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Fractal dimension of the breast cancer cells resistant and sensitive to doxorubicin activity

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The main purpose of this research is examining and comparing of the fractal dimension (FD) and quantity of biogenic magnetic nanoparticles (BMN) of the resistant and sensitive MCF-line breast cancer cells to doxorubicin activity. The FD of boundaries of sensitive cells is greater than the FD of the boundaries of resistant cells. The quantity of BMN per one cell in sensitive and resistant cells do not differ. The FD of the cell boundaries can be an additional marker for the determination of cell sensitivity to the doxorubicin activity. The part of the tumor cell surface containing one BMN can be also the potential marker for the determination of the sensitivity to the doxorubicin action as well. **Keywords:** fractal dimension; doxorubicin; MCF-line breast cancer cells; BMN.

Основной целью этого исследования является изучение и сравнение фрактальной размерности (ФР) и количества биогенных магнитных наночастиц (БМН) резистентных и чувствительных к действию доксорубицина клеток рака молочной железы линии MCF. В результате проведенного исследования обнаружено, что ФР контура чувствительных клеток больше, чем резистентных клеток. Количество БМН на одну клетку в чувствительных и резистентных клетках практически не отличается. ФР контура клеток может служить дополнительным маркером для определения их чувствительности к действию доксорубицина, как и средняя площадь поверхности клетки, которая приходится на одну БМН.

Фрактальна розмірність резистентних та чутливих до дії доксорубіцину клітин раку молочної залози. *О.В.Медвєдєв, О.Ю.Горобець, В.Ф.Чехун, С.В.Горобець, І.В.Дем'яненко, К.О.Бутенко.*

Основною метою даного дослідження є вивчення і порівняння фрактальної розмірності (ФР) та кількості біогенних магнітних наночастинок (БМН) резистентних і чутливих до дії доксорубіцину клітин раку молочної залози лінії MCF. В результаті проведеного дослідження виявлено, що ФР контуру чутливих клітин є більшою за ФР резистентних клітин. Кількість БМН на одну клітину у чутливих та резистентних клітинах практично не відрізняється. ФР контуру клітин може бути додатковим маркером для визначення їх чутливості до дії доксорубіцину, як і середня площа поверхні клітини, що припадає на одну БМН.

The latest studies in the field of medicine and biotechnology showed the existence of distinctions in morphological properties of the surfaces of normal cells as well as sensitive and resistant to the action of antitumor agents tumor cells [1, 2]. By the end of 1990's it was suggested, that the tumor cells and tumors appear to be "self-similar"

figures in structure called fractals. Fractal dimension is the best characteristic for the quantitative description of the curve or a surface branching and self-similarity properties [3]. In this way fractal-like structures are inherent for the living systems at all levels of their organization (corals, starfishes and sea hedgehogs, flowers and plants, fruits, tops and roots of trees, leaves of plants, vascular system and bronchial tubes of the people and animals etc.) [4]. In general, the fractal is the figure with the small parts comparable to the whole figure in the arbitrary magnification. The term "fractal" was introduced in 1975 by Benua Mandelbrot [3]. The number of studies is devoted to the testing of a hypothesis about fractal properties of the tumor cells [5–8]. It was proved that the fractal dimension could be used in differentiating phases of shoot organogenesis [9] and may be successfully applied for differentiation between anal intraepithelial neoplasia stages [10]. It is detected that normal cells and epithelial tumor cells of the uterine cervix show different fractal dimensions within the nanoscales [6]. The fractal figure was also discovered in the process of measuring of the force with which the probe of an atomic force microscope interacts with its surface in each point of a cell and then the data can be visualized in a form of the specific map [6, 7]. Thus, the dimensions of this fractal are significantly different for normal and tumor cells [6–8]. It was found on the basis of the tumor and normal cells image analysis with application of the atomic force microscopy (AFM) that there is a fractal-like surface of normal and tumor cell in the indicated scale range (40–300 nm) but their fractal dimensions are the same. Space distributions of the adhesive force on the normal and tumor cells surface also turned to be fractal and demonstrated essential divergence in the fractal dimension [11]. That particular parameter helps to distinguish the tumor cells from the normal ones. In this case the term fractal does not mean the surface of the cell but the figure created from the AFM data of the adhesive force on its surface [11]. This fact can give a possibility to distinguish normal and tumor cells by the AFM image.

