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FRACTAL DIMENSION OF THE RESISTANT AND SENSITIVE TO DOXORUBICIN BREAST CANCER CELLS, EXPOSED IN THE MAGNETIC FIELD

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Abstract. *In this paper the fractal dimension of the resistant and sensitive to doxorubicin MCF-line breast cancer cells, exposed in the magnetic field, is examined. It is revealed, that the resistant and sensitive to doxorubicin MCF-line breast cancer cells have different value ranges of the fractal dimension. Thereby, the main purpose of the present research is to check the possibility of using the fractal dimension as a marker for sensitivity determining of MCF-line breast cancer cells, exposed in the magnetic field.*

Keywords: atomic force microscopy; fractal dimension; doxorubicin; magnetic force microscopy; magnetic field; resistant and sensitive MCF-line breast cancer cells.

1. Introduction

For the first time the term "fractal" was mentioned in the scientific literature in 1975 by Benoit Mandelbrot. The broad scientific use of the term "fractal geometry" was introduced through his book "The Fractal Geometry of Nature". Mandelbrot defined a fractal as a structure, consisting of parts that are similar overall. Dimension of this structure is not equal to it's topological dimension and can be a noninteger value [1]. This dimension is called Hausdorff-Besicovitch dimension or fractal dimension. In a short time fractal geometry has become extremely widespread in technical and natural sciences. Fractal analysis allows us to represent complex processes in a simple and convenient for analysis form [2]. The use of fractal geometry in biological research are also very important, so that all parts of living organisms are built on quasi-fractal principle, they contain the traits of self-similarity, and many biological structures have real fractal forms [3, 4].

The morphology of the cells is determined by the organization of the cytoskeleton (cytomatrix) as a fractal cluster. Fractal geometry is the geometry of the hierarchical random processes and structures, that is used to describe cellular morphology, ranging from sub-cellular level to cellular, tissular and organismal levels [5]. The cells and their complexes can be described quantitatively by determining the fractal dimension (D). It shows the complexity of spatial fractal organization [6].

2. Analysis of the latest research and publications

In 1997, Austrian scientists formed the hypothesis according to which the growth of tumor cells can be described, assuming that these entities are fractals. In 2010, the team of scientists from the US in the Physical Review Letters journal shows that cancer really generates fractal structures [7]. The research was performed on cervical epithelial cells (approximately 300) received from 12 individuals during biopsy, half of which had malignant tumors and half were completely healthy. The images of cells surface (topography) and adhesion maps were obtained using an atomic force microscope. Analysis of fractal behavior of surfaces and their adhesion maps was conducted in 40-300 nm scale. The resulting images were analyzed using mathematical methods to check whether they are fractals and if so, what is their dimension. The surface of normal and tumor cells are fractal-like in the specified range (40-300 nm) but their fractal dimensions are almost similar. The spatial distribution of adhesion force on the surface of normal and tumor cells were also fractal-like and demonstrated significant differences in the fractal dimension values. This parameter allows to distinguish tumor and healthy cells. It makes possible to distinguish normal and tumor cells using the images of an atomic force microscopy [7]. In the other research, scientists of the Max Planck Institute for Intelligent Systems found some differences between the fractal dimensions of two individual cell lines of human pancreatic cancer [8].

3. Aim of the work

The main purpose of the present research is to check the possibility of using the fractal dimension as a marker for sensitivity determining of MCF-line breast cancer cells, exposed in the magnetic field

4. Cell lines and cultivation conditions.

The objects of the study were MCF-line breast cancer human cells sensitive (MCF-7S) and resistant to doxorubicin (MCF-7/Dox). Resistant line was obtained from the original (sensitive) line at the department of mechanisms of anticancer therapies of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine.

Doxorubicin is a drug used in cancer chemotherapy and derived by chemical semisynthesis from a bacterial species [9]. It is an anthracycline antitumor antibiotic closely related to the natural product daunomycin and like all anthracyclines, it works by intercalating DNA, with the most serious adverse effect being life-threatening heart damage. It is commonly used in the treatment of a wide range of cancers, including hematological malignancies (blood cancers, like leukaemia and lymphoma), many types of carcinoma (solid tumors) and soft tissue sarcomas [10].

MCF-7S and MCF-7/Dox cells were cultured in Dulbecco ISCOV (Sigma, USA) culture medium supplemented with 10% fetal calf serum (Sigma, USA) in a humidified atmosphere of 5% CO_2 at 37°C within 24 hours.

