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Abstract

[1]I. M. Lukavenko,
[1]A. V. Kolnoguz,
[2]M. O. Kyrychenko,
[1]O. V. Ataman,
[1]V. Yu. Harbuzova,
[1]Sumy State University, Sumy, Ukraine;
[2]Municipal Non-Commercial Organization "Clinical Hospital № 5" of Sumy City Council

ASSOCIATION BETWEEN THE RS801460-POLYMORPHISM IN THE SRA1 GENE AND THYROID NODULES AMONG UKRAINIAN WOMEN WITH PROLIFERATIVE TYPE OF BENIGN BREAST DYSPLASIA WITHOUT ATYPIA

Introduction. As it was revealed, the greater part of the human genome is represented by non-coding sequences. They also include long non-coding RNAs (lncRNAs). SRA1 is one of its representatives. This lncRNA affects steroid hormones receptors by activating their transcriptional activity. Thereby, SRA1 can be involved in pathogenesis of steroid-responsive tissues tumors.

Purpose. To study the association between the *SRA1* rs801460-polymorphism and the development of thyroid nodules in Ukrainian females with proliferative type of benign breast dysplasia (BBD) without atypia.

Materials and methods. 117 patients with proliferative type of BBD without atypia were enrolled into the study. They were divided into two comparison groups: 12 subjects with thyroid nodules and 105 subjects with no thyroid nodules. All patients were diagnosed on an outpatient basis by a licensed surgeon (license AG No. 600519). The polymerase chain reaction-restriction fragment length polymorphism analysis was used for *SRA1* rs801460-polymorphism genotyping. Statistical data processing was performed using Statistical Package for Social Science software (SPSS, version 25.0, Chicago, IL, USA).

Results. The distribution of CC-homozygotes, CT-heterozygotes and TT-homozygotes in patients with thyroid nodules was 1 (8.3 %), 11 (91.7 %) and 0 (0 %), respectively. The distribution of genotypes in the group without thyroid nodules was 38 (36.2 %), 51 (48.6 %) and 16 (15.2 %), respectively. Significant difference was found in genotypes distribution between patients with and without nodules (P = 0.017). Multivariable logistic regression has shown that CT-genotype has lower thyroid nodules risk compared to CC- and TT-genotypes (P = 0.020, OR = 0.083, 95% CI = 0.010-0.681).

Conclusion. A statistically significant association was found between *SRA1* rs801460-polymorphism and thyroid nodules occurrence in Ukrainian females with proliferative type of BBD without atypia. Individuals with CT-genotype have less risk of thyroid nodules development compared to homozygotes (CC and TT).

Keywords: SRA1, rs801460-polymorphism, benign breast disease, thyroid nodule.

Corresponding author: *kolnoguz.aliona@ukr.net*

Резюме

[1]І. М. Лукавенко,
[1]А. В. Колногуз,
[2]М. О. Кириченко,
[1]О. В. Атаман,
[1]В. Ю. Гарбузова,
[1]Сумський державний університет, м. Суми, Україна;
[2]Комунальне некомерційне підприємство «Клінічна лікарня № 5» Сумської міської ради

ЗВ'ЯЗОК ПОЛІМОРФІЗМУ RS801460 ГЕНУ SRA1 З ВИНИКНЕННЯМ ВУЗЛІВ ЩИТОВИДНОЇ ЗАЛОЗИ СЕРЕД УКРАЇНСЬКИХ ЖІНОК З ПРОЛІФЕРАТИВНИМ ТИПОМ ДОБРОЯКІСНОЇ ДИСПЛАЗІЇ МОЛОЧНОЇ ЗАЛОЗИ БЕЗ АТИПІЇ

Вступ. Як було виявлено, більша частина людського геному представлена некодуючими послідовностями. Вони включають також і довгі некодуючі РНК (днРНК), представником яких є SRA1. Ця днРНК взаємодіє з рецепторами стероїдних гормонів, активуючи їх транскрипційну активність. Таким чином, SRA1 може брати участь у патогенезі пухлин гормон-чутливих тканин.

Мета. Вивчити зв'язок між поліморфізмом rs801460 гена SRA1 та розвитком вузлів щитовидної залози серед українських жінок з проліферативним типом доброякісної дисплазії молочної залози (ДДМЗ) без атипії.

Матеріали та методи. У дослідження було включено 117 пацієнтів з проліферативним типом ДДМЗ без атипії. Вони були поділені на дві групи порівняння: 12 об'єктів з вузлами щитовидної залози та 105 – без. Обстеження та лікування усіх пацієнтів проводив ліцензований хірург (ліцензія AG № 600519). Полімеразна ланцюгова реакція з аналізом довжин рестрикційних фрагментів була використана для генотипування *SRA1* rs801460-поліморфізму. Статистичну обробку даних проводили за допомогою програмного забезпечення «Статистичний пакет для соціальних наук» (SPSS, версія 25.0, Чикаго, Іллінойс, США).