As a result the problem is actual to find the differences of fractal dimensions of the tumor cells with different biological properties (metastatic potential, migration ability, adhesiveness, sensitivity to the action of anticancer drugs etc.). The possibility of the correlation of fractal dimension with biological properties of cells requires the creation of statistic database of the value ranges of fractal dimensions of tumor cells especially with the different sensitivities to the action of anticancer drugs. However there is an interesting issue if there the value ranges of the fractal dimension are different for the sensitive and resistant to the action of anticancer drugs tumor cells which are calculated on the basis of the analysis of surface topography of the cells, cell adhesion maps etc. There are several geometric characteristics, namely all the intrinsic volumes (i.e. volume, surface area, Euler characteristic, etc.), that can be used to estimate the fractal dimension of sets from digital images [12]. There are also several investigations that proved using of cell contour fractal dimension as a separate cell type characteristic [13–16]. Thereby it was counted the mean value of normal T-lymphocytes contour fractal dimensions (D = 1.20±0.05) [17], CD3 T-lymphocytes (D = 1.20±0.03), CD4 T-lymphocytes (D = 1.16±0.03), CD8 T-lymphocytes (D = 1.23±0.03) and CD19 B-lymphocytes (D = 1.12±0.03) [18]. In this paper, the fractal dimension of the cell contour of sensitive and resistant to doxorubicin breast cancer cells is determined.

The present paper contains the possibility to use the value of the fractal dimension of the cell contour at the subject glass as a potential marker of the resistance to the anticancer drugs unlike the paper [11] in which the fractal dimension of the cell surface does not give us the possibility to distinguish the normal and tumor cells. Specifically, in this paper the fractal dimensions of boundaries of the resistant and sensitive to doxorubicin activity MCF-line breast cancer cells are examined. Doxorubicin is one of the antracyclic antibiotics. It is a cytostatic drug, which has been known from the 1960s. It is manufactured in a semisynthetic way and it is produced by *Streptomyces coeruleorubidus* or *Streptomyces peucetius* microorganisms. Doxorubicin has an anticancer activity and is used in chemotherapy of cancer [19, 20]. The medicine can used for the treatment of such diseases as soft tissue sarcoma, osteogenic sarcoma, Hodgkin's disease and non-Hodgkin's lymphoma, acute lymphoblastic leukemia, acute myeloid leukemia, thyroid cancer, breast cancer, ovarian cancer, bladder cancer, small cell lung cancer, neuroblastoma etc. [12]. The low level of selectivity as well

as primary and acquired in the course of the treatment resistance of tumor cells to the doxorubicin activity are the most principal issues that essentially limit the anticancer chemotherapy efficiency [21, 22].

It has been also established that tumor cells contain the magnetically ordered phase in the form of the BMN [23, 24]. They were detected in different types of the oncological and neurodegenerative diseases such as the breast cancer, ovarian cancer, prostate cancer, Alzheimer's, Parkinson's and Huntington's disease and epilepsy [23, 24]. According to a large number of papers the biogenic magnetic nanoparticles concentration in the tumor tissues is one order of magnitude greater than in the normal ones. One hypothesis of the BMN synthesis in the tumor cells is a metabolic iron disturbance in the organism because the increase of the nontransferrin-bound iron and ferritin concentration is present in cases of neurodegenerative and oncological diseases. But the experimental studies had not detected any correlation between the quantity of BMN and the ferritin in tissues. Furthermore it is established by means of the bioinformatic methods that the ferritin is not obligatory for the biomineralization of BMN. Still there is an open question whether there are distinctions in quantity and space distribution of BMN in tumor cells resistant and sensitive to the action of anticancer drugs. In this paper, therefore the quantity of BMN is examined in resistant and sensitive to doxorubicin activity MCF-line breast cancer cells. Magnetic force microscopy is one of BMN detection methods in the cells [25, 26]. This particular method is used in this paper for the identification of the BMN quantity and their spatial localization in the resistant and sensitive MCF-line breast cancer cells to the doxorubicin activity.

