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Signalling networks in focus

Impact of FOXL2 mutations on signaling in ovarian granulosa cell tumors

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ABSTRACT

Granulosa cell tumors (GCT) are unique sex-cord stromal tumors which account for ~8% of all ovarian malignancies. They exhibit morphological, biochemical and hormonal features similar to proliferating granulosa cells of the preovulatory follicle, including estrogen and inhibin synthesis. A somatic missense mutation in the forkhead box L2 (*FOXL2*) gene (C134W) is unique to adult GCT, and absent in other ovarian cancers. FOXL2 is a transcription factor that plays a critical role in ovarian function, in particular, proliferation and differentiation of granulosa cells. The molecular mechanisms underlying the pathogenicity of the mutant FOXL2 remain unresolved. Here we review the molecular alterations known to be associated with mutant FOXL2 and the potential signaling implications. Several studies suggest that dysregulated FOXL2 function may alter cell cycle progression and apoptosis. Further insights into the molecular mechanism of GCT pathophysiology may identify therapeutic targets for the treatment of these tumors.

1. Introduction

Forkhead box (FOX) proteins are a family of evolutionarily conserved transcription factors. The characteristic, highly conserved 80–100 amino acid DNA binding domain, known as the 'forkhead' domain, binds to a consensus DNA sequence to regulate transcription. It was first described in *Drosophila* in 1990 and since then more than 170 *FOX* genes have been identified in 14 species, including 50 in the human genome. The *FOX* genes are categorized into 19 subfamilies, from *FOXA* to *FOXS*, based on the degree of sequence conservation (Jackson et al., 2010). Their modes of action as pioneer factors, transcription factors, or both, are quite diverse. They participate in a wide spectrum of physiological processes including development, stem cell differentiation, metabolism and immunity. This review focuses on the forkhead box protein L2 (FOXL2), a critical transcription factor in sex determination. FOXL2 is expressed in the ovary (Schmidt et al., 2004), endometrium (Governini et al., 2014), pituitary (Schmidt et al., 2004) and the developing eyelid (Crisponi et al., 2001). In addition to the forkhead domain, FOXL2 contains an alanine/proline-rich domain in the C-terminus which is responsible for transcriptional regulation (Crisponi et al., 2001).

Here we review the function of FOXL2 in ovarian development and the molecular alterations associated with mutant FOXL2 in a subset of ovarian cancer, the granulosa cell tumors (GCT). The adult form of GCT is characterized by a somatic missense mutation in the *FOXL2* gene which has been shown to potentially impact FOXL2 signaling.

2. Functions, cascades and key molecules

2.1. FOXL2 in sex determination and early ovarian development

FOXL2 is the earliest marker of ovarian development. In mice, it is detected from 12.5 days post-coitum, and its expression is sustained in the granulosa cells (GC) and stromal cells of ovarian follicles throughout female reproductive life (Schmidt et al., 2004). The sexually dimorphic pattern of gene expression of *FOXL2* in the ovaries, or *SRY* and *SOX9* in the testes, determines the fate of the bi-potential gonad in development. The importance of *FOXL2* expression extends beyond this initial sex determination stage. It is required to maintain the ovarian phenotype. Ablation of *FOXL2* alone in sexually mature female mice leads to structural changes suggesting sex-reversal, including oocyte degeneration and the occurrence of cells morphologically similar to Sertoli cells (Uhlenhaut et al., 2009) (Fig. 1). In addition, SOX9 expression and other Sertoli cell markers were markedly increased.

In the postnatal ovary, FOXL2 regulates GC differentiation and supports follicular growth. In $FOXL2^{-/-}$ mice, GC failed to complete

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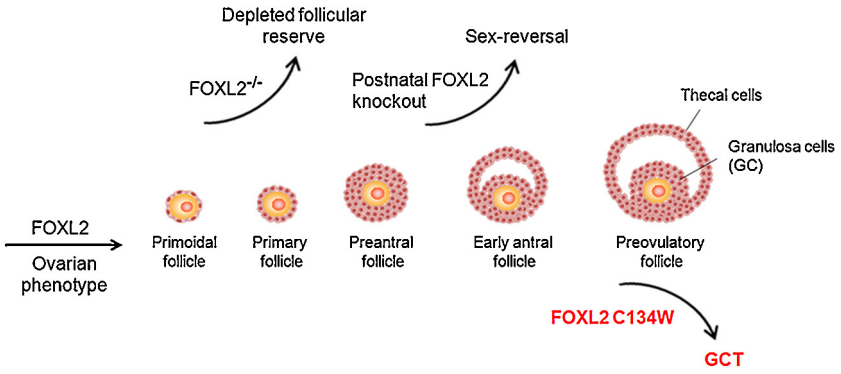

Fig. 1. Roles of FOXL2 in ovarian biology. Expression of FOXL2 is required to drive ovarian development and maintain ovarian phenotype. In the postnatal ovary, FOXL2 regulates differentiation of squamous to cuboidal GC and support follicular growth. In FOXL2$^{-/-}$ mice, lack of cuboidal GC blocks follicular growth, oocytes undergo atresia and follicular reserve is depleted. In postnatal female mice, ablation of FOXL2 alone is sufficient to induce sex-reversal phenotypes. The *FOXL2*^{C134W} mutation is unique to aGCT and is arguably pathonognomonic for these cancers.

the squamous-to-cuboidal transition which represents as a gateway to GC proliferation (Schmidt et al., 2004) (Fig. 1). Without the support of cuboidal GC, follicular growth was blocked. Atresia was observed in most of the oocytes, with no maturation into secondary follicles.

