual behavior. Indeed, follicular atresia in *Foxl2*$^{-/-}$ ovaries suggested that FoxL2 acted as an anti-apoptotic agent (Schmidt et al., 2004; Uda et al., 2004), whereas *in vitro* data has suggested it to be pro-apoptotic (Lee et al., 2005a,b). This rather ambivalent behavior is illustrated by its ability to upregulate several pro- and anti-apoptotic regulators in KGN cells overexpressing it (Batista et al., 2007). Such ambivalence has been described for other forkhead factors, and might be cell- or condition-dependent (Brunet et al., 1999; Burgering and Medema, 2003). Here, we hypothesize that, in the event of a severe stress, FOXL2 would likely promote granulosa cell apoptosis and thus elimination of follicles damaged beyond repair. In the cases of milder stresses, we propose that FOXL2 would rather promote ROS detoxification, the repair of genotoxic lesions and a return to pre-stress conditions. By promoting cell protection against ROS damage (thus protecting the reserve of functional follicles) and by preventing cell proliferation (and thus the recruitment of follicles into active folliculogenesis, which is accompanied by increased division rates), FOXL2 should also be able to promote ovarian longevity. Of course, one of the main "regular" roles of FOXL2 is to preserve the ovarian identity of granulosa cells and prevent their transdifferentiation into Sertoli-like testis cells. The direction of the action of FOXL2 is indicated by a red arrow (when promoting the process) or a bar (when inhibiting the process).

the recruitment of follicles into active folliculogenesis, which is accompanied by increased division rates), FOXL2 should also promote female reproductive longevity.

Acknowledgments

Our work is supported by Centre National de la Recherche Scientifique, La fondation pour la recherche médicale, La ligue nationale contre le Cancer (Comité de Paris), l'Université Paris Diderot-Paris7 and Institut Universitaire de France.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mce.2011.06.019.

References

Agarwal, A., Gupta, S., Sharma, R.K., 2005. Role of oxidative stress in female reproduction. Reprod. Biol. Endocrinol. 3, 28.

Al-Agha, O.M., Huwait, H.F., Chow, C., Yang, W., Senz, J., Kalloger, S.E., Huntsman, D.G., Young, R.H., Gilks, C.B., 2011. FOXL2 is a sensitive and specific marker for sex cord-stromal tumors of the ovary. Am. J. Surg. Pathol. 35, 484–494.

Baron, D., Cocquet, J., Xia, X., Fellous, M., Guiguen, Y., Veitia, R.A., 2004. An evolutionary and functional analysis of FoxL2 in rainbow trout gonad differentiation. J. Mol. Endocrinol. 33, 705–715.

Batista, F., Vaiman, D., Dausset, J., Fellous, M., Veitia, R.A., 2007. Potential targets of FOXL2, a transcription factor involved in craniofacial and follicular development, identified by transcriptomics. Proc. Natl. Acad. Sci. USA 104, 3330–3335.

Benayoun, B.A., Caburet, S., Dipietromaria, A., Bailly-Bechet, M., Fellous, M., Vaiman, D., Veitia, R.A., 2008. The identification and characterization of a FOXL2 response element provides insights into the pathogenesis of mutant alleles. Hum. Mol. Genet. 17, 3118–3127.

Benayoun, B.A., Batista, F., Auer, J., Dipietromaria, A., L'Hote, D., De Baere, E., Veitia, R.A., 2009a. Positive and negative feedback regulates the transcription factor FOXL2 in response to cell stress: evidence for a regulatory imbalance induced by disease-causing mutations. Hum. Mol. Genet. 18, 632–644.

Benayoun, B.A., Dipietromaria, A., Bazin, C., Veitia, R.A., 2009b. FOXL2: at the crossroads of female sex determination and ovarian function. In: Maiese, K. (Ed.), Forkhead Transcription Factors: Vital Elements in Biology and Medicine. Landes Biosciences, Austin, TX, USA, pp. 207–226.

Benayoun, B.A., Kalfa, N., Sultan, C., Veitia, R.A., 2009c. The forkhead factor FOXL2: a novel tumor suppressor? Biochim. Biophys. Acta 1805, 1–5.

Benayoun, B.A., Caburet, S., Dipietromaria, A., Georges, A., D'Haene, B., Pandaranayaka, P.J., L'Hote, D., Todeschini, A.L., Krishnaswamy, S., Fellous, M., De Baere, E., Veitia, R.A., 2010. Functional exploration of the adult ovarian granulosa cell tumor-associated somatic FOXL2 mutation p. Cys134Trp (c.402C>G). PLoS One 5, e8789.

