

MicroRNA (221,429) Correlates with Lymphocytes of Axillary Lymph Nodes in Experimental Breast Cancer

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I. INTRODUCTION

Breast cancer (BC) often revealed in women worldwide [4]. At the present time microRNA used as marker for diagnosis of tumor growth and metastasis. MicroRNA (18– 25 nucleotides) small non-coding RNA which involved in regulation of the proliferation, differentiation, and apoptosis cells include cancer cells [6], for example BC [1, 7]. Data obtained on basis of the interlink of pathological signs in lymph node with the levels of microRNA 21, 221, 222, and 429 may be used for correction of chemotherapy in cancer. Aim obtained possible correlation of the pathological signs in axillary lymph node with the levels of microRNA in rat BC model.

II. MATERIALS AND METHODS

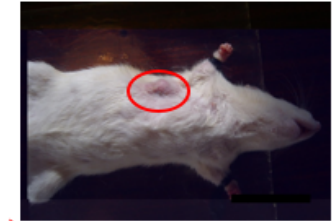
Wistar female rats weighting 250-300 g was divided into 2 groups: control (n=20) and BC-group (n=20). BC was induced by injection of N-methyl-N-nitrosourea (MNU) (Sigma, USA) subcutaneously into the area of the 2nd mammary gland on the right (5 injection with an interval of 7 days). 24-weeks late the tissue from normal mammary gland, malignant mammary gland, cord blood [5], thoracic duct lymph was collected [2]. Standard histology of axillary lymph nodes was done [3]. Total RNA from blood serum, lymph, and mammary gland (normal and malignant) tissue specimens were extracted using kit reagent (Vector-Best, Russia). To obtain the cDNA was carried out reverse transcription on the matrix of microRNAs. To determine the number of microRNA (21, 221, 222, and 429) in samples, QT-PCR was performed on the amplifier CFX96 (Bio-Rad, USA), small RNA U6 (Vector-Best, Russia) was used as a comparison gene. Statistical comparisons

of obtained data were done by Mann-Whitney U-test. Data are expressed as the median (Me), lower (Q1) and upper (Q3) quartiles, differences were considered significant at $p \leq 0.05$.

DESIGN OF EXPERIMENT

Wistar female rats
weighting 250-300 g

Induction of breast cancer



0 8 weeks 12 weeks 16 weeks 20 weeks 24 weeks

BC was induced by injection of N-methyl-N-nitrosourea (MNU) subcutaneously into the area of the 2nd mammary gland on the right (5 injection with an interval of 7 days).

Collected materials

- Cord blood
- Lymph
- Mammary gland
- Breast cancer
- Axillary lymph nodes

Groups:

1. Control – 20
2. Breast cancer – 20

III. RESULTS

The microRNA-429 in malignant mammary gland spices significantly decreased compare with normal mammary gland ($p < 0.05$). In the thoracic duct lymph of the BC animal increased levels of the microRNA-221 (pro- oncogenic microRNA) compared with control-group was done ($p < 0.05$). While was estimated decreased levels of the microRNA-429 in thoracic duct lymph on BC-group compared with control-group ($p < 0.05$). In BC-group in axillary lymph node occurred changes of the number of some cell type compared with control-group. For example, we estimated decreased number of the medium size lymphocytes ($p = 0.0005$), and the number of the mature plasma B-cells ($p = 0.013$) compared with control-group. While the number of the small size lymphocytes ($p = 0.0001$), and the number of reticula cells ($p = 0,0001$) in medullar zone of the axillary lymph node were increased compared with control-group. Correlation analysis revealed interlink between the number of mature plasma B-cells in medullar zone pf axillary lymph node and level of the microRNA-429 in malignant mammary gland tissue ($r = 0.51$, $p = 0.0033$); between the number of medium size lymphocytes in medullar zone of the axillary lymph node and levels of the thoracic duct lymph microRNA-221 ($r = -0.62$, $p = 0.028$)⁴ between the number of medium size lymphocytes in medullary zone of the axillary lymph node and levels of the thoracic duct lymph microRNA-429 ($r = 0,62$. $p = 0.028$) in BC-group.

IV. CONCLUSION

In summary was estimated some correlations between microRNA levels from malignant mammary gland and levels of the thoracic duct lymph with the number of lymphocytes from medullar zone of the axillary lymph node in rat model of breast cancer.

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