

Immunohistochemical (Ki-67 Marker) Examination of Morphological Changes in the Lungs as a Result of Breast Cancer

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Abstract: In recent years, the incidence of breast cancer and death from this disease have increased dramatically around the world. In this study, morphological changes in the lungs of white rats with experimental mammary gland cancer were investigated by immunohistochemical method. Micropreparations prepared from lung tissue structures isolated from them were examined with immunohistochemical marker Ki-67 and the results were presented depending on the level of expression of this marker.

Keywords: Ki-67 marker, Breast cancer, cell proliferation, proliferation index, immunohistochemical analysis, lung.

The purpose of the study: to determine the level of expression of the Ki-67 marker in lung tissue as a result of experimental breast cancer.

Material and methods: 60 rats of white breed were used as the object of the study in normal vivarium conditions of 6 months. In the experimental groups, the cancer of the mammary gland of rats was induced by means of the carcinogen 7,12-dimethylbenzanthracene. A 68.9% success rate was achieved, i.e., 42 rat mammary cancers were induced by subcutaneous injection of the carcinogen 7,12-dimethylbenzanthracene at a dose of 0.1 mg in the breast of 60 female rats.

1- Table. Distribution of animals depending on the content of the experiment

Groups (n-control, t-experiment)	Content of experience	Youngs of animals	Total number of animals (*dead rats number)
		9 month	
I c	Group 1 - healthy white rats in standard vivarium conditions	30	30
II e	2- group - an experimental group, non-white rats, in which mammary gland cancer was induced in rats by the carcinogen 7,12-dimethylbenzanthracene	30(6*)	30(6*)
Total		60	60(6*)

We prepared micropreparations from the lung tissue of these 45 rats. Tissue sections for immunohistochemical staining were cut with a microtome to a thickness of 4-6 μm , placed on a slide and covered with a coverslip. Obtained tissues were made using the method of avidin-biotin immunoperoxidase, using the method of dehydration and deparaffinization of sections.

To block endogenous peroxidase, the drug was placed in a 3% hydrogen peroxide solution for 10 minutes. After washing the drug with Tris-NaCl-buffer pH 7.6 solution, Ki-67 marker was used to determine the proliferative activity of the cells to determine the expression, and they were stained with DAB+ chromogenic method. Stained preparations were examined using a microscope. To evaluate the immunohistochemical results, morphometric examinations were performed using the QuPhat 4.4.0 program in 5 fields of view with a magnification of 200-400 times.

The positively expressed cells in the extracted area were calculated as a percentage of the total cells in the total area. The level of expression was evaluated as 20% (low expression), 20-60% (medium expression) and more than 60% (high expression).



Picture1. Lungs of 6-month-old white rats in the experimental group macroscopic appearance.

