Poster P1- 60

Insight into the Human Ovarian Sex Development Network

Leila Bouazzi, Mariangela Franco, Wassim Eid, Monika Meyer, Alexander Maret, Anna Biason-Lauber
University of Fribourg, Fribourg, Switzerland

Background and Objective

In ovary differentiation network, no single female-determining factor has been identified to be an equivalent of SRY or SOX9 in the testis. Recently, data suggested Chromobox homolog 2 (CBX2) is a pioneer regulator promoting testis development (4). The exact position of CBX2 in sex development cascade is still unknown and the role of CBX2 in ovarian development and maintenance is yet to be explored. The main goal is to gain insights the ovarian determination process to identify a regulatory network in which CBX2 takes part. We looked into the molecular interaction relating CBX2 to female determining genes i.e. FOXL2, RSPO1 and WNT4 and identified novel CBX2-specific targets in ovarian granulosa cells.

Methods

We evaluated the effects of CBX2 isoforms on female-markers RSPO1, FOXL2, WNT4 by RT-qPCR following CBX2.1 and CBX2.2 forced-expression and knock-down assay. DamID (DNA adenine methyltransferase identification) has been performed under forcing expression and down regulation of CBX2 isoforms to screen for new female-determining genes on ovarian granulosa cells (KGN). Notably, validation of experiments were performed using RT-qPCR. All graphs are the average of 3 independent experiments, error bars represent SD and values are expressed as relative to control =1. Differences were considered statistically significant when p-value < 0.05. ****:P<0.0001; ***:P<0.001; **: P < 0.01; *:P <0.05.

Results

1. Interaction between CBX2 and female-specific markers

CBX2.1 showed a repressive effect on all female markers (figure1) and under female genes forced expression CBX2.1 was found to be down regulated (figure 2). When knocking-down CBX2.1, FOXL2 and RSPO1 showed a significant up regulation (figure 1).

CBX2.2 seems to activate RSPO1 (figure 1). Reciprocally, we observed a positive regulation of CBX2.2 expression following RSPO1 and WNT4 forcing expression (figure 2). Similarly to CBX2.1, CBX2.2 repressed WNT4 and FOXL2 expression (figure1). WNT4 and FOXl2 found to be significantly up regulated after CBX2.2 inhibition (figure1).