Результати. Розподіл CC-гомозигот, CT-гетерозигот та TT-гомозигот у пацієнтів із вузлами щитовидної залози становив 1 (8.3%), 11 (91.7%) та 0 (0%) відповідно. Розподіл генотипів у групі пацієнтів без вузлів щитовидної залози становив 38 (36.2%), 51 (48.6%) та 16 (15.2%) відповідно. Встановлено статистично значущу різницю у розподілі генотипів між пацієнтами з вузлами та без них (P = 0.017). Мультиваріабельна логістична регресія показала, що CT-генотип має нижчий ризик виникнення вузлів щитовидної залози порівняно з CC- та TT-генотипами (P = 0.020, OR = 0.083, 95% ДІ = 0.010-0.681).

Висновок. Було виявлено статистично значущий зв'язок між *SRA1* rs801460-поліморфізмом та появою вузлів щитовидної залози серед українських жінок з проліферативним типом ДДМЗ без атипії. Особи з CT-генотипом мають менший ризик розвитку вузлів щитовидної залози порівняно з гомозиготами (CC та TT).

Ключові слова: SRA1, rs801460-поліморфізм, доброякісна дисплазія молочної залози, вузол щитовидної залози.

Автор, відповідальний за листування: *kolnoguz.aliona@ukr.net*

Introduction

It was reported that only 2 % of the human genome encodes proteins. The remaining part can be represented by non-coding sequences. One of the key molecular factors of tumorigenesis is exactly non-coding RNAs. The steroid receptor RNA activator 1 (SRA1) is a long non-coding RNA that can act through the steroid hormone receptors transactivation [1, 2]. Thus, a potential role for *SRA1* in the pathogenesis of hormone-sensitive tissue tumors is suggested, for example, mammary gland.

According to the World Health Organization (WHO), breast cancer (BC) is the first most common cause of cancer mortality among females in Ukraine.

Therefore, it is very important to prevent the development of this pathology in time and early diagnosis is critical [3]. Due to this, pre-cancerous mammary conditions are detected, for example, benign breast dysplasia (or benign breast disease).

BBD is a heterogeneous group of lesions that includes all non-cancerous conditions of the breast [4]. It is one of the most important risk factors for BC. BBD is divided into non-proliferative disease, a proliferative disease without atypia, and proliferative disease with atypia. The presence of proliferation and atypia increases the risk of BC [5].

Luo et al. and Schonfeld et al. have found that BBD can be associated with a thyroid cancer increased risk [4, 6]. Also, it has been identified that patients with history of a thyroid cancer have an increased risk of BC [7]. This may mean that a common mechanism lies in the pathogenesis of BBD and thyroid cancer. However, which ones have not yet been established. These authors studied postmenopausal women and thyroid cancer. Unlike them, we decided to check whether BBD in women, regardless of menopause, is associated with thyroid nodules. Thus, the purpose of our study is to establish whether there is an association between the *SRA1* rs801460-polymorphism and the development of thyroid nodules in Ukrainian females with a proliferative type of BBD without atypia.

Materials and methods

Study population

The whole venous blood of 117 Ukrainian women with a proliferative type of BBD without atypia (mean age [±SD] 30.43 ± 9.22) was used for the study. 12 subjects with thyroid nodules (mean age 33.92 ± 14.18) were identified among them (Table 1).

Table 1 – Clinical characteristics of the BBD patients with and without thyroid nodules

Parameter	With (n = 12)	Without (n = 105)	P	F
Age, years	33.92 ± 14.18	30.03 ± 8.48	0.167	10.449
Weight, kg	66.92 ± 14.93	58.11 ± 9.05	0.004	10.575
Growth, cm	170.67 ± 10.5	165.95 ± 6.24	0.024	9.755
BMI, kg/m^2	22.83 ± 3.93	21.09 ± 2.99	0.146	2.147

BBD – benign breast dysplasia; n – number of cases; BMI – body mass index; P – indicator of statistical significance; F - the ratio of the between-class scatter to the within-class scatter. Quantitative variables were compared by *t*-test.

Each BBD patient was diagnosed on an outpatient basis by a licensed surgeon (license AG No. 600519). The study did not include subjects with non-proliferative benign breast disease, no features of genetic predisposition to breast diseases, or the patients who refused to participate. All patients underwent surgical treatment at the clinical sites of the Department of Surgery with a Course of Pediatric Surgery and Urology of the Sumy Regional Oncology Center. The Scientific Laboratory of Molecular Genetic Research of the Sumy State University performed molecular genetic research. All subjects with thyroid nodules had TI-RADS 3 (Thyroid Imaging Reporting and Data Systems classification).