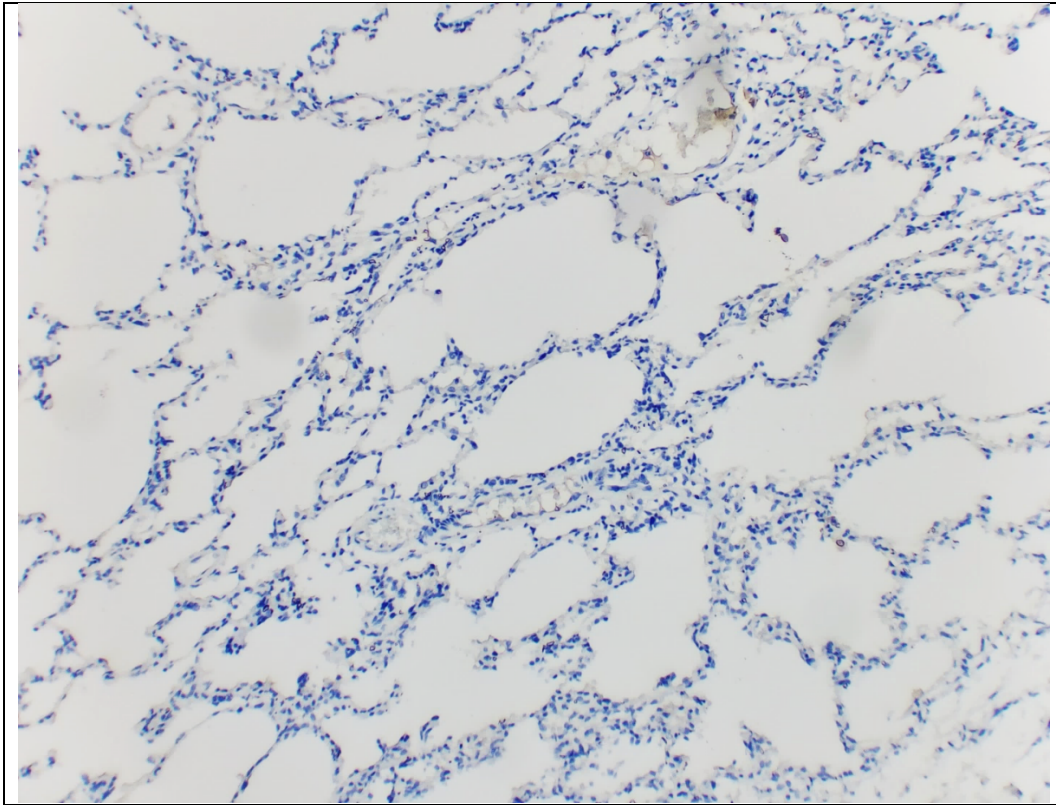
Results and conclusions

Immunohistochemical marker Ki-67 is used to evaluate the proliferative activity of cells. The proliferative activity of the cell depends on different periods of the cell cycle. The cell cycle is considered as the time of existence of this cell, which lasts from the time of the cell's emergence from the mother cell to the end of its life.

This phenomenon can be explained in such a way that the period from the appearance of the cell to its death is taken into account. The cell cycle can be divided into the following phases: the presynthetic period (G1), the synthetic period (S), the postsynthetic period (G2) and the period of cell division, i.e., the mitosis period. The presynthetic phase lasts longer and in this phase the cell grows, develops, synthesizes RNA and forms organelles. Synthetic period (S) is the period during which DNA replication takes place. In the (G2) phase, the cell prepares for division, and proteins and ATf necessary for division are synthesized.

The period of mitosis is the period from cell division to the next cell division. Mainly immunohistochemical marker Ki-67 is also used to evaluate the proliferative activity of cells in tumor processes. This marker is evaluated in percentages and shows the level of cell division activity.

