Upregulation of the lncRNAs steroid receptor RNA activator (SRA) and c-terminal binding protein 1 antisense (CTBP1-AS) in the granulosa cells of patients with polycystic ovary syndrome

Ali Ruba Mohamed

Academy of Biology and Biotechnology named after D I Ivanovsky, Southern Federal University, 194/1 Stachki Avenue, Rostov on Don 344090, Russian Federation, ruba.m.ali@mail.ru (A. R. M.)

DOI: 10.18522/2308-9709-2023-46-7

Abstract

This study investigates the involvement of two long non-coding RNAs (lncRNAs), Steroid Receptor RNA Activator (SRA) and C-Terminal Binding Protein 1 Antisense (CTBP1-AS), in the context of polycystic ovary syndrome (PCOS). SRA, known to regulate steroid receptors and implicated in various diseases, including obesity and cancer, has been linked to PCOS-associated comorbidities. Similarly, CTBP1-AS, an androgen receptor modulator, has relevance in androgen metabolism and prostate cancer. We measured SRA and CTBP1-AS expression in granulosa cells from PCOS patients and controls undergoing IVF, revealing significantly higher levels in PCOS patients. Correlations were explored between the SRA and CTBP1-AS expression levels and hormone levels in the follicular fluid of PCOS patients, indicating a significant association between SRA expression and estradiol levels in follicular fluid. These findings, aligning with previous research on SRA's impact on hormone regulation, suggest potential roles for SRA and CTBP1-AS in the mechanisms underlying PCOS development, particularly in hyperandrogenism.

Keywords: Polycystic ovary syndrome; PCOS; LncRNAs; SRA; CTBP1-AS.

Повышенная экспрессия длинных некодирующих РНК: РНК активатора стероидных рецепторов (*SRA*) и антисмыслового С-концевого связывающего белка 1 (*CTBP1-AS*) в гранулезных клетках пациентов с синдромом поликистозных яичников

Али Руба Мохамед

Академия биологии и биотехнологии имени Д.И. Ивановского, Южный федеральный университет, проспект Стачки, 194/1, Ростов-на-Дону 344090, Российская Федерация, ruba.m.ali@mail.ru (А. Р. М.)

DOI: 10.18522/2308-9709-2023-46-7

Аннотация

В данном исследовании мы изучили участие двух длинных некодирующих РНК (lncRNAs) - РНК активатора стероидных рецепторов (SRA) и антисмыслового С-концевого связывающего белка 1 (CTBP1-AS), - в развитии синдрома поликистозных яичников (СПКЯ). SRA, известный как регулятор стероидных рецепторов и связанный с различными заболеваниями, включая ожирение и рак, был ассоциирован с сопутствующими заболеваниями, связанными с СПКЯ. Аналогично, CTBP1-AS, модулятор рецепторов андрогена, имеет отношение к метаболизму андрогена и раку простаты. Мы измерили экспрессию SRA и CTBP1-AS в клетках гранулезы пациентов с СПКЯ и контрольной группы, проходивших процедуру ЭКО, и выявили значительно более высокие уровни у пациентов с СПКЯ. Была изучена корреляция между уровнями экспрессии SRA и CTBP1-AS и уровнями гормонов в фолликулярной жидкости пациентов с СПКЯ, что указывает на значительную ассоциацию между экспрессией SRA и уровнем эстрадиола в фолликулярной жидкости. Эти результаты, согласующиеся с предыдущими исследованиями влияния SRA на гормональную регуляцию, позволяют предположить потенциальную роль SRA и CTBP1-AS в механизмах, лежащих в основе развития СПКЯ, в частности в гиперандрогении.

Ключевые слова: Синдром поликистозных яичников; СПКЯ; LncRNAs; SRA; CTBP1-AS.

Ali R. M., Upregulation of the lncRNAs steroid receptor RNA activator (SRA) and c-terminal binding protein 1 antisense (CTBP1-AS) in the granulosa cells of patients with polycystic ovary syndrome // «Живые и биокосные системы». – 2023. – № 46; URL: https://jbks.ru/archive/issue-46/article-7; DOI: 10.18522/2308-9709-2023-46-7

Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial endocrinological disorder, that affects an estimated 8–13% of reproductive-aged women, as per the World Health Organization's 2023 statistics. PCOS is highly heterogeneous and presents with different clinical symptoms and phenotypes, but it is diagnosed depending on three important characteristics, which are hyperandrogenism, the polycystic morphology of the ovaries, and oligo- or anovulation. PCOS stands as a primary contributor to female infertility related to ovulation issues (Cunha and Póvoa 2021). Recent studies have shown that the principal determinants of PCOS are hyperandrogenism and insulin resistance (Ding et al. 2021). The mechanisms of androgen actions in the ovary, a complex interplay commencing in fetal development and extending into adulthood, involve numerous cell-specific and follicle stage-specific factors, many of which remain unidentified. The long non-coding RNA (lncRNA) steroid receptor RNA activator (SRA), which is encoded by *SRA1* gene at 5q31.3, was the first lncRNA determined to regulate steroid receptors, including the androgen, estradiol, and progesterone receptors (Lanz et al. 1999). *SRA*1 is a bifunctional gene that is transcribed not only into several lncRNA isoforms sharing a core sequence of 687 base pairs, but also as a messenger RNA encoding the conserved steroid receptor RNA activator protein, known as SRAP. SRA is a multifunctional molecule that regulates hundreds of genes directly and indirectly by binding to coactivator or corepressor complexes. SRA functions have been revealed in various biological processes including mammary gland development, adipocyte differentiation, myogenesis and steroidogenesis (C. Liu et al. 2016). SRA is implicated in the pathogenesis of breast and prostate cancer, in addition to its role in the development of obesity, insulin resistance and cardiovascular diseases (C. Liu et al. 2016), which are highly associated with and are co-morbidities of PCOS. LncRNA c-terminal binding protein 1 antisense (*CTBP1-AS*)—

an androgen receptor modulator—localizes in the antisense (AS) region of CTBP1 in chr4 p16.3. Previous studies have revealed the involvement of CTBP1-AS in the regulation of androgen metabolism and its association with the androgen receptor (AR) signaling pathway in prostate cancer (Takayama et al. 2013). The differential expression of AR modulators such as *SRA* and CTBP1-AS may contribute to the development of PCOS.

The aim of this study is to evaluate the expression level of SRA and CTBP1-As in the follicular fluid of PCOS patients and the control group.

Materials and methods

1. Study subjects

Participants in this study included 12 women diagnosed with PCOS and 15 controls who underwent assisted reproductive technologies (ART) treatments at the Center for Human Reproduction and IVF in Rostov-on-Don, Russia. The diagnosis of PCOS followed the Rotterdam criteria, while the control group comprised women seeking IVF/ICSI due to tubal factor infertility or male infertility. Exclusion criteria for both groups encompassed endocrinological pathologies such as hyperprolactinemia, Cushing's disease, adrenal hyperplasia, or ovarian tumors. All participants provided informed consent, and the study adhered to the ethical principles of the Helsinki Declaration, approved by the local bioethics committee at Southern Federal University (Protocol No. 2 of 17.01.2018).

2. Biochemical and hormonal analysis

Blood samples collected after a 12-14 hour fasting period underwent centrifugation, the serum was separated and stored at -20°C for subsequent analysis. Follicular fluid samples, devoid of red blood cell contamination, were also centrifuged and stored at -20°C. Granulosa cell samples from follicular fluid were collected and stored at -80°C in

RNA stabilizing solution. Biochemical and hormonal analyses followed established protocols (Lomteva et al. 2022).

3. Quantitative real-time PCR

The total RNA was extracted from GCs using the "ExtractRNA" kit from (Evrogen, Russia). Synthesis of cDNA from the total RNA was carried out using the reverse transcription kit "MMLV RT kit" from (Evrogen, Russia). Following the manufacturer's instructions, a total of 20 µl reaction solution containing 6 µl RNA, 1 µl Random primer, and 0.5 µl MMLV revertase. The reaction mix was prepared using RNase-free water. Then the samples were incubated at 40 °C for 40 min, and finally, to stop the reaction, the mixture was incubated at 70 °C for 10 min.