The study protocol and final morphological diagnosis complied with the European Convention of Human Rights and Biomedicine, the Declaration of Helsinki of the World Medical Association on Ethical Principles for Medical Research Involving Human Subjects and the Order of the Ministry of Health of Ukraine No. 616 dated 03.08.2012.

The voluntary informed written consent was received from all patients.

Genotyping

The whole venous blood of 117 subjects was used in the study. DNA extraction was performed using the GeneJET Whole Blood Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). The polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) was used for the *SRA1* rs801460 single nucleotide polymorphism (SNP) genotyping. The essence of *SRA1* rs801460-polymorphism is the replacement of Cytosine (C) to Thymine (T) in the 140,552,345th position of chromosome 5 [8]. The following specific primers were selected: forward – 5`(140552495)TTT TTA GTA GAG ACA GGG TTT TGC C(140552471)3` and reverse – 5`(140552318)ACT CTA CGC CAG ACA ATA TGC TAT G(140552342)3`.

To separate the restriction products, the horizontal electrophoresis (10 V/cm) in 2.5% agarose

gel with the addition of a bromide ethidium solution (10 mg/ml) was used.

Statistical analysis

The Statistical Package for Social Science software (SPSS, version 25.0, Chicago, IL, USA) was used to perform all calculations. Kolmogorov–Smirnov test confirmed the normal distribution. It was used chi-square (χ^2) test and two-tailed Student's t-test in the study. The logistic regression estimated the odds ratio (OR) and 95% confidence interval (CI) in the framework of additive, dominant and over-dominant models of inheritance. It was used multivariable logistic regression for age and BMI adjustments. It was accepted $P < 0.05$ value as significant.

Results

There were 12 subjects (mean age 33.92 ± 14.18) with and 105 subjects (mean age 30.03 ± 8.48) without thyroid nodules in patients with a proliferative type of BBD without atypia. No significant differences were found in mean age as well as in BMI in comparison groups ($P > 0.05$). At the same time, significant difference was found in average weight ($P = 0.004$) and growth ($P = 0.024$) between these two groups (Table1).

It was revealed no significant differences in height of the glandular and fibroglandular parts of the breast ($P > 0.05$) (Table 2).

Table 2 – Breast structure of the BBD patients with and without thyroid nodules

Parameter	With (n = 12)	Without (n = 105)	P	F
Height of the glandular part of the breast (mm)	17.17 ± 4.63	15.10 ± 4.02	0.752	0.100
Height of the fibroglandular part of the breast (mm)	21.58 ± 4.25	19.07 ± 4.29	0.991	< 0.001

BBD – benign breast dysplasia; n – number of cases; P – indicator of statistical significance; F - the ratio of the between-class scatter to the within-class scatter. Quantitative variables were compared by *t*-test.

The differences in the *SRA1* rs801460 SNP genotype distribution in individuals with a proliferative type of BBD without atypia depending on the thyroid nodules occurrence are shown in Table 3. There were 11 estimated cases (91.7 %) of CT-genotype, 1 case (8.3 %) of CC-genotype and 0 – of TT-genotype in patients with thyroid nodules. Fifty-one subjects (48.6 %) of CT-heterozygotes, 38 subjects (36.2 %) of CC-homozygotes and 16 (15.2 %) – of TT-homozygotes were revealed among patients without thyroid nodules. It was found a statistically significant differences ($\chi^2 = 8.110$; $P = 0.017$).

Table 3 – The presence of thyroid nodules in patients with proliferative type of BBD without atypia depending on the *SRA1* rs801460 SNP genotypes

Genotype	With, n (%)	Without, n (%)
T/T	0 (0)	16 (15.2)
C/T	11 (91.7)	51 (48.6)
C/C	1 (8.3)	38 (36.2)
Together	12 (100)	105 (100)

$\chi^2 = 8.110$; $P = 0.017$

BBD – benign breast dysplasia; n – number of cases; P – indicator of statistical significance. Categorical variables were compared by $\chi 2$-test

The results of the association analysis between the *SRA1* rs801460-polymorphism and the thyroid nodules development in patients with a proliferative type of BBD without atypia are summarized in Table 4. Significant association between *SRA1* rs801460 SNP and thyroid nodules was revealed under over-dominant ($P = 0.021$, OR = 0.086, 95% CI = 0.011-0.689) and additive ($P = 0.048$, OR = 0.122, 95% CI = 0.015-0.986 – for CT- and CC-genotypes) models of inheritance. After adjusting for covariates of age and BMI genotypic association of rs801460 SNP remained only under over-dominant ($P = 0.020$, OR = 0.083, 95% CI = 0.010-0.681) model. According to this, the CT-genotype has lower thyroid nodule risk compared to CC- and TT-genotypes in patients with a proliferative type of BBD without atypia.