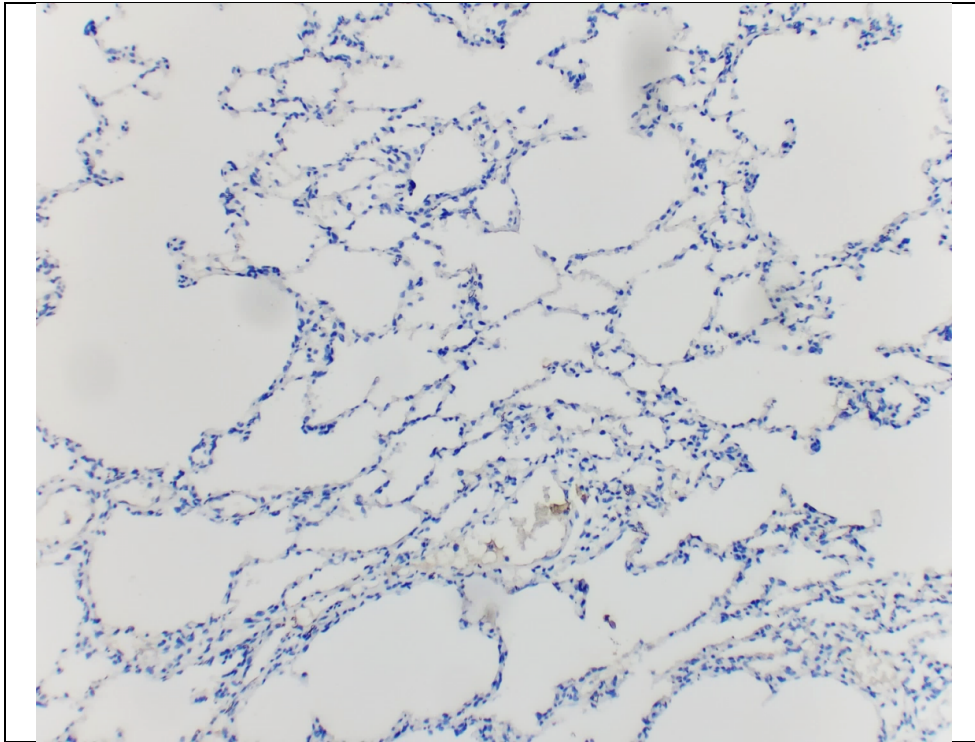
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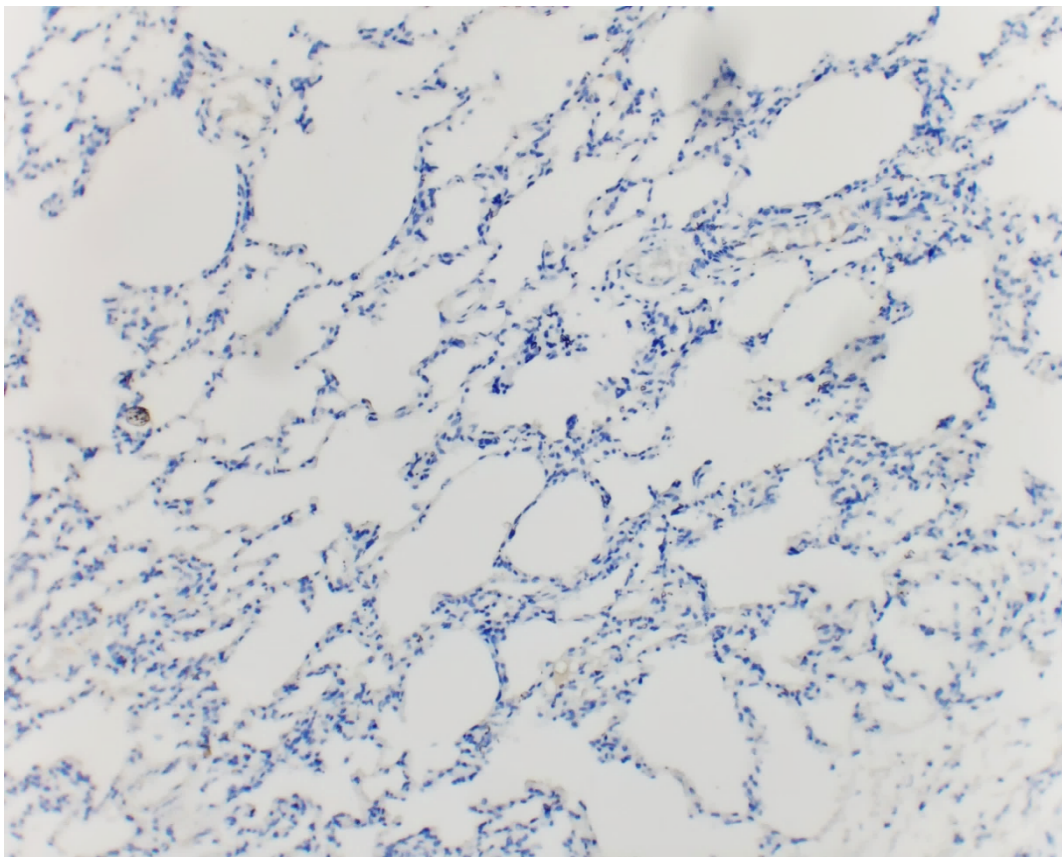
total number of cells	950
Positive cells	401
Negative Cells	500
Positive Expression	44,90 %
Total area	927700 px ²

Pic. 2. Expression indicators of immunohistochemical marker Ki 67 in the lung tissue of 6-month-old white rats with experimental mammary gland cancer.

Using the immunohistochemical method, it is possible to assess the response of tumor cells to treatment after chemotherapy. At the same time, it is widely used to evaluate other morphological changes besides tumor cells. The special protein expressing the Ki 67 marker is located in the nucleus of the cell and is one of the materials important for cell proliferation. Determining this protein is an important indicator for assessing the effectiveness of treatment based on the stage of cell division, how active and fast it is, the growth rate of tumor cells, and the risk of metastasis.



total number of cells	170
Positive cells	92
Negative Cells	69
Positive Expression	50,83 %
Total area	165600 px ²



total number of cells	990
Positive cells	460

Negative Cells	530
Positive Expression	47,83 %
Total area	958600 px ²

Pic. 3,4. Expression indicators of immunohistochemical marker Ki 67 in the lung tissue of 6-month-old white rats with experimental mammary gland cancer.

For the study, micropreparations isolated from 45 white outbred rats with mammary gland cancer induced by the carcinogen 7,12-dimethylbenzanthracene were studied with Ki-67 marker immunohistochemical results. In the obtained results, it was determined that the immunohistochemical Ki 67 marker was expressed in the lung tissue.

Summary:

1. In the experiment, it was found that Ki-67 marker expression was at a medium level in almost all of the materials obtained from white outbred rats with mammary gland cancer, i.e., the proliferative index was 47.6%.

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