Benayoun, B.A., Georges, A.B., L'Hote, D., Andersson, N., Dipietromaria, A., Todeschini, A.L., Caburet, S., Bazin, C., Anttonen, M., Veitia, R.A., 2011. Transcription factor FOXL2 protects granulosa cells from stress and delays cell cycle: role of its regulation by the SIRT1 deacetylase. Hum. Mol. Genet. 20, 1673–1686.

Beysen, D., Raes, J., Leroy, B.P., Lucassen, A., Yates, J.R., Clayton-Smith, J., Ilyina, H., Brooks, S.S., Christin-Maitre, S., Fellous, M., Fryns, J.P., Kim, J.R., Lapunzina, P., Lemyre, E., Meire, F., Messiaen, L.M., Oley, C., Splitt, M., Thomson, J., Van de Peer, Y., Veitia, R.A., De Paepe, A., De Baere, E., 2005. Deletions involving long-range conserved nongenic sequences upstream and downstream of FOXL2 as a novel disease-causing mechanism in blepharophimosis syndrome. Am. J. Hum. Genet. 77, 205–218.

Beysen, D., De Jaegere, S., Amor, D., Bouchard, P., Christin-Maitre, S., Fellous, M., Touraine, P., Grix, A.W., Hennekam, R., Meire, F., Oyen, N., Wilson, L.C., Barel, D., Clayton-Smith, J., de Ravel, T., Decock, C., Delbeke, P., Ensenauer, R., Ebinger, F., Gillessen-Kaesbach, G., Hendriks, Y., Kimonis, V., Laframboise, R., Laissue, P., Leppig, K., Leroy, B.P., Miller, D.T., Mowat, D., Neumann, L., Plomp, A., Van Regemorter, N., Wieczorek, D., Veitia, R.A., De Paepe, A., De Baere, E., 2008.