Real time-PCR was performed to measure the relative expression of *SRA* and *CTBP1-AS* in GCs using the SYBR Green master mix " 5X qPCRmix-HS SYBR" from (Evrogen, Russia) and the QuantStudio Real-Time PCR Systems (Applied Biosystems, Thermo Fisher Scientific, United States). The 25 µl PCR reaction solution contains 5 µl (1x) qPCRmix-HS SYBR, 1 µl of each primer forward and reverse primers, 2 µl cDNA templates and 16 µl Nuclease-Free Water. PCR conditions were as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 10 s, and 72 °C for 20 s, followed by high-resolution melting curves of PCR amplicons with temperatures ranging from 60 °C to 95 °C. As internal control, we used GAPDH. All samples were conducted in duplicate. Fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers were as follow: GAPDH forward primer 5′- GGGAAACTGTGGCGTGAT -3′, reverse primer 5′- GAGTGGGTGTCGCTGTTGA -3′; *SRA* forward primer 5′- CCTGGACGTGTCTCAACTGG -3′, reverse primer 5′- CCCGGAACTCCACTGTTAGC -3′; *CTBP1-AS* forward primer 5′- ACAACACAAAGCCCCGGAA -3′, reverse primer 5′- AGTGAAGAATGGTCTCGCCC -3′.

4. Statistical analysis

Statistical analysis employed GraphPad Prism 7, utilizing two-tailed unpaired t-tests (or Unpaired t test with Welch's correction) for variable comparisons and Pearson's correlation coefficient test for correlation analyses. Results were expressed as means ± SEM for each group, with significance set at $p < 0.05$.

Results

1. Follicular fluid hormones profile in PCOS patients and controls

We examined testosterone, estradiol, and progesterone levels in the follicular fluid of both PCOS patients and the control group, presenting the data in Table 1. Our findings highlighted significant differences: notably higher testosterone levels ($p = 0.0005$) and significantly lower progesterone levels ($p < 0.0001$) in PCOS patients compared to the control group. While the increase in FF estradiol levels for PCOS patients wasn't statistically significant ($p = 0.0716$), the E2/T ratio showed a substantial variance between the groups ($p < 0.0001$). Specifically, this ratio was approximately halved in PCOS patients in comparison to the control group.

Table 1 — Hormonal levels in follicular fluid of PCOS patients and controls

	PCOS Mean ± SD	**Control Mean ± SD**	**P value**
Testosterone (ng/ml)	118.4 ± 14.94	46.47 ± 5.57	**0.0005**
Estradiol (ng/ml)	15134 ± 1512	11715 ± 920	**0.0716**
E2/T	527.2 ± 81.2	1039 ± 75.94	**<0.0001**
Progesterone (ng/ml)	5589 ± 1161	15884± 1184	**<0.0001**

2. SRA and CTBP1-AS expression levels in granulosa cells

Our findings indicated a notable elevation in *SRA* and *CTBP1-AS* expression within GCs of PCOS patients compared to the control group ($p = 0.0018$, $p = 0.003$, respectively) Fig. 1. Additionally, we explored potential correlations between *SRA* and *CTBP1-AS*

expression levels in PCOS patients' GCs and both follicular fluid and serum hormone levels. While a positive correlation between *SRA* and follicular fluid testosterone was observed, it did not reach statistical significance (p = 0.0707). Nevertheless, a significant positive correlation emerged between *SRA* expression and follicular fluid estradiol levels (p = 0.0350) Fig. 2. Conversely, no significant correlations were found between *CTBP1-AS* expression in PCOS patients' GCs and testosterone, estradiol, or the E2/T ratio in blood serum or follicular fluid.

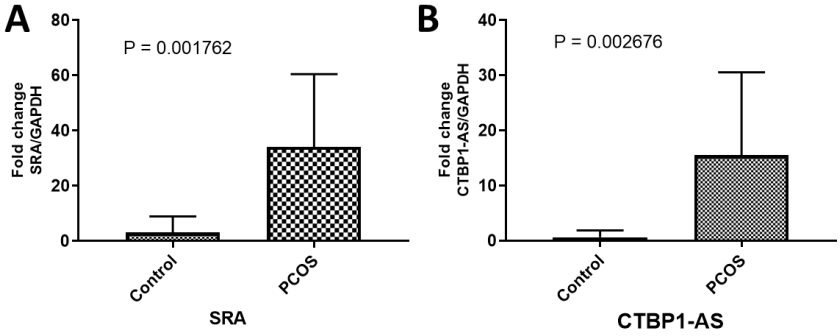

Figure 1 — The relative expression levels of SRA (A), and CTBP1-AS (B) in GCs of PCOS patients and controls.

