DOI 10.18699/SBB-2022-448

Functional study of rs2072580 (*SART3* and *ISCU* genes) associated with breast cancer

Degtyareva A.[1]*, Antontseva E.[1], Merkulova T.[1, 2]

[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*

[2] *Novosibirsk State University, Novosibirsk, Russia*

* *degtyareva_rso@mail.ru*

Key words: rSNPs, *SART3*, *ISCU*, EMSA, breast cancer

Motivation and Aim: Single nucleotide polymorphism (SNP) rs2072580 T>A, located in the overlapping promoter regions of the *SART3* and *ISCU* genes, was identified by us as potentially regulatory (rSNP) using a bioinformatics approach developed in the laboratory for the analysis of genome-wide allele specific ChIP-seq and RNA-seq data [1]. An association of this polymorphism with an increased risk of developing breast cancer in the case of the A allele was also established [2]. The aim of this work is a functional analysis of the regulatory potential of rs2072580 to understand its significance in the molecular mechanisms of breast cancer development.

Methods and Algorithms: Electrophoretic mobility shift assay (EMSA) – method of DNA-probes retention in the gel by nuclear extract proteins was used to study the ability of this SNP to destroy or create binding sites for certain transcription factors (TFs). For its implementation, 31 bp double-stranded oligonucleotides V1 (T-allele) and V2 (A-allele) corresponding to allelic variants of the SNP location in the genome were used. Human cell lines HepG2, MCF7 were chosen as a source of nuclear extract proteins. Using the MotifbreakR software package, five TFs were selected, the binding sites of which, with a high probability, could change their affinity as a result of the rs2072580 T>A substitution. These are CREB1, AP1, FOXK1, PAX3 and REST. Oligonucleotides containing experimentally confirmed binding sites for these TFs were used in EMSA for competitive analysis. To evaluate the effect of the rs2072580 substitution on the expression of the Luc2 luciferase reporter gene, plasmid constructs containing a large promoter region (~1500 bp) both in the orientation of the *SART3* gene and in the orientation of the *ISCU* gene were created. Allelic variants of the promoter region were obtained by site-directed mutagenesis. The pGL3-Basic plasmid was used as a vector. The HepG2 cell line was used for transfection.

Results: The method of DNA-probes retention in gel with proteins of the nuclear extract of HepG2 cells showed that V1 forms more intense delay bands than V2, which was confirmed during cross-competition of these oligonucleotides, i.e. the substitution T>A leads to a decrease in the affinity of the given region for certain TFs. As a result of competitive analysis, it turned out that oligonucleotides containing the binding sites of CREB1, PAX3 and AP1 specifically compete with V1 and V2 for protein binding, since they attenuate certain bands on radioautograph. Moreover, the use of the CREB1-mut oligonucleotide as a competitor, with the destroyed site of this TF, did not lead to any change in the binding pattern. Thus, the presence of rs2072580 T>A affects the affinity of the transcription factors CREB1, PAX3, and AP1 for this region and, as a result, can determine the level of expression of nearby genes. It should be noted that, according to

the data of the ENCODE project, this SNP falls into the ChiP-seq peaks of the same TFs obtained on HepG2 and MCF7 cell lines.

The regulatory potential of rs2072580 in the *in vivo* system was studied in a reporter system on the HepG2 cell line. It revealed that the T>A substitution affects the expression of the Luc2 luciferase gene. Using data from the GTEx portal (https://www.gtexportal.org/), which contains information on the association of SNPs with the level of gene expression in postmortem samples of various human tissues, it was found that rs2072580 is associated with changes in the expression level of the *FICD* and *ISCU* genes in some tissues.

Conclusion: Thus, rs2072580 associated with breast cancer, localized in the common promoter region of the *SART3* and *ISCU* genes, has a regulatory potential and can affect the expression of target genes and finally to phenotype. In addition, the analysis of literature data revealed that SART3 and ISCU are shown to be involved in carcinogenesis processes [3, 4].

Acknowledgements: This work was supported by the State Budget Project FWNR-2022-0016.

References

1. Korbolina E.E. et al. Novel approach to functional SNPs discovery from genome-wide data reveals promising variants for colon cancer risk. *Hum Mutat*. 2018;39(6):851-854. doi: 10.1002/humu.23425.
2. Degtyareva A.O. et al. rs2072580T>A Polymorphism in the overlapping promoter regions of the SART3 and ISCU genes associated with the risk of breast cancer. *Bull Exp Biol Med*. 2020;169(1):81-84.
3. Whitmill A. et al. Tip110: Physical properties, primary structure, and biological functions. *Life Sci*. 2016;149:79-95.
4. Ullmann P. et al. Hypoxia-responsive miR-210 promotes self-renewal capacity of colon tumor-initiating cells by repressing ISCU and by inducing lactate production. *Oncotarget*. 2016;7(40):65454-65470.