Identification of 34 novel and 56 known FOXL2 mutations in patients with blepharophimosis syndrome. Hum. Mutat. 29, E205–E219.
Blount, A.L., Schmidt, K., Justice, N.J., Vale, W.W., Fischer, W.H., Bilezikjian, L.M., 2009. FoxL2 and Smad3 coordinately regulate follistatin gene transcription. J. Biol. Chem. 284, 7631–7645.
Brunet, A., Bonni, A., Zigmond, M.J., Lin, M.Z., Juo, P., Hu, L.S., Anderson, M.J., Arden, K.C., Blenis, J., Greenberg, M.E., 1999. Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. Cell 96, 857–868.
Burgering, B.M., Medema, R.H., 2003. Decisions on life and death: FOXO forkhead transcription factors are in command when PKB/Akt is off duty. J. Leukoc. Biol. 73, 689–701.
Caburet, S., Demarez, A., Moumne, L., Fellous, M., De Baere, E., Veitia, R.A., 2004. A recurrent polyalanine expansion in the transcription factor FOXL2 induces extensive nuclear and cytoplasmic protein aggregation. J. Med. Genet. 41, 932–936.
Calnan, D.R., Brunet, A., 2008. The FoxO code. Oncogene 27, 2276–2288.
Carlsson, P., Mahlapuu, M., 2002. Forkhead transcription factors: key players in development and metabolism. Dev. Biol. 250, 1–23.
Chan, I.T., Kutok, J.L., Williams, I.R., Cohen, S., Kelly, L., Shigematsu, H., Johnson, L., Akashi, K., Tuveson, D.A., Jacks, T., Gilliland, D.G., 2004. Conditional expression of oncogenic K-ras from its endogenous promoter induces a myeloproliferative disease. J. Clin. Invest. 113, 528–538.
Chida, D., Miyoshi, K., Sato, T., Yoda, T., Kikusui, T., Iwakura, Y., 2010. The role of glucocorticoids in pregnancy, parturition, lactation, and nurturing in melanocortin receptor 2-deficient mice. Endocrinology 152, 1652–1660.
Cocquet, J., Pailhoux, E., Jaubert, F., Servel, N., Xia, X., Pannetier, M., De Baere, E., Messiaen, L., Cotinot, C., Fellous, M., Veitia, R.A., 2002. Evolution and expression of FOXL2. J. Med. Genet. 39, 916–921.
Corpuz, P.S., Lindaman, L.L., Mellon, P.L., Coss, D., 2010. FoxL2 is required for activin induction of the mouse and human follicle-stimulating hormone {beta}-subunit genes. Mol. Endocrinol. 24, 1037–1051.
Crisponi, L., Deiana, M., Loi, A., Chiappe, F., Uda, M., Amati, P., Bisceglia, L., Zelante, L., Nagaraja, R., Porcu, S., Ristaldi, M.S., Marzella, R., Rocchi, M., Nicolino, M., Lienhardt-Roussie, A., Nivelon, A., Verloes, A., Schlessinger, D., Gasparini, P., Bonneau, D., Cao, A., Pilia, G., 2001. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. Nat. Genet. 27, 159–166.
D'Angelo, E., Mozos, A., Nakayama, D., Espinosa, I., Catasus, L., Munoz, J., Prat, J., 2011. Prognostic significance of FOXL2 mutation and mRNA expression in adult and juvenile granulosa cell tumors of the ovary. Mod. Pathol., doi:10.1038/modpathol.2011.95.
D'Haene, B., Attanasio, C., Beysen, D., Dostie, J., Lemire, E., Bouchard, P., Field, M., Jones, K., Lorenz, B., Menten, B., Buysse, K., Pattyn, F., Friedli, M., Ucla, C., Rossier, C., Wyss, C., Speleman, F., De Paepe, A., Dekker, J., Antonarakis, S.E., De Baere, E., 2009. Disease-causing 7.4 kb cis-regulatory deletion disrupting conserved non-coding sequences and their interaction with the FOXL2 promotor: implications for mutation screening.. PLoS Genet. 5, e1000522.
D'Haene, B., Nevado, J., Pugeat, M., Pierquin, G., Lowry, R.B., Reardon, W., Delicado, A., Garcia-Minaur, S., Palomares, M., Courtens, W., Stefanova, M., Wallace, S., Watkins, W., Shelling, A.N., Wieczorek, D., Veitia, R.A., De Paepe, A., Lapunzina, P., De Baere, E., 2010. FOXL2 copy number changes in the molecular pathogenesis of BPES: unique cohort of 17 deletions. Hum. Mutat. 31, E1332–E1347.
De Baere, E., Beysen, D., Oley, C., Lorenz, B., Cocquet, J., De Sutter, P., Devriendt, K., Dixon, M., Fellous, M., Fryns, J.P., Garza, A., Jonsrud, C., Koivisto, P.A., Krause, A., Leroy, B.P., Meire, F., Plomp, A., Van Maldergem, L., De Paepe, A., Veitia, R., Messiaen, L., 2003. FOXL2 and BPES: mutational hotspots, phenotypic variability, and revision of the genotype–phenotype correlation. Am. J. Hum. Genet. 72, 478–487.
