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IMMUNOCYTOCHEMICAL METHOD IN STUDYING THE BASIC MORPHOLOGICAL CHARACTERISCTICS OF TUMOR CELLS (ИММУНОЦИТОХИМИЧЕСКИЙ МЕТОД В ИЗУЧЕНИИ ОСНОВНЫХ МОРФОЛОГИЧЕСКИХ ХАРАКТЕРИСТИК ОПУХОЛЕВЫХ КЛЕТОК)

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Immunocytochemical methods have become an integral part of the clinical laboratory, as well as the research setting. This article provides the main features of transplantable cell lines that are used in cell biotechnology, respectively.

Immunocytochemistry (ICC), by definition, is the identification of a tissue constituent *in situ* by means of a specific antigen-antibody interaction where the antibody has been tagged with a visible label [5]. Cell staining is a powerful method to demonstrate both the presence and subcellular location of a particular molecule of interest. Initial attempts to label antibodies with ordinary dyes were unsatisfactory because the label was not sufficiently visible under the microscope. A. H. Coons first introduced immunofluorescence in 1941, using specific antibodies labeled with a fluorescent dye to localize substances in tissues [4]. This technique was considered difficult, and its potential was not widely realized for nearly 20 yr. Early attempts focused on labeling the specific antibody itself with a fluorophore. In this method, the specific antibody, bound to the antigen, was detected with a secondary reagent, usually another antibody that had been tagged with either a fluorophore or an enzyme. Fluorochrome-labeled anti-immunoglobulin antibodies are now widely used in immunocytochemistry, flow cytometry and hybridoma screening. The availability of fluorophores with different emission spectra has also made it possible to detect two or more antigens on the same cell or tissue section [1].

Cultures of cells, tissues or organs have been widely used and are now being used in scientific research. They are a method of maintaining viability or growing outside the body of individual cells, as well as organized structures (tissues, organs, embryos) that retain their differentiation. Since the scientists found a way to isolate pure cell lines, the cell culture method has become an ideal way to study the structure and properties of living cells. Cell cultures obtained by modern methods are homogeneous populations of genetically homogeneous cells growing under constant conditions [2].

1. Materials and methods

As an object of research, cell lines from the collection of the Research platform of agricultural biotechnology were used: *HeLa, FLK, MA-104*; nutrient medium *DMEM*, fetal bovine serum, *PBS 1x*, *Tween-20*, 96% ethanol, 70% ethanol, 1% and 0.1% *BSA*, *Alexafluor 488* with *Antirabbit, At Nestin, A tFSP, At β-tubulmrabbit*, trypan blue, double-distilled water, distilled water, disposable pipettes, plastic petri dishes, cotton wool, parafilm, glass slides, coverslips, tablets, vials, dimethyl sulfoxide, test tubes, tips.

The following equipment was used for the research: 5% CO2 incubator, laminar box, refrigerator, water bath, dispensing gun, inverted microscope, fluorescence microscope, autoclave, bactericidal UV lamp and centrifuge**.**

The basis of the ICC method is the immunological reaction of antigen and antibody. It is also possible to localize and identify cellular and tissue antigens based on their binding to antibodies [3].

For the ICC method, cell lines *FLK, HeLa*, and *MA-104* were used. Each line was sown in 3 cups for a total of 9 cups. Next, the following procedures were performed: the medium was washed, washed with *PBS* in 3 ml for 5 minutes 3 times. 700 ml of 96% ethanol were added to each well and placed in a CO2 incubator for 30 minutes. Next, the wells were washed 3 times with *PBST* solution, 500 ml per well, for 5 minutes. 1 ml of 1% *BSA* was added and incubated for 1 hour in a CO2 incubator. Prepared a working concentration of primary antibodies: *FSP* 1: 500, *Nestin* 1: 500 and *β-tubulmrabbit* 1: 500. That amounted to 2 ml of antibody +1 ml of 0.1% *BSA*. After that, 1 ml of a solution of antibodies of three types was added in three lines and incubated at room temperature, leaving overnight. The next day, they were washed 3 times in a 2 ml *PBST* solution, stirring vigorously for 5 minutes. Next, secondary antibodies of 6 ml *Alexafluor 488* with *Antirabbit* + 6 ml of 1% *BSA* were prepared and 500 ml were added to each well, followed by incubation on a stirrer in a dark place at room temperature for 2 hours. After 2 hours, the secondary antibodies were removed and washed with a *PBST* solution of 500 ml in each well 3 times, for 5 minutes. Then the coverslips with the samples were dried and fixed.