Investigation of surface morphology was performed on cytocentrifuged preparations of tumor cells. The drop of supernatant fluid of cell suspension (10^6 cells/ml) MCF-7S and MCF-7/Dox was applied onto a piece of glass for the manufacture of specimens. Slides were placed on a special stand and spun up to speed 900 r/min for 5 minutes.

Obtained preparations were exposed in the magnetic field with the induction of 160 millitesla for 1.5 hours.

5. Research methodology with the application of the images of atomic force microscopy tumor cells.

The atomic force microscopy images obtained using a scanning probe microscope SOLVER PRO-M were used for the researching of the tumor cells morphology (Fig.1, Fig. 2). In this paper, the fractal dimension of the MCF-line breast cancer human cells was counted using the program with the algorithm, based on box-counting method, that is widely used for calculating of fractal dimension of different biological objects [11, 12, 13, 14, 15].

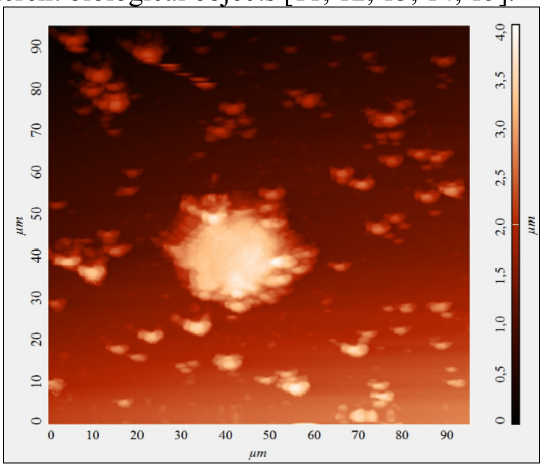

Fig. 1. The topography of the surface of tumor cells of breast cancer MCF-7/Dox, resistant to doxorubicin obtained using atomic force microscopy

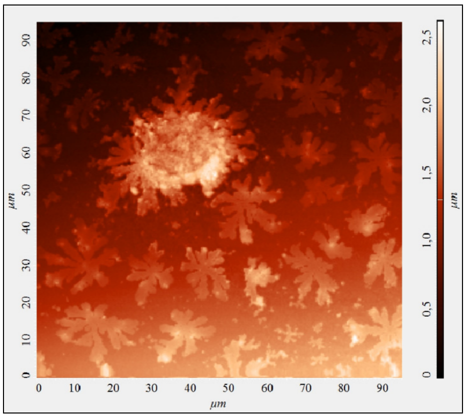

Fig. 2. The topography of the surface of tumor cells of breast cancer MCF-7S, sensitive to doxorubicin obtained using atomic force microscopy

6. Dimension calculation

The blocks of the algorithm of the program for fractal dimension calculation:

- Image extraction procedure;
- loading of the atomic force microscopy tumor cells image in the bmp format;
- determination of the variables for the recalculation of the X,Y and Z Cartesian coordinates of the cell surface in the microns;
- creation of the filter for extraction of the 2D curve determining the cell contour (fig. 3);
- calculation of fractal dimension;
- program testing on the modeling fractal called the Koch's curve.

The program block for determination of cell contour fractal dimension consists of construction of diagram of a logarithm dependence of square box quantity necessary for a cell contour covering to a logarithm of the dimension of such a box. The line diagram of such dependence is a confirmation of the fractal-like structure of the cell contour at the defined scale range (fig. 3).

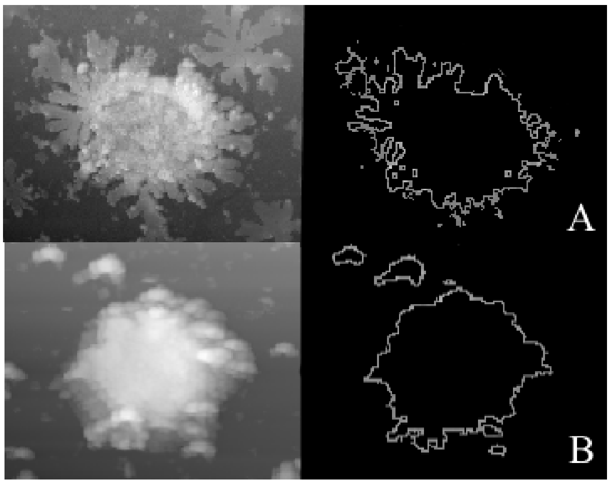

Fig. 3. Extraction of the 2D curve determining the cell contour with a help of the MathCAD program block (A – for sensitive cell, B – for resistant cell)

The formula for fractal dimension calculation is of the form [16]:

$$D = \frac{-\log N(L)}{\log L},$$

where L is the box size required for the cell contour coverage (fig. 3), $N(L)$ is the quantity of the L-size boxes required for the cell contour coverage [16].