de Ru, M.H., Gille, J.J., Nieuwint, A.W., Bijlsma, J.B., van der Blij, J.F., van Hagen, J.M., 2005. Interstitial deletion in 3q in a patient with blepharophimosis–ptosis–epicanthus inversus syndrome (BPES) and microcephaly, mild mental retardation and growth delay: clinical report and review of the literature. Am. J. Med. Genet. A 137, 81–87.
Dipietromaria, A., Benayoun, B.A., Todeschini, A.L., Rivals, I., Bazin, C., Veitia, R.A., 2009. Towards a functional classification of pathogenic FOXL2 mutations using transactivation reporter systems. Hum. Mol. Genet. 18, 3324–3333.
Dupont, S., Krust, A., Gansmuller, A., Dierich, A., Chambon, P., Mark, M., 2000. Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. Development 127, 4277–4291.
Ellsworth, B.S., Burns, A.T., Escudero, K.W., Duval, D.L., Nelson, S.E., Clay, C.M., 2003. The gonadotropin releasing hormone (GnRH) receptor activating sequence (GRAS) is a composite regulatory element that interacts with multiple classes of transcription factors including Smads, AP-1 and a forkhead DNA binding protein. Mol. Cell. Endocrinol. 206, 93–111.
Ellsworth, B.S., Egashira, N., Haller, J.L., Butts, D.L., Cocquet, J., Clay, C.M., Osamura, R.Y., Camper, S.A., 2006. FOXL2 in the pituitary: molecular, genetic, and developmental analysis. Mol. Endocrinol. 20, 2796–2805.
Fleming, N.I., Knower, K.C., Lazarus, K.A., Fuller, P.J., Simpson, E.R., Clyne, C.D., 2010. Aromatase is a direct target of FOXL2:C134W in granulosa cell tumors via a single highly conserved binding site in the ovarian specific promoter. PLoS One 5, e14389.
Garcia-Ortiz, J.E., Pelosi, E., Omari, S., Nedorezov, T., Piao, Y., Karmazin, J., Uda, M., Cao, A., Cole, S.W., Forabosco, A., Schlessinger, D., Ottolenghi, C., 2009. Foxl2 functions in sex determination and histogenesis throughout mouse ovary development. BMC Dev. Biol. 9, 36.
Gersak, K., Harris, S.E., Smale, W.J., Shelling, A.N., 2004. A novel 30 bp deletion in the FOXL2 gene in a phenotypically normal woman with primary amenorrhoea: case report. Hum. Reprod. 19, 2767–2770.
Gershon, R., Aviel-Ronen, S., Korach, J., Daniel-Carmi, V., Avivi, C., Bar-Ilan, D., Barshack, I., Meirow, D., Ben-Baruch, G., Cohen, Y., 2011. FOXL2 C402G mutation detection using MALDI-TOF-MS in DNA extracted from Israeli granulosa cell tumors. Gynecol. Oncol. June 2 (Epub ahead of print).
Gillian, A.L., Svaren, J., 2004. The Ddx20/DP103 dead box protein represses transcriptional activation by Egr2/Krox-20. J. Biol. Chem. 279, 9056–9063.
Glidewell-Kenney, C., Weiss, J., Hurley, L.A., Levine, J.E., Jameson, J.L., 2008. Estrogen receptor alpha signaling pathways differentially regulate gonadotropin subunit gene expression and serum follicle-stimulating hormone in the female mouse. Endocrinology 149, 4168–4176.
Govoroun, M.S., Pannetier, M., Pailhoux, E., Cocquet, J., Brillard, J.P., Couty, I., Batellier, F., Cotinot, C., 2004. Isolation of chicken homolog of the FOXL2 gene and comparison of its expression patterns with those of aromatase during ovarian development. Dev. Dyn. 231, 859–870.
Grundhoff, A.T., Kremmer, E., Tureci, O., Glieden, A., Gindorf, C., Atz, J., Mueller-Lantzsch, N., Schubach, W.H., Grasser, F.A., 1999. Characterization of DP103, a novel DEAD box protein that binds to the Epstein-Barr virus nuclear proteins EBNA2 and EBNA3C. J. Biol. Chem. 274, 19136–19144.
Hanahan, D., Weinberg, R.A., 2000. The hallmarks of cancer. Cell 100, 57–70.
Harris, S.E., Chand, A.L., Winship, I.M., Gersak, K., Aittomaki, K., Shelling, A.N., 2002. Identification of novel mutations in FOXL2 associated with premature ovarian failure. Mol. Hum. Reprod. 8, 729–733.