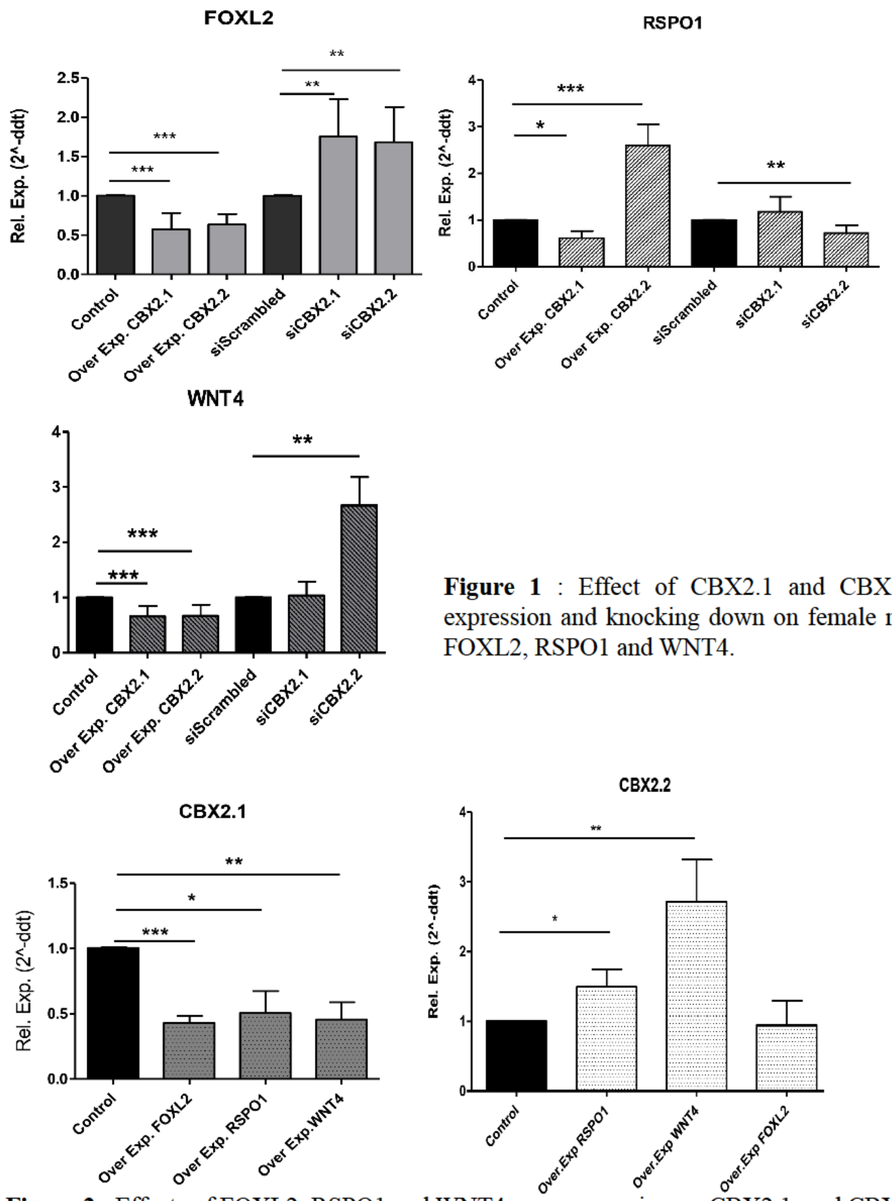


Figure 1 : Effect of CBX2.1 and CBX2.2 over expression and knocking down on female markers : FOXL2, RSPO1 and WNT4.

Figure 2 : Effects of FOXL2, RSPO1 and WNT4 over expression on CBX2.1. and CBX2.2 markers.

2. CBX2 and developmental stages

We explored the impact of CBX2 isoforms on specific ovarian developmental-stage markers as OCT4 and AMH, early expressed indicators and ER a late markers (scheme 2). **CBX2.1** has been found to repress OCT4 and AMH expression (figure 4). However, **CBX2.2** seems to activate them (figure4).

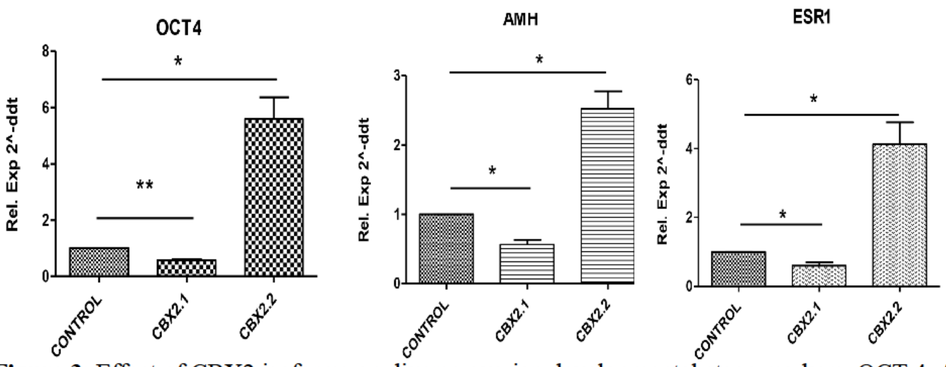
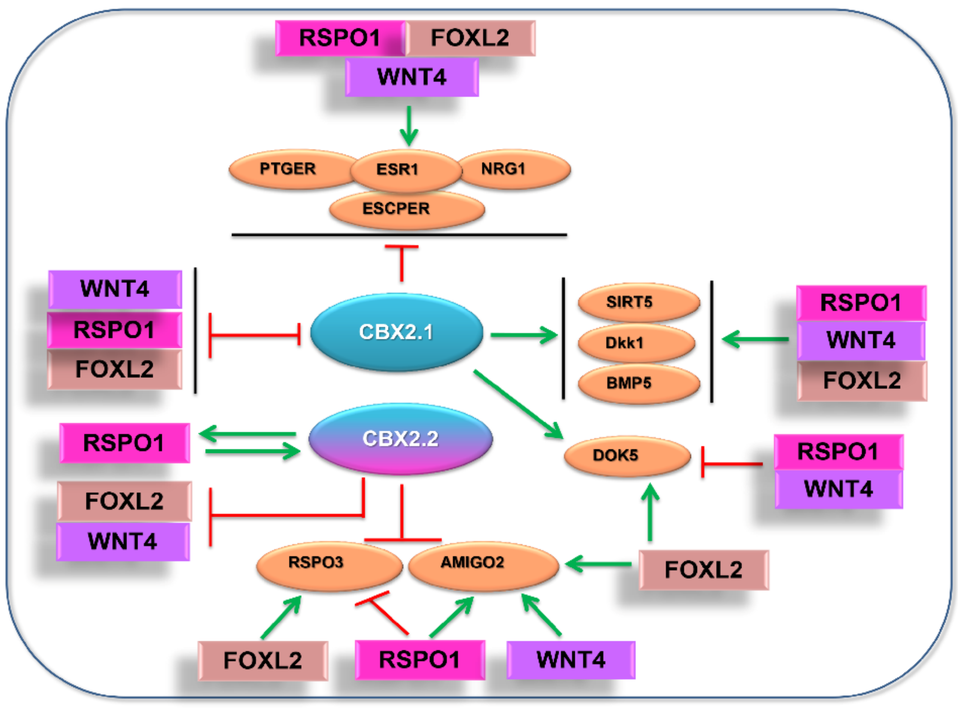


