DOI 10.18699/BGRS/SB-2020-278

Micrornas (221, 429) are correlation with lymphocytes of axillary lymph nodes in experimental breast cancer

Aleksey Kabakov
RICEL-Branch of
IC&G SB RAS,
Novosibirsk, Russia
kabakov_av85@mail.ru

Alexandr Lykov
RICEL-Branch of
IC&G SB RAS,
Novosibirsk, Russia
aplykov2@mail.ru

Oleg Kazakov
RICEL-Branch of
IC&G SB RAS,
Novosibirsk, Russia
kazakoff_oleg@mail.ru

Alexandr Poveshchenko
RICEL-Branch of
IC&G SB RAS,
Novosibirsk, Russia
poveshchenkoa200@mail.ru

Olga Poveshchenko
RICEL-Branch of
IC&G SB RAS,
Novosibirsk, Russia
poveschenkoov@yandex.ru

Dmitriy Strunkin
RIMBB FRC FTM
Novosibirsk, Russia
strunkind@mail.ru

Lyudmila Gulyaeva
RIMBB FRC FTM
Novosibirsk, Russia
gulyaeva@soramn.ru

Andrey Letyagin,
RICEL-Branch of
IC&G SB RAS,
Novosibirsk, Russia
letyaginay@bionet.nsc.ru

Vladimir Konenkov
RICEL-Branch of
IC&G SB RAS,
Novosibirsk, Russia
vikonenkov@gmail.com

Abstract — **The study of concentration miRNA (21, 221, 222, and 429) in serum, lymph of the thoracic duct and breast cancer tissue, as well as the cellular composition of axillary lymph node in Wistar female rats in chemically induced breast cancer. Concentration microRNA (-221, -429) in the tissues breast cancer and lymph are correlated with a decrease in number of lymphocytes in medullary cords of axillary lymph nodes.**

Keywords - microRNA, breast cancer, lymphocytes

INTRODUCTION

In women all over the world, breast cancer (BC) is one of most common pathologies [4]. One of new important diagnostic markers of tumor growth, attracting the attention of researchers, are microRNAs. MicroRNAs (18–25 nucleotides) small non-coding RNAs take part in regulation of vital functions of cells (proliferation, differentiation, apoptosis), expression of which is often disturbed during pathological processes in organism, including cancer pathology [6], for example, breast cancer [1, 7]. The study of microRNA correlations (-21, -221, -222, -429) in breast cancer with morphological changes in regional lymph nodes of a tumor is of scientific interest and can serve as a basis for the development of new diagnostic methods and therapy.

Purpose of the study identification of correlation relationships of microRNA levels in serum, lymph and breast tissue with axillary lymph node cells in breast cancer in Wistar rats.

MATERIALS AND METHODS

In the experiment were used rats-female Wistar 10-12 week-old, weighted 250-300 g. 1st group - control (20 intact animals), 2nd group - breast cancer. Breast cancer (BC) was induced in 20 Wistar female rats at the age of 3 months by 5-fold administration with an interval of 7 days of N-methyl-N-nitrosourea (MNU) (Sigma, USA) subcutaneously into the area of the 2nd mammary gland on the right. 24-weeks after administration MNU breast tissue, BC tissue, blood [5], and lymph specimens (thoracic duct lymph) were collected [2]. For histological examination, axillary lymph nodes were taken (caudal of 4 nodes) [3]. Total RNA from blood serum, lymph and breast tissue specimens was extracted using a kit of reagents (Vector-Best, Russian Federation) according to the instruction. To obtain the cDNA was carried out reverse transcription on the matrix of microRNAs. To determine the number of microRNAs-21, -221, -222, -429 in biological samples, QT-PCR was performed in real time using reagents on the amplifier CFX96 (Bio-Rad Lab., USA), small RNA U6 (Vector-Best reagents, Russian Federation) was used as a comparison gene. Statistical data processing was performed using the Statistica 10 software package, central tendency and dispersion measures were described by the median (Me), lower (Q1) and upper (Q3) quartiles, the significance of the difference was calculated using the Mann-Whitney U-test, and was taken with $p < 0.05$. The correlation relationships between the studied parameters were estimated by the Spearman rank correlation coefficient.

RESULTS

Induction of breast cancer led to a decrease concentration of tumor-suppressive microRNA-429 in tumor ($p<0.05$). In lymph of animals with BC, concentration of pro-oncogenic microRNA-221 increased in comparison with intact animals ($p<0.05$). Concentration of tumor-suppressive microRNA-429 in lymph in female rats with BC was decreased compared of intact animals ($p<0.05$).

In study of the cellular composition of axillary lymph nodes in Wistar rats with BC revealed changes compared with intact animals. In study of the cellular composition of axillary lymph nodes in Wistar rats with BC revealed changes compared with intact animals. It was shown that in medullary cords, there was a decrease in number of medium lymphocytes

($p = 0.0005$) and mature plasma cells ($p = 0.013$), an increase in number of small lymphocytes ($p = 0.0001$) and reticular cells ($p = 0.0001$).

When analyzing the correlation relationships in animals with BC, the number of mature plasma cells of the axillary lymph node medullary cords was correlated with concentration of miR-429 in tumor tissues ($R = 0.51$; $p = 0.0033$). In lymph of animals with BC, the number of medial lymphocytes of medullary cords of axillary lymph nodes had a reverse correlation with concentration of microRNA-221 in the lymph ($R = –0.62$; $p = 0.028$). At the same time, in Wistar rats with BC the number of medium lymphocytes of medullary cords of axillary lymph nodes had a direct and moderate grade of correlation with concentration of miR-429 in the lymph ($R = 0.62$; $p = 0.028$).

CONCLUSION

Thus, concentration of microRNA (-221,-429) in breast cancer tissue and lymph are correlated with a decrease in number of lymphocytes in medullary cords of axillary lymph nodes.

REFERENCES

[1] Grishina K.A., Khailenko V.A., Khailenko D.V., Karpukhin A.V. Role of microRNA in the development of breast cancer and their potential as biomarkers of this disease. // Tumors of the female reproductive system. - 2018. - T. 15. - № 3. - P. 40-47.

[2] Kuznetsov, A.V. New method of collecting lymph from animals. // Bulletin of experimental biology and medicine. - 1993. - № 9. - p. 329–331.

[3] Nozdrachev A.D., Polyakov E.L. Rat Anatomy (Laboratory animals). - SPb .: Lan, 2001. - 464 p.

[4] Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebelo M., Parkin D.M., Forman D., Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. // Int J Cancer. – 2015. – No. 136(5): E359-86. doi: 10.1002/ijc.29210. Epub 2014 Oct 9.

[5] Harikai N., Fujii T., Onodera S., Tashiro S. Increases in plasma dihydroxyphenylacetic acid levels in decapitated mice after exposure to various stresses. // Biol. Pharm. Bull. – 2002. – Vol. 25. – No. 7. – P. 823–826.

[6] Li Z., Branham W.S., Dial S.L., Wang Y., Guo L., Shi L., Chen T. Genomic analysis of microRNA time-course expression in liver of mice treated with genotoxic carcinogen N-ethyl-N-nitrosourea. // BMC Genomics. – 2010. – Vol. 11. – P. 609. http://www.biomedcentral.com/1471-2164/11/609.

[7] Waters P.S., McDermott A.M., Wall D., Heneghan H.M., Miller N., Newell J., Kerin MJ., Dwyer R.M. Relationship between circulating and tissue microRNAs in a murine model of breast cancer. // PLoS One. – 2012. – Vol. 7, no. 11. e50459. doi:10.1371/journal.pone.0050459.