2. Experimental

Cell lines and cultivation conditions. The objects of the study were breast cancer human cells sensitive (MCF-7S) and resistant to the influent of doxorubicin (MCF-7/Dox). Resistant line was obtained from the original (sensitive) line at the department of mechanisms of anticancer therapies of R.Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine [27].

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 h.

Investigation of surface morphology and localization of magnetically ordered phase 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 min.

Research methodology with the application of the images of AFM tumor cells and magnetic force microscopy (MFM). The localization of magnetically ordered phase in the form of the BMN was studied in the tumor cells by means of the scanning probe microscope SOLVER PRO-M [22, 26] in the AFM and MFM modes. The double-scanning semi-contact methodology was used in the SOLVER PRO-M for the investigation of the samples. The AFM image of the topography of the sample surface (AFM mode) was received during the first scanning by a magnetic probe (Fig. 1, 2). This topography is remembered and the phase shift of cantilever vibration is examined characterizing the magnetic dipole interaction of the magnetic probe with the magnetically ordered phase in the examined tumor cells at the constant distance between the probe and the surface sample. BMN can represent separate nanoparticles and/or their clusters in the cells.

In this paper for the first time fractal dimension of breast cancer human cells was applied to determine their sensitivity to doxorubicin. It 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 [17, 18, 28–30].

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

* Image extraction procedure;
* loading of the AFM 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

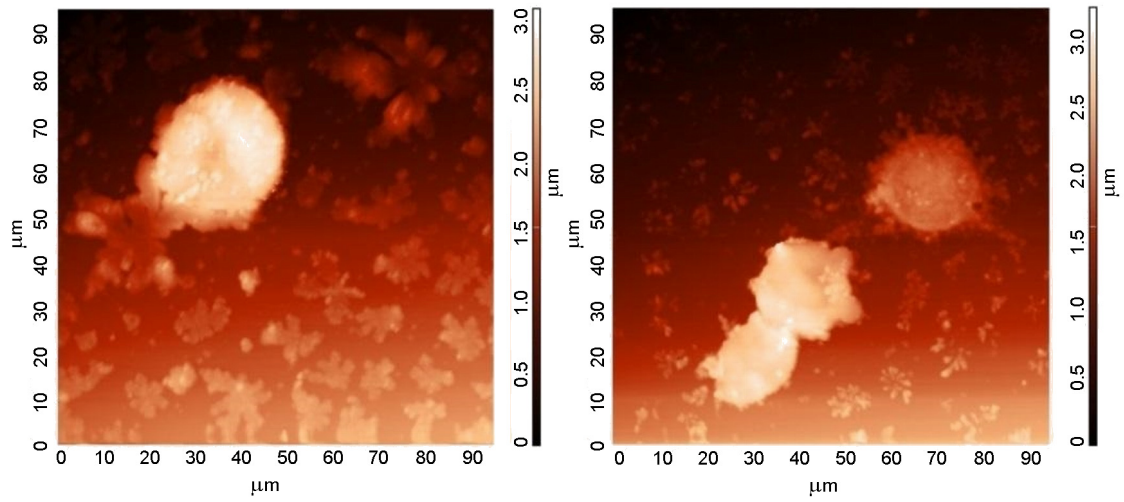

Fig. 1. The topography of the surface of tumor cells of breast cancer MCF-7S, sensitive to doxorubicin obtained using AFM.

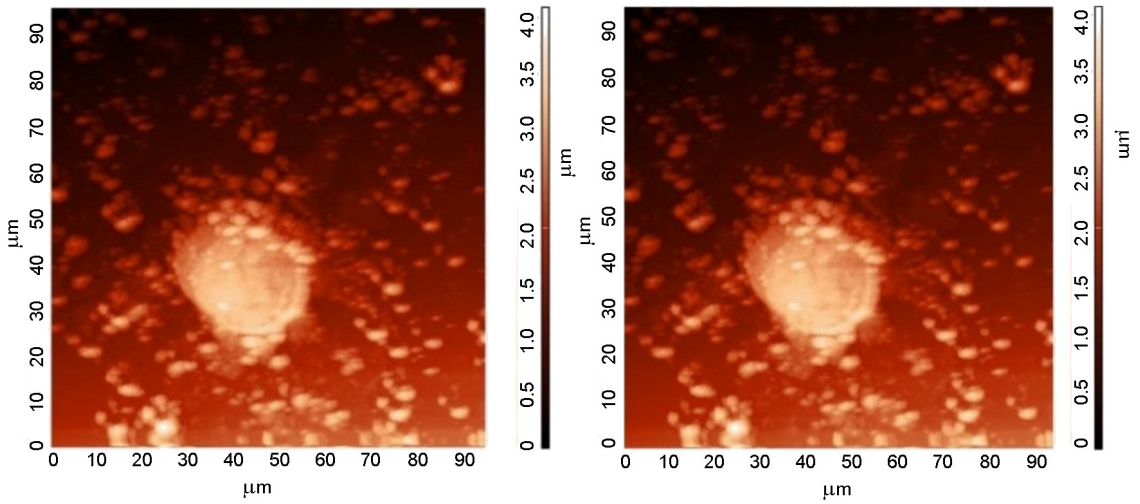

Fig. 2. The topography of the surface of tumor cells of breast cancer MCF-7/Dox, resistant to doxorubicin obtained using AFM.

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) [3].

The formula for fractal dimension calculation is of the form [31]:

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

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 [31].

Just like the tumor cells have a definite fractal dimension value range, so it is necessary to estimate the fractal dimension on the set of data. The fractal dimension calculation is very sensitive to noise in the experimental data especially to the limitations of data amount [31]. Therefore for the fractal dimension calculation was carried out for 30 resistant to the doxorubicin breast cancer cells and for the similar quantity of the sensitive to the doxorubicin MCF-line breast cancer cells (Fig. 4).

30 resistant and 30 sensitive to the doxorubicin MCF-line breast cancer cells were selected for the BMN research. The surface area and the quantity of BMN within each of the chosen cells were calculated. Calculations of the area of the cells were carried out by means of the Gwyddion modular program for image analysis (Fig. 5) [32].

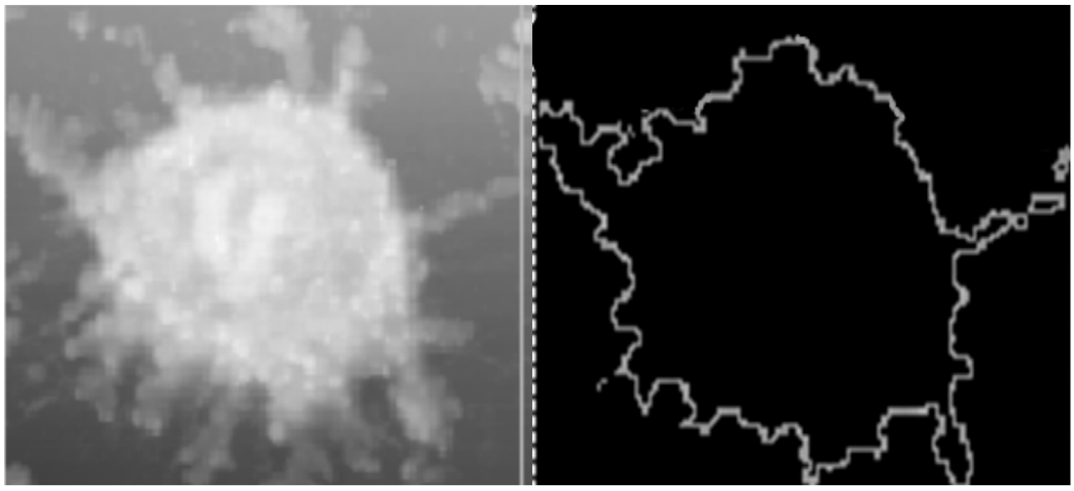

Fig. 3. Extraction of the 2D curve determining the cell contour with a help of the MathCAD program block.

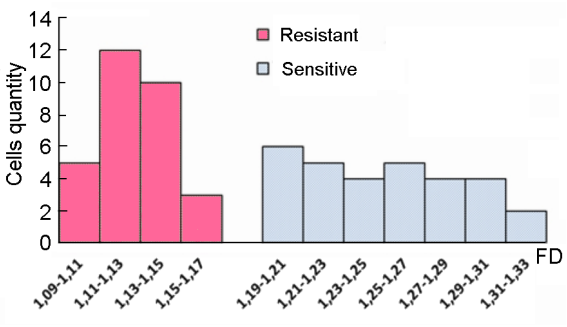

Fig. 4. The density of a cumulative distribution function of resistant and sensitive MCF-line breast cancer cells to the doxorubicin activity by the fractal dimension (FD).

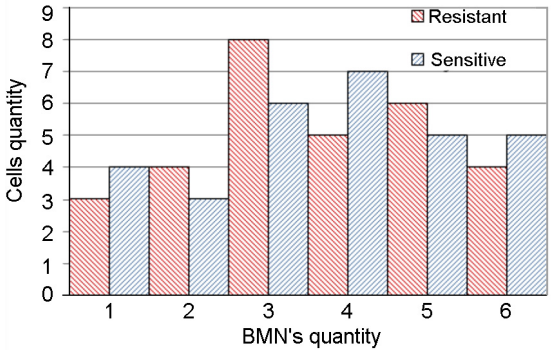

Fig. 5. The density of a cumulative distribution function of resistant and sensitive breast cancer cells to the doxorubicin activity by the amount of BMN.

3. Results and discussion

Results of the calculation of the fractal dimension of the resistant and sensitive to the doxorubicin cancer cells. The received results were represented in a graphic form (Fig. 4). The diagram accurately visualizes that the fractal dimension of sensitive cells is greater than the fractal dimension of the resistant cells and the value ranges of this characteristics are not intersected for sensitive and resistant cells. 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 the doxorubicin activity is $<Dr> = 1.126\pm0.003$ and it is $<Dnr> = 1.250\pm0.007$ for the sensitive ones.

The quantity of BMN in the resistant and sensitive MCF-line breast cancer cells. The received results for BMN quantity in the cells are represented in a graphic form (Fig. 5). The diagram accurately visualizes that the quantity of BMN per one cell does not vary for the resistant and sensitive MCF-line breast cancer cells. Thus the values of the projection areas on the specimen glass are different for the resistant and sensitive MCF-line breast cancer cells.

Additionally, it is calculated that there is one BMN on average per the 0.223 ± 0.004 μm^2 of the cell surface for resistant MCF-line breast cancer cells and one BMN per $0.247\pm+0.003$ μm^2 for sensitive ones.

4. Conclusions

Summarizing the mentioned above it is possible to draw a conclusion that the value ranges of the fractal dimension of boundaries of the resistant and sensitive MCF-line breast cancer cells were calculated and compared. The conducted research established that the fractal dimension of boundaries of sensitive cells is greater than the fractal dimension of the boundaries of resistant

cells and the value ranges of these characteristics for sensitive and resistant cells do not overlap. It testifies the more "branched" structure of the sensitive cells boundaries than of the resistant ones.

The quantity of BMN is also compared in the resistant and sensitive MCF-line breast cancer cells to the doxorubicin activity on the basis of the image analysis received by the MFM method. The quantity of BMN per one cell is not a marker of determination of cell sensitivity to the doxorubicin activity. Though one BMN is accounted for the smaller part of the cell surface for the resistant cells than for the sensitive ones.

Thus, the fractal dimension of the cell boundaries can be an additional marker for the determination of cell sensitivity