Hes, O., Vanecek, T., Petersson, F., Grossmann, P., Hora, M., Perez Montiel, D.M., Steiner, P., Dvorak, M., Michal, M., 2011. Mutational analysis (c.402C>G) of the FOXL2 gene and immunohistochemical expression of the FOXL2 protein in testicular adult type granulosa cell tumors and incompletely differentiated sex cord stromal tumors. Appl. Immunohistochem. Mol. Morphol. 19, 347–351.
Huang da, W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44–57.
Jamieson, S., Butzow, R., Andersson, N., Alexiadis, M., Unkila-Kallio, L., Heikinheimo, M., Fuller, P.J., Anttonen, M., 2010. The FOXL2 C134W mutation is characteristic of adult granulosa cell tumors of the ovary. Mod. Pathol. 23, 1477–1485.
Jeyasuria, P., Ikeda, Y., Jamin, S.P., Zhao, L., De Rooij, D.G., Themmen, A.P., Behringer, R.R., Parker, K.L., 2004. Cell-specific knockout of steroidogenic factor 1 reveals its essential roles in gonadal function. Mol. Endocrinol. 18, 1610–1619.
Kim, J.H., Yoon, S., Park, M., Park, H.O., Ko, J.J., Lee, K., Bae, J., 2010a. Differential apoptotic activities of wild-type FOXL2 and the adult-type granulosa cell tumor-associated mutant FOXL2 (C134W). Oncogene 30, 1653–1663.
Kim, M.S., Hur, S.Y., Yoo, N.J., Lee, S.H., 2010b. Mutational analysis of FOXL2 codon 134 in granulosa cell tumour of ovary and other human cancers. J. Pathol. 221, 147–152.
Kim, S.Y., Weiss, J., Tong, M., Laronda, M.M., Lee, E.J., Jameson, J.L., 2009. Foxl2, a forkhead transcription factor, modulates nonclassical activity of the estrogen receptor-alpha. Endocrinology 150, 5085–5093.
Kioussi, C., O'Connell, S., St.-Onge, L., Treier, M., Gleiberman, A.S., Gruss, P., Rosenfeld, M.G., 1999. Pax6 is essential for establishing ventral–dorsal cell boundaries in pituitary gland development. Proc. Natl. Acad. Sci. USA 96, 14378–14382.
Kruse, J.P., Gu, W., 2008. SnapShot: p53 posttranslational modifications. Cell 133, 930–930 e1.
Kuo, F.T., Bentsi-Barnes, I.K., Barlow, G.M., Bae, J., Pisarska, M.D., 2009. Sumoylation of forkhead L2 by Ubc9 is required for its activity as a transcriptional repressor of the steroidogenic acute regulatory gene. Cell Signal. 21, 1935–1944.
Laissue, P., Lakhal, B., Benayoun, B.A., Dipietromaria, A., Braham, R., Elghezal, H., Philibert, P., Saad, A., Sultan, C., Fellous, M., Veitia, R., 2009. Functional evidence implicating FOXL2 in non syndromic premature ovarian failure and in the regulation of the transcription factor OSR2. J. Med. Genet. 46, 455–457.
Lamba, P., Fortin, J., Tran, S., Wang, Y., Bernard, D.J., 2009. A novel role for the forkhead transcription factor FOXL2 in activin A-regulated follicle-stimulating hormone beta subunit transcription. Mol. Endocrinol. 23, 1001–1013.
Lee, K., Pisarska, M.D., Ko, J.J., Kang, Y., Yoon, S., Ryou, S.M., Cha, K.Y., Bae, J., 2005a. Transcriptional factor FOXL2 interacts with DP103 and induces apoptosis. Biochem. Biophys. Res. Commun. 336, 876–881.
Lee, M.B., Lebedeva, L.A., Suzawa, M., Wadekar, S.A., Desclozeaux, M., Ingraham, H.A., 2005b. The DEAD-box protein DP103 (Ddx20 or Gemin-3) represses orphan nuclear receptor activity via SUMO modification. Mol. Cell. Biol. 25, 1879–1890.
Li, W.X., Wang, X.K., Sun, Y., Wang, Y.L., Lin, L.X., Tang, S.J., 2005. A novel mutation in the FOXL2 gene in a Chinese family with blepharophimosis, ptosis, and epicanthus inversus syndrome. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 22, 372–375.
Lin, L., Philibert, P., Ferraz-de-Souza, B., Kelberman, D., Homfray, T., Albanese, A., Molini, V., Sebire, N.J., Einaudi, S., Conway, G.S., Hughes, I.A., Jameson, J.L., Sultan, C., Dattani, M.T., Achermann, J.C., 2007. Heterozygous missense mutations in steroidogenic factor 1 (SF1/Ad4BP, NR5A1) are associated with 46, XY disorders of sex development with normal adrenal function. J.Clin. Endocrinol. Metab. 92, 991–999.
Marongiu, M., Deiana, M., Meloni, A., Marcia, L., Puddu, A., Cao, A., Schlessinger, D., Crisponi, L., 2010. The forkhead transcription factor Foxl2 is sumoylated in both