2. Results and discussions

Antibodies *FSP, β-tubulmrabbit* and *Nestin* were selected for the immunocytochemical method because staining of the cytoplasm using these antibodies to *Antirabbit* is considered a positive result.

All available samples were viewed through a fluorescence microscope and the results of the studies were obtained. According to the results of the study, not all cells showed cytoplasmic antigens. Antigens specific for the selected antibody were labeled with dye *Alexafluor 488*.

Below are the results of the ICC method. Of the nine cups, six showed a positive result:

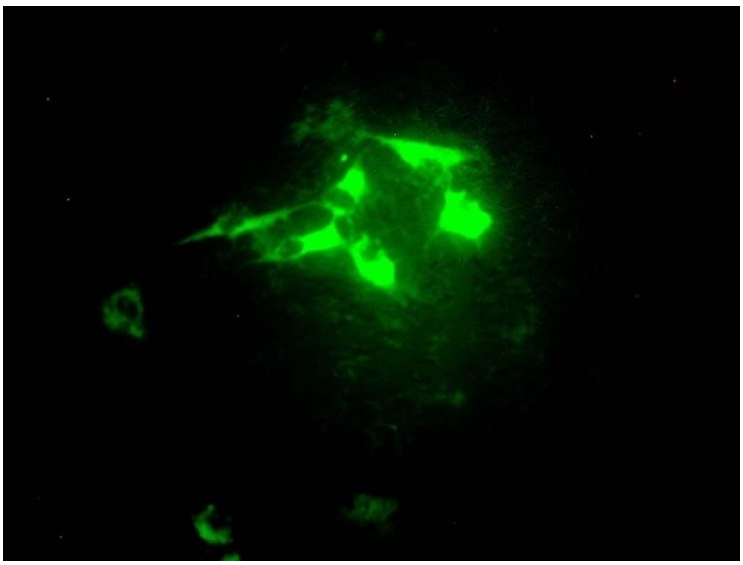


Figure 1. *FLK* cell line with *β-tubulmrabbit* antibodies, 40x magnification

In this figure, the cytoplasm of the cells are displayed brightly. In this connection, rounded black nuclei are clearly observed. In this case, *FLK* cells were associated with antibodies and showed a positive result.