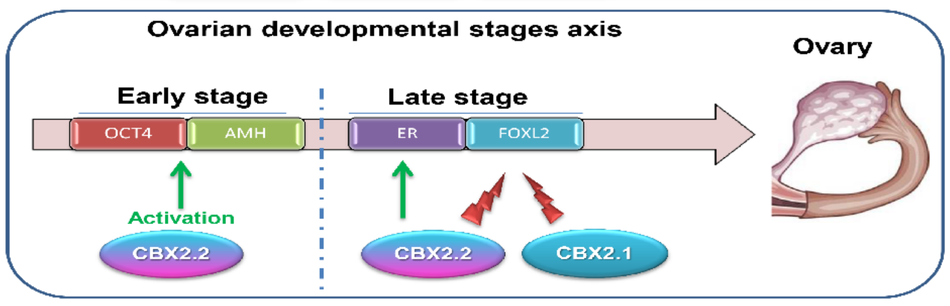
Figure 3: Effect of CBX2 isoforms on diverse ovarian developmental stage markers OCT-4, AMH, ER

3. Novel CBX2 targets/partners

524 targets of CBX2.1 and 835 targets associated to CBX2.2 were identified by DamID. We selected 10 genes basing on their potential links to sex development and their differential expression in the ovary. The selected candidates ESR1, AMIGO, NRG1, BMP5, RSPO3, PTRGER, SCAPER, SIRT5 DKK1, and DOK5 showed expression changes towards female markers and CBX2 isoforms (Scheme1). ESR1, SCAPER, PTGER and NRG1were negatively controlled by CBX2.1 but up regulated by female markers. DOK5 showed a positive responsiveness towards CBX2.1 and FOXL2 and found altered by RSPO1 and WNT4. DKK1, a mice pro-male marker (2), turned out to be preferentially expressed in human KGN and up regulated by female genes and CBX2.1. AMIGO2 and RSPO3 (CBX2.2 targets) seem to be alerted by CBX2.2. Interestingly, AMIGO2 found to be positively regulated by the three specific ovarian markers. RSPO3 showed to be enhanced by FOXL2 and repressed by RSPO1.



Scheme 1: A schematic representation of the new genes regulatory networks in human granulosa cells involving CBX2 isoforms, female markers and the novel DamID candidates (orange color). Green arrows indicates positive regulation, red lines indicates inhibitory effects.



Scheme 2: Ovarian developmental stage axis showing the effects of CBX2.1 and CBX2.2 on early (OCT-4, AMH) and late (ER, Foxl2) ovarian developmental markers.

Conclusion:

We confirmed that CBX2.1 is a pro-male factor (1,3) also in female gonads. We observed a bidirectional antagonistic interaction opposing CBX2.1 to FOXL2 reminding the famed repressive relationship facing SOX9 Vs. FOXL2 (5). We assumed a positive alliance between CBX2.2 and RSPO1 suggesting a CBX2.2 putative pro-female role. As repressive factors, both CBX2 isoforms might contribute to the fine-tuning of the cellular WNT4 activity and possibly function as a cell guardian to secure the proper cell growth in the ovary.

During development, CBX2.2 might be produced in the very early fetal indifferent gonads and CBX2.1 plausibly could be expressed afterwards to silence embryonic markers and launch gonads differentiation.

Using DamID technique, we identified novel putative sex development markers regulated by CBX2 factor and interacting with female markers. This selected list might contribute to gain new insights into the sex determination cascade.

References:
1. Biason-Lauber *et al*. Ovaries and Female Phenotype in a Girl with 46,XY Karyotype and Mutations in the CBX2 Gene.t.The Am . Jour. of Hum Genet 658–663, 2009.
2. Combes AN *et al*. Expression and functional analysis of *dkk1* during early gonadal development. Sex Dev 5:124-130 (2011).
3. Eid W *et al*. Genome-wide identification of CBX2 targets: insights in the human sex development network. Mol Endocrino. 2015 Feb;29(2):247-57.
4. Katoh-Fukui Y, *et al*. Cbx2, a polycomb group gene, is required for Sry gene expression in mice. Endocrinology. 2012 Feb;153(2):913-24.
5. Veita RA. FOXL2 versus SOX9: a lifelong "battle of the sexes". Bioessays. 2010 May;32(5):375-80.

DOI: 10.3252/pso.eu.54espe.2015