human and mouse: sumoylation affects its stability, localization, and activity. PLoS One 5, e9477.
Mouillet, J.F., Yan, X., Ou, Q., Jin, L., Muglia, L.J., Crawford, P.A., Sadovsky, Y., 2008. DEAD-box protein-103 (DP103, Ddx20) is essential for early embryonic development and modulates ovarian morphology and function. Endocrinology 149, 2168–2175.
Moumne, L., Batista, F., Benayoun, B.A., Nallathambi, J., Fellous, M., Sundaresan, P., Veitia, R.A., 2008a. The mutations and potential targets of the forkhead transcription factor FOXL2. Mol. Cell. Endocrinol. 282, 2–11.
Moumne, L., Dipietromaria, A., Batista, F., Kocer, A., Fellous, M., Pailhoux, E., Veitia, R.A., 2008b. Differential aggregation and functional impairment induced by polyalanine expansions in FOXL2, a transcription factor involved in cranio-facial and ovarian development. Hum. Mol. Genet. 17, 1010–1019.
Nakamoto, M., Matsuda, M., Wang, D.S., Nagahama, Y., Shibata, N., 2006. Molecular cloning and analysis of gonadal expression of Foxl2 in the medaka, *Oryzias latipes*. Biochem. Biophys. Res. Commun. 344, 353–361.
Oshima, Y., Uno, Y., Matsuda, Y., Kobayashi, T., Nakamura, M., 2008. Molecular cloning and gene expression of Foxl2 in the frog *Rana rugosa*. Gen. Comp. Endocrinol. 159, 170–177.
Ottolenghi, C., Omari, S., Garcia-Ortiz, J.E., Uda, M., Crisponi, L., Forabosco, A., Pilia, G., Schlessinger, D., 2005. Foxl2 is required for commitment to ovary differentiation. Hum. Mol. Genet. 14, 2053–2062.
Ottolenghi, C., Pelosi, E., Tran, J., Colombino, M., Douglass, E., Nedorezov, T., Cao, A., Forabosco, A., Schlessinger, D., 2007. Loss of Wnt4 and Foxl2 leads to female-to-male sex reversal extending to germ cells. Hum. Mol. Genet. 16, 2795–2804.
Ou, Q., Mouillet, J.F., Yan, X., Dorn, C., Crawford, P.A., Sadovsky, Y., 2001. The DEAD box protein DP103 is a regulator of steroidogenic factor-1. Mol. Endocrinol. 15, 69–79.
Pailhoux, E., Vigier, B., Chaffaux, S., Servel, N., Taourit, S., Furet, J.P., Fellous, M., Grosclaude, F., Cribiu, E.P., Cotinot, C., Vaiman, D., 2001. A 11.7-kb deletion triggers intersexuality and polledness in goats. Nat. Genet. 29, 453–458.
Pailhoux, E., Vigier, B., Vaiman, D., Servel, N., Chaffaux, S., Cribiu, E.P., Cotinot, C., 2002. Ontogenesis of female-to-male sex-reversal in XX polled goats. Dev. Dyn. 224, 39–50.
Pannetier, M., Servel, N., Cocquet, J., Besnard, N., Cotinot, C., Pailhoux, E., 2003. Expression studies of the PIS-regulated genes suggest different mechanisms of sex determination within mammals. Cytogenet. Genome Res. 101, 199–205.
Pannetier, M., Fabre, S., Batista, F., Kocer, A., Renault, L., Jolivet, G., Mandon-Pepin, B., Cotinot, C., Veitia, R., Pailhoux, E., 2006. FOXL2 activates P450 aromatase gene transcription: towards a better characterization of the early steps of mammalian ovarian development. J. Mol. Endocrinol. 36, 399–413.
Park, M., Shin, E., Won, M., Kim, J.H., Go, H., Kim, H.L., Ko, J.J., Lee, K., Bae, J., 2010. FOXL2 interacts with steroidogenic factor-1 (SF-1) and represses SF-1-induced CYP17 transcription in granulosa cells. Mol. Endocrinol. 24, 1024–1036.
Parker, K.L., 2004. Tissue-specific knockouts of steroidogenic factor 1. Endocr. Res. 30, 855.
Pisarska, M.D., Bae, J., Klein, C., Hsueh, A.J., 2004. Forkhead l2 is expressed in the ovary and represses the promoter activity of the steroidogenic acute regulatory gene. Endocrinology 145, 3424–3433.
Pisarska, M.D., Kuo, F.T., Bentsi-Barnes, I.K., Khan, S., Barlow, G.M., 2010. LATS1 phosphorylates forkhead L2 and regulates its transcriptional activity. Am. J. Physiol. Endocrinol. Metab. 299, E101–E109.