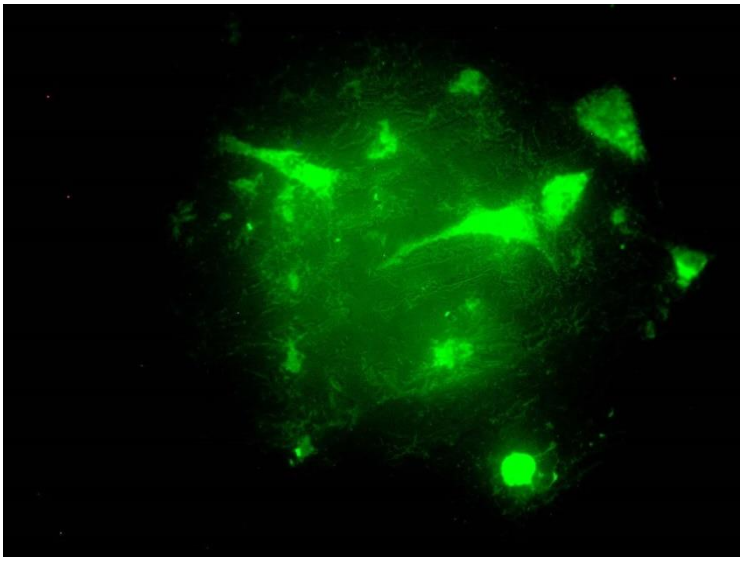
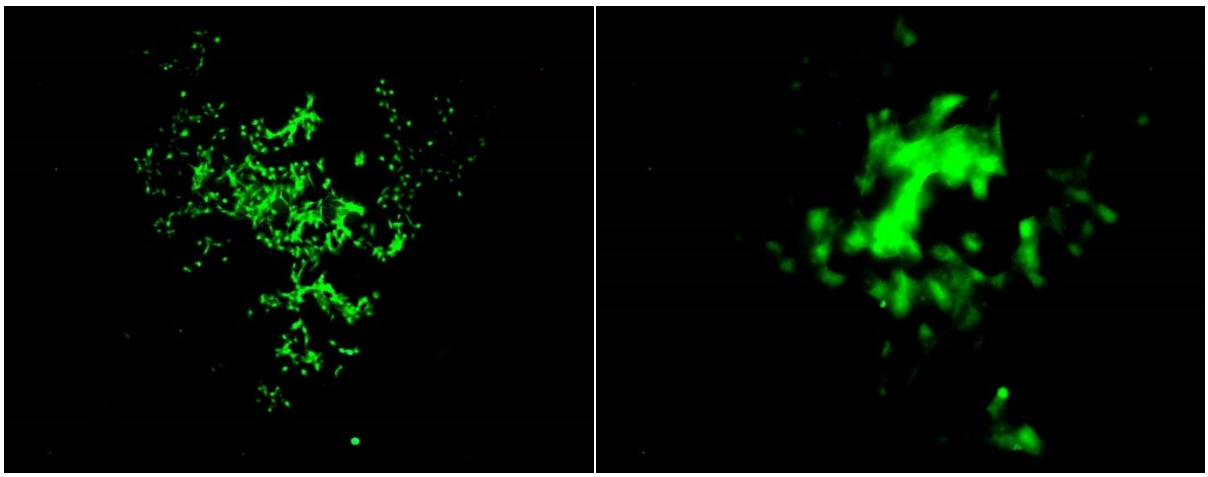


Figure 2. *FLK* cell line with *Nestin* antibodies, magnification 40x

Figure 2 shows the relationship of antigens with antibodies. In this case, immunocytochemical analysis gave a positive result. The expanding tissue of the cells is clearly noticeable, and the cytoplasm of the cells is visible and shows the antigenic ability to target antibodies.



a) magnification 20x b) magnification 40x

Figure 3. *HeLa* cell line with *FSP* antibodies

In this figure it can be seen how successfully passed the reaction, since the scope marks cytoplasm shows positive binding glue-accurate *HeLa* line with *FSP* antibodies.

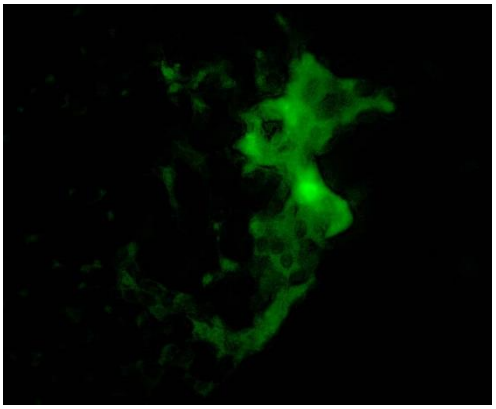


Figure 4. *HeLa* cell line with *β-tubulmrabbit* antibodies, magnification 40x

In this case, *HeLa* cells showed antigenic ability with *β-tubulmrabbit* antibodies. Black nuclei of cells are clearly observed in the figure, since *Alexafluor 488* tags have the ability to stain only the cytoplasm of cells.