7. Results

The received results were represented in a graphic form (fig. 4). The diagram accurately visualizes that the fractal dimension of sensitive cells, exposed in the magnetic field, is greater than the fractal dimension of the resistant cells, exposed in the magnetic field, and the value ranges of this characteristics are not intersected for sensitive and resistant cells, exposed in the magnetic field. It testifies the more "branched" structure of the sensitive cells surface than of the resistant ones.

The mean value of the fractal dimension of the resistant MCF-line breast cancer cells to doxorubicin, exposed in the magnetic field, is <Dr> = 1,160 ± 0,003 and it is <Dnr> = 1,273 ± 0,007 for the sensitive ones.

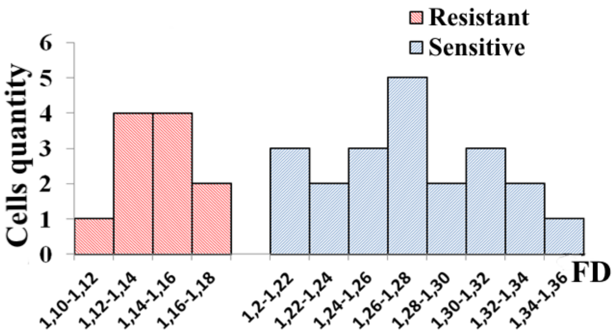

Fig. 4. The density of a cumulative distribution function of resistant and sensitive MCF-line breast cancer cells, exposed in the magnetic field, by their fractal dimension (FD)

8. Conclusion

The conducted research established that the fractal dimension of boundaries of sensitive cells, exposed in the magnetic field, is greater than the fractal dimension of the boundaries of resistant cells, exposed in the magnetic field, and the value ranges of these characteristics for sensitive and resistant cells, exposed in the magnetic field, do not overlap. It testifies the more "branched" structure of the sensitive cells boundaries than of the resistant ones.

Thus, the fractal dimension of the cells, exposed in the magnetic field, can be an additional marker for the determination of their sensitivity to doxorubicin.

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Фрактальна розмірність резистентних та чутливих до доксорубіцину клітин раку молочної залози, експонованих у магнітному полі
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У роботі досліджується фрактальна розмірність резистентних та чутливих до дії доксорубіцину клітин раку молочної залози, експонованих у магнітному полі. Виявлено, що резистентні та чутливі до дії доксорубіцину клітини раку молочної залози лінії MCF мають різне значення фрактальної розмірності. Таким чином, головна мета цього дослідження полягає в перевірці можливості використання фрактальної розмірності як маркеру для визначення чутливості до дії доксорубіцину клітин раку молочної залози лінії MCF, експонованих у магнітному полі.

Ключові слова: атомно-силова мікроскопія; доксорубіцин; магнітне поле магнітно-силова мікроскопія; резистентні та чутливі клітини раку молочної залози лінії MCF; фрактальна розмірність.

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Фрактальная размерность резистентных и чувствительных к доксорубицина клеток рака молочной железы, представляемых в магнитном поле

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В работе исследуется фрактальная размерность резистентных и чувствительных к действию доксорубицина клеток рака молочной железы, представляемых в магнитном поле. Выявлено, что резистентные и чувствительные к действию доксорубицина клетки рака молочной железы линии MCF имеют разное значение фрактальной размерности. Таким образом, главная цель этого исследования заключается в проверке возможности использования фрактальной размерности как маркера для определения чувствительности к действию доксорубицина клеток рака молочной железы линии MCF, представляемых в магнитном поле.

Ключевые слова: атомно-силовая микроскопия; доксорубицин; магнитное поле; магнитно-силовая микроскопия; резистентные и чувствительные клетки рака молочной железы линии MCF; фрактальная размерность.

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