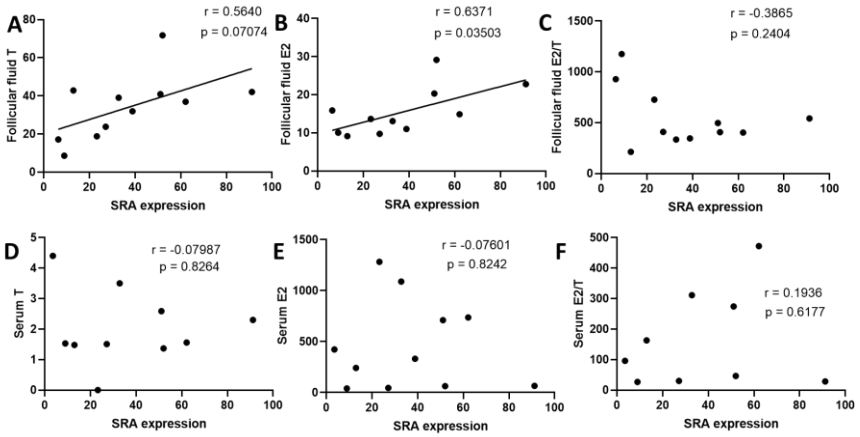

Figure 2 — Correlation of SRA expression levels in GCs of PCOS patients with follicular fluid testosterone levels, estradiol levels, and Estradiol/Testosterone (E2/T) (A, B, C), respectively; and with serum testosterone levels, estradiol levels, and Estradiol/Testosterone (E2/T) (D, E, F), respectively.

Discussion

Polycystic ovary syndrome encompasses a complex interplay of factors, prominently featuring hyperandrogenism as a diagnostic hallmark, prevalent in 60%-80% of cases (Ye et al. 2021). Both elevated androgen levels and increased androgen receptor activation contribute to this characteristic. While traditional genetic studies have mainly focused on protein-coding genes, the exploration of lncRNAs has unveiled novel dimensions in understanding PCOS etiology. In our investigation into lncRNAs, specifically *SRA* and *CTBP1-AS*, we identified compelling alterations in their expression profiles among PCOS patients. Notably, our study revealed a substantial elevation in *SRA* expression within GCs of PCOS patients, suggesting its involvement in PCOS pathogenesis. Intriguingly, heightened *SRA* expression positively correlates with estradiol levels in follicular fluid, indicating a potential role in estradiol dysregulation, often implicated in PCOS hormonal imbalances. These findings support prior research demonstrating increased *SRA* expression in peripheral blood leukocytes of PCOS patients and a positive correlation between *SRA* and body mass index (BMI) (Z Liu et al. 2015). Noteworthy is the consequential effect of suppressing lncRNA *SRA* in mice with PCOS, where it led to notable alterations in insulin production, mitigation of ovarian damage, and a decrease in the production of angiogenic factors. These findings collectively underscore the diverse impact of SRA in modulating critical facets of PCOS pathology, offering promising avenues for therapeutic interventions. Similarly, *CTBP1-AS*, recognized for its regulatory impact on androgen receptor (AR) activity, exhibited notably elevated expression in GCs of PCOS individuals, consistent with previous reports of its increased presence in peripheral blood leukocytes of PCOS patients (Wen et al. 2022). Our findings align with earlier studies from China and India, establishing the association between *CTBP1-AS* expression levels and PCOS, particularly its increased expression

correlated with elevated total testosterone levels (Zhenteng Liu et al. 2015; Wen et al. 2022; Nabi et al. 2021).

Synthesizing our findings with existing literature, the intricate roles of SRA and CTBP1-AS in PCOS emerge as focal points. These lncRNAs, influencing critical facets of PCOS pathophysiology including hormonal imbalances and androgen receptor modulation, hold promise as prospective therapeutic targets. Understanding their intricate mechanisms not only enriches our comprehension of PCOS but also opens avenues for innovative interventions directed at these specific lncRNAs, potentially revolutionizing approaches to managing this complex syndrome.

Conclusion

Our investigation unveiled noteworthy elevations in *SRA* and *CTBP1-AS* expression levels within GCs of PCOS patients, compared to the control group. These lncRNAs, with their discernible influence on pivotal aspects of PCOS pathophysiology such as hormonal imbalances and androgen receptor modulation, emerge as promising candidates for future therapeutic interventions.

Acknowledgment

This study was funded by the Russian Science Foundation (RSF) grant № 23-15-00464.

References

Cunha, Anita, and Ana Margarida Póvoa. 2021. Infertility Management in Women with Polycystic Ovary Syndrome: A Review. https://doi.org/10.1097/j.pbj.0000000000000116.