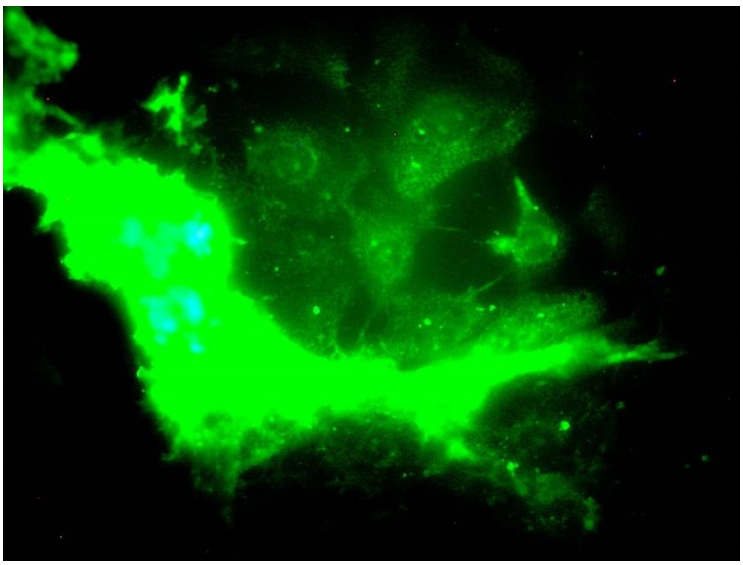


Figure 5. *MA-104* cell line with *Nestin* antibodies, magnification 40x

Figure 5 shows single cells and a positive result of the binding of *MA-104* cell lines to *Nestin* antibodies, as well as a clear decomposition of fibroblasts between cytoplasms, moreover nuclei are noticeable.

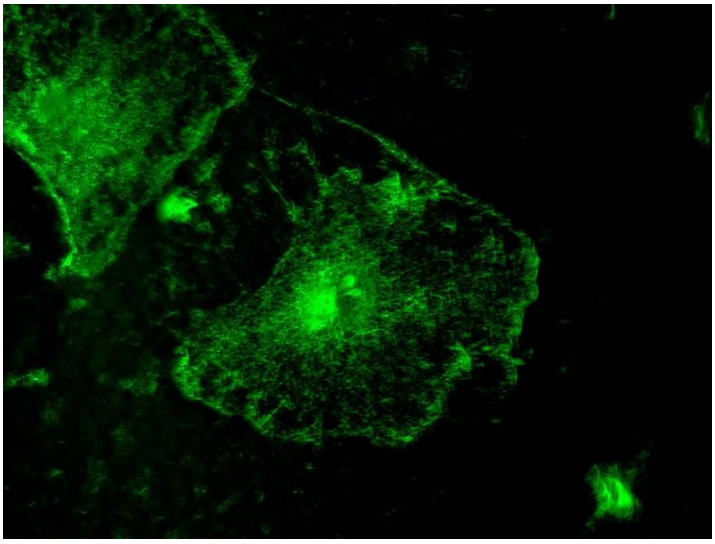


Figure 6. *MA-104* cell line with *FSP* antibodies, magnification 40x

Figure 6 shows how *MA-104* cells contacted and tested positive for FSP antibodies. Here, the cells grew on top of each other, so a clear display of the cytoplasm is not visible, but a bright green color of the cytoplasm is seen under the fibroblasts. In this case, the antigenic ability of the cell was revealed.

3. Conclusion

Thus, 3 cell samples for immunocytochemistry gave negative results: the *FLK* cell line with *FSP* antibodies; *MA-104* with *β-tubulmrabbit* antibodies; *HeLa* with *Nestin* antibodies. When studying the cells with a fluorescence microscope, the above cells with primary antibodies were not labeled and did not contact

Antirabbit with the label *Alexafluor 488*. This indicates the non-specificity of the above cells with the given antibodies.

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