Table 4 – Analysis of the association between the *SRA1* rs801460-polymorphism and the thyroid nodules in patients with proliferative type of BBD without atypia

Model	P_c	OR_c (95% CI)	P_a	OR_a (95% CI)
Dominant	0.085	0.160 (0.020-1.290)	0.096	0.168 (0.020-1.371)
Over-dominant	0.021	0.086 (0.011-0.689)	0.020	0.083 (0.010-0.681)
Additive	0.048	0.122 (0.015-0.986)	0.059	0.131 (0.016-1.079)

BBD – benign breast dysplasia; P_c: crude P value; ORc: crude odds ratio; CI: confidence interval; P_a: P value adjusted for age and BMI; OR_a: adjusted odds ratio

Discussion

The association between *SRA1* rs801460-polymorphism and the development of thyroid nodules in patients with a proliferative type of BBD without atypia was checked in this study. The *SRA1* is located at reverse strand of chromosome 5 (5q31.3) and has a 687 bp core sequence [8, 9].

SRA1 was discovered in 1999 by Lanz et al. [10]. They concluded that the main mechanism of its action is the coactivation of steroid hormone receptors. Therefore, a potential role of SRA1 in the pathogenesis of hormone-sensitive tissue tumors was suggested. After that, these scientists generated a transgenic-mouse model with *SRA1* overexpression, whereby cell proliferation and apoptosis were found. Nevertheless, they elicited that its overexpression does not lead to tumor progression, because increased cell proliferation causes apoptosis [1].

Cooper et al. reported that *SRA1* overexpression has a statistically significant association with breast tumors with higher progesterone receptor contents. In contrast to Lanz et al., they found that the SRA1 may be involved in tumorigenesis and breast cancer progression [11].

Among all the *SRA1* polymorphisms, we chose rs801480, the essence of which is the C to T replacement in the 140,552,345th position of the (+)chain of chromosome 5 [8]. Yan et al. studied the association between *SRA1* rs10463297 and rs801460 SNPs and breast cancer development. They identified rs801460 GA-, AA-, and GA- + AA-genotypes, as well, as rs10463297 TC- and TC- + CC-genotypes associated with estrogen receptor (ER) positivity in BC patients [2].

Interestingly, tumor processes in mammary and thyroid glands are associated. Luo et al. investigated the possible association between BBD and thyroid cancer development. They established that postmenopausal females with BBD had an increased risk of thyroid cancer than females without BBD [4]. The same conclusion was made by Schonfeld et al. [6]. Patients with a thyroid cancer history also can have an increased risk of BC. Thyroid cancer survivors were found by Kuo et al. to have a higher risk for breast cancer development than the general population [7].

Conclusions

1. There is an association between *SRA1* rs801460-polymorphism and the development of thyroid nodules in Ukrainian females with proliferative type of BBD without atypia.

2. The *SRA1* rs801460 CT-heterozygotes have lower thyroid nodules risk compared to CC- and TT-homozygotes.

Prospects for future research

There are not so many studies on SRA1 and its role in various diseases. Therefore, we need to keep exploring this, as well, as common mechanisms of thyroid and benign breast diseases pathogenesis. Also, further studies are necessary to expand the comparison groups and explore other *SRA1* polymorphisms.

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Conflict of interest

The authors declare no conflict of interest.

Відомості про авторів/Information about the authors

Лукавенко Іван Михайлович, к. мед. н., асистент кафедри хірургії, травматології, ортопедії та фтизіатрії, Сумський державний університет, вул. Римського-Корсакова, 2, м. Суми, Україна (e-mail: i.lukavenko@med.sumdu.edu.ua , тел.: +380-95-535-43-84).

Колногуз Альона Владиславівна, студентка Медичного інституту Сумського державного університету, вул. Римського-Корсакова, 2, м. Суми, Україна (e-mail: kolnoguz.aliona@ukr.net, тел.: +380-50-880-50-90).

Кириченко Микола Олександрович, лікар-інтерн КНП "КЛ5" СМР, вул. Марко Вовчок, 2, м. Суми, Україна (e-mail: kiri7337@gmail.com, тел.: +380-66-541-86-25)

Атаман Олександр Васильович, д. мед. н., професор, завідувач кафедри фізіології та патофізіології з курсом медичної біології, Сумський державний університет, вул. Римського-Корсакова, 2, м. Суми, Україна (e-mail: ataman@med.sumdu.edu.ua, тел.: (+380542) 65-40-63).

Гарбузова Вікторія Юріївна, д. біол. н., професор кафедри фізіології та патофізіології з курсом медичної біології, Сумський державний університет, вул. Римського-Корсакова, 2, м. Суми, Україна (e-mail: v.garbuzova@med.sumdu.edu.ua, тел.: (+38(0542) 65-40-63).