Qian, X., Shu, A., Qin, W., Xing, Q., Gao, J., Yang, J., Feng, G., He, L., 2004. A novel insertion mutation in the FOXL2 gene is detected in a big Chinese family with blepharophimosis–ptosis–epicanthus inversus. Mutat. Res. 554, 19–22.
Rodgers, R.J., Irving-Rodgers, H.F., Russell, D.L., 2003. Extracellular matrix of the developing ovarian follicle. Reproduction 126, 415–424.
Schmidt, D., Ovitt, C.E., Anlag, K., Fehsenfeld, S., Gredsted, L., Treier, A.C., Treier, M., 2004. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. Development 131, 933–942.
Schrader, K.A., Gorbatcheva, B., Senz, J., Heravi-Moussavi, A., Melnyk, N., Salamanca, C., Maines-Bandiera, S., Cooke, S.L., Leung, P., Brenton, J.D., Gilks, C.B., Monahan, J., Huntsman, D.G., 2009. The specificity of the FOXL2 c. 402C.G somatic mutation: a survey of solid tumors. PLoS One 4, e7988.
Shah, S.P., Kobel, M., Senz, J., Morin, R.D., Clarke, B.A., Wiegand, K.C., Leung, G., Zayed, A., Mehl, E., Kalloger, S.E., Sun, M., Giuliany, R., Yorida, E., Jones, S., Varhol, R., Swenerton, K.D., Miller, D., Clement, P.B., Crane, C., Madore, J., Provencher, D., Leung, P., Defazio, A., Khattra, J., Turashvili, G., Zhao, Y., Zeng, T., Glover, J.N., Vanderhyden, B., Zhao, C., Parkinson, C.A., Jimenez-Linan, M., Bowtell, D.D., Mes-Masson, A.M., Brenton, J.D., Aparicio, S.A., Boyd, N., Hirst, M., Gilks, C.B., Marra, M., Huntsman, D.G., 2009. Mutation of FOXL2 in granulosa-cell tumors of the ovary. N. Engl. J. Med. 360, 2719–2729.
St. John, M.A., Tao, W., Fei, X., Fukumoto, R., Carcangiu, M.L., Brownstein, D.G., Parlow, A.F., McGrath, J., Xu, T., 1999. Mice deficient of Lats1 develop soft-tissue sarcomas, ovarian tumours and pituitary dysfunction. Nat. Genet. 21, 182–186.
Stocco, D.M., 2001. StAR protein and the regulation of steroid hormone biosynthesis. Annu. Rev. Physiol. 63, 193–213.
Treier, M., Gleiberman, A.S., O'Connell, S.M., Szeto, D.P., McMahon, J.A., McMahon, A.P., Rosenfeld, M.G., 1998. Multistep signaling requirements for pituitary organogenesis in vivo. Genes Dev. 12, 1691–1704.
Uda, M., Ottolenghi, C., Crisponi, L., Garcia, J.E., Deiana, M., Kimber, W., Forabosco, A., Cao, A., Schlessinger, D., Pilia, G., 2004. Foxl2 disruption causes mouse ovarian failure by pervasive blockage of follicle development. Hum. Mol. Genet. 13, 1171–1181.
Uhlenhaut, N.H., Jakob, S., Anlag, K., Eisenberger, T., Sekido, R., Kress, J., Treier, A.C., Klugmann, C., Klasen, C., Holter, N.I., Riethmacher, D., Schutz, G., Cooney, A.J., Lovell-Badge, R., Treier, M., 2009. Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell 139, 1130–1142.
Wang, D.S., Kobayashi, T., Zhou, L.Y., Paul-Prasanth, B., Ijiri, S., Sakai, F., Okubo, K., Morohashi, K., Nagahama, Y., 2007. Foxl2 up-regulates aromatase gene transcription in a female-specific manner by binding to the promoter as well as interacting with ad4 binding protein/steroidogenic factor 1. Mol. Endocrinol. 21, 712–725.
Yan, X., Mouillet, J.F., Ou, Q., Sadovsky, Y., 2003. A novel domain within the DEAD-box protein DP103 is essential for transcriptional repression and helicase activity. Mol. Cell. Biol. 23, 414–423.
Yang, W.H., Gutierrez, N., Wang, L., Ellsworth, B.S., Wang, C.M., 2010. Synergistic activation of the Mc2r promoter by FOXL2 and NR5A1 in mice. Biol. Reprod. 83, 842–851.
Zlotogora, J., Sagi, M., Cohen, T., 1983. The blepharophimosis, ptosis, and epicanthus inversus syndrome: delineation of two types. Am. J. Hum. Genet. 35, 1020–1027.