The transcriptional regulator CBX2 and ovarian function: A whole-genome and whole transcriptome approach

Leila Bouazzi

It is now well established that CBX2 is indispensable for testis development in humans and mice. However, the role of this polycomb protein in the ovary remains largely undefined. To address this issue and understand its function in human ovaries, I firstly adopted a hypothesis-driven approach and evaluated the expression of known female genes, i.e. FOXL2, RSP01 and WNT4 in human pre-granulosa cells after forced expression and RNA interference of CBX2 isoforms and vice-versa. I also conducted a comprehensive and unbiased genome-wide analysis based-Dam methylase identification (DamID) and RNA sequencing strategies to identify CBX2 responsive candidate genes in the ovary, as a hypothesis-generating approach. The two isoforms appeared to be functionally distinct: CBX2.1 has been confirmed to be a pro-male factor, whereas CBX2.2 might be partly pro-female most likely via the interaction with RSP01 and through the regulation of ovary developmental markers (i.e. OCT-4, AMH and ESR2). Besides having a sex-determining role, both CBX2 isoforms might act as regulatory agents protecting granulosa cells from uncontrolled growth and proliferation by influencing WNT4 and RSP01 signaling pathways. In the second part of this project, CBX2 regulated-genes were identified, but focus was on gonads-specific genes reportedly involved in sex development and disorders of sex development (DSD). Functional enrichment analysis revealed CBX2’s involvement in several molecular pathways in developmental processes. Notably, I found out that CBX2.1 and CBX2.2 are ahead of genes contributing to folliculogenesis and steroidogenesis (i.e. ESR1, NRG1, PTGER2, TGFB, BMP2, FSHR, STARD6 and NTRK1/2). In addition, CBX2-related-genes involved in PCOS like AMH, AKR1C1, RSP02 and DKK1 were confirmed. Other genes were linked to premature ovarian failure (POF) (POF1B, BMP15 and HOXA13) and pituitary hormone deficiency (i.e. LHX4 and KISS1). PcG proteins have been identified as being important proteins in tumorigenesis due to their potential to repress tumor suppressor genes and regulate genes related to stemness and differentiation. This is not surprising given the well-proven tight connection between development and cancer. In accordance to what was hypothesized above, CBX2 isoforms might protect granulosa cells from uncontrolled growth by affecting oncogenic markers like NRG1, FZD7, TGFa, AMIGO2 and RSPO3. Consistent with this, our findings identified group of genes with specific actions in the networks regulating sex development and eventually helped clarifying the rank of CBX2 regulator in the female regulatory events.

Jury:

Prof. Anna Lauber-Biason, University of Fribourg (Thesis Supervisor)
Prof. Christa Flück, University of Bern (External Expert)
Prof. Serge Nef, University of Geneva (External Expert)
Prof. Zhihong Yang, University of Fribourg (President of the Jury)