Ding, Haigang, Juan Zhang, Feng Zhang, Songou Zhang, Xiaozhen Chen, Wenqing Liang, and Qiong Xie. 2021. Resistance to the Insulin and Elevated Level of Androgen: A Major Cause of Polycystic Ovary Syndrome. Frontiers in Endocrinology 12 (20): 741764. https://doi.org/10.3389/FENDO.2021.741764/BIBTEX.

Lanz, Rainer B., Neil J. McKenna, Sergio A. Onate, Urs Albrecht, Jiemin Wong, Sophia Y. Tsai, Ming Jer Tsai, and Bert W. O'Malley. 1999. A Steroid Receptor Coactivator, SRA, Functions as an RNA and Is Present in an SRC-1 Complex. Cell 97 (1): 17–27. https://doi.org/10.1016/S0092-8674(00)80711-4.

Liu, Chan, Hong Tao Wu, Neng Zhu, Ya Ning Shi, Zheng Liu, Bao Xue Ao, Duan Fang Liao, Xi Long Zheng, and Li Qin. 2016. Steroid Receptor RNA Activator: Biologic Function and Role in Disease. Clinica Chimica Acta 459 (August): 137–46. https://doi.org/10.1016/J.CCA.2016.06.004.

Liu, Z, C Hao, X Huang, N Zhang, H Bao, and Q Qu. 2015. Peripheral Blood Leukocyte Expression Level of LncRNA Steroid Receptor RNA Activator (SRA) and Its Association with Polycystic Ovary Syndrome: A Case Control Study. Gynecological Endocrinology : The Official Journal of the International Society of Gynecological Endocrinology 31 (5): 363–68. https://doi.org/10.3109/09513590.2014.999763.

Liu, Zhenteng, Cuifang Hao, Dehua Song, Ning Zhang, Hongchu Bao, and Qinglan Qu. 2015. Androgen Receptor Coregulator CTBP1-AS Is Associated With Polycystic Ovary Syndrome in Chinese Women: A Preliminary Study. Reproductive Sciences 22 (7): 829. https://doi.org/10.1177/1933719114565037.

Lomteva, S.V., T.P. Shkurat, E.S. Bugrimova, O.S. Zolotykh, Alexandrova A A, and Karantysh G V. 2022. Violation of the Hormonal Spectrum in Polycystic Ovaries in Combination with Insulin Resistance. What Is the Trigger: Insulin Resistance or Polycystic Ovary Disease? Baghdad Science Journal 19 (5): 990–98. https://doi.org/10.21123/bsj.2022.6317.

Nabi, Mudasar, Syed Mudasir Andrabi, Shayaq Ul Abeer Rasool, Sairish Ashraf, Imran Majid, and Shajrul Amin. 2021. Androgen Receptor Coregulator Long Noncoding RNA CTBP1-AS Is Associated with Polycystic Ovary Syndrome in Kashmiri Women. Endocrine 75 (2): 614–22. https://doi.org/10.1007/S12020-021-02894-9.

Takayama, Ken Ichi, Kuniko Horie-Inoue, Shintaro Katayama, Takashi Suzuki, Shuichi Tsutsumi, Kazuhiro Ikeda, Tomohiko Urano, et al. 2013. Androgen-Responsive Long Noncoding RNA CTBP1-AS Promotes Prostate Cancer. The EMBO Journal 32 (12): 1665–80. https://doi.org/10.1038/EMBOJ.2013.99.

Wen, Mingxiao, Xiaoqing Dou, Shuzhen Zhang, Bin Wang, Jiangyan Xu, Wenchao Zhang, and Feifei Wang. 2022. CTBP1-AS Upregulation Is Associated with Polycystic Ovary Syndrome and Can Be Effectively Downregulated by Cryptotanshinone. Molecular Medicine Reports 26 (1). https://doi.org/10.3892/MMR.2022.12761.

Ye, Wenting, Tingting Xie, Yali Song, and Lili Zhou. 2021. The Role of Androgen and Its Related Signals in PCOS. Journal of Cellular and Molecular Medicine 25 (4): 1825–37. https://doi.org/10.1111/JCMM.16205.

Ali R. M., Upregulation of the lncRNAs steroid receptor RNA activator (SRA) and c-terminal binding protein 1 antisense (CTBP1-AS) in the granulosa cells of patients with polycystic ovary syndrome // «Живые и биокосные системы». – 2023. – № 46; URL: https://jbks.ru/archive/issue-46/article-7; DOI: 10.18522/2308-9709-2023-46-7