2.2. FOXL2 in the hypothalamic–pituitary–gonadal axis

The hypothalamic–pituitary–gonadal axis is a critical pathway in development and reproduction. Major components include gonadotropin-releasing hormone (GnRH) which is secreted by the hypothalamus, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) that are produced by the anterior pituitary upon GnRH stimulation. The regulation of FSH production is multifactorial. In particular, FOXL2 and the homolog of *Drosophila* Small Mothers Against Decapentaplegic 3 (SMAD3) synergistically induce transcription of the *FSHβ* gene in response to the transforming growth factor (TGF)-β superfamily member, activin (Roybal et al., 2014). Transcriptional regulation of *FSHβ* requires physical interaction of both transcription factors. In line with this process, FOXL2 expression has been described in the adult mouse pituitary, where it co-localizes with FSH in the gonadotropes and thyrotropes. Activin induces the phosphorylation of SMAD3, which then translocates to the nucleus to stimulate transcription of *FSHβ*, which is rate-limiting for the formation of the dimeric biologically active FSH molecule. The FOXL2-SMAD-activin-induced FSH promotes GC differentiation, induces LH receptors and stimulates aromatization; all of which are essential to foster follicular development.

SMAD3 is also essential in FOXL2-induced follistatin transcription in GC (Nonis et al., 2013) (Fig. 2). The FOXL2-SMAD3 complex acts in synergy with growth differentiation factor-9 (GDF-9) to increase follistatin transcription. Follistatin is predominantly expressed in the ovary, especially GC of the developing follicles. It binds to activin to attenuate activin-induced GC proliferation (Cheng et al., 2014).

3. FOXL2 as determinant of folliculogenesis

Overexpression of FOXL2 induces apoptosis in normal rat GC, an essential mechanism in folliculogenesis. The anti-proliferative function of FOXL2 involves the regulation of activin and follistatin in TGF-β signaling. It does so by promoting cleavage of

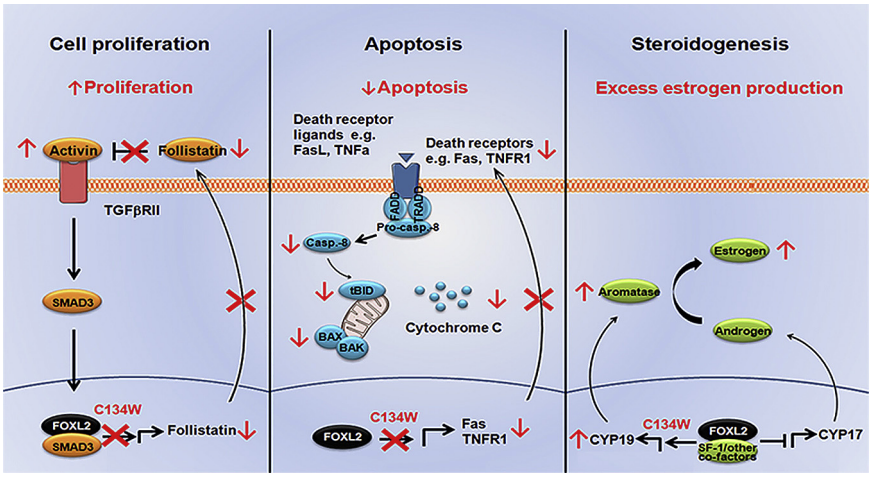

Fig. 2. FOXL2 in cell proliferation, apoptosis and steroidogenesis. FOXL2 is a transcription factor that plays a critical role in ovarian function, in particular, proliferation, apoptosis and steroidogenesis. The potential impact of the *FOXL2*^{C134W} mutation is indicated in red. The FOXL2-SMAD3 complex stimulates follistatin gene transcription, and subsequently modulates activin-induced proliferation. Mutant FOXL2^{C134W} suppresses follistatin gene transcription and subsequently reduces the inhibitory effect of follistatin on activin, leading to an increase in activin-induced proliferation. The anti-proliferative function of FOXL2 involves the transcriptional regulation of death receptors *e.g.* Fas and TNFR1. Cells expressing FOXL2^{C134W} showed reduced transcription of death receptors, diminished activation of caspases, BID, BAX, BAK and cytochrome C production and as a result, a decrease in apoptosis. In steroidogenesis, the FOXL2-SF-1 complex regulates *CYP17* and *CYP19* gene transcription. FOXL2-SF-1-regulated *CYP19* and *CYP17* gene transcription is disrupted by the C134W mutation. *CYP19* transcription is upregulated while repression of *CYP17* transcription is lost. These events increase aromatization and consequently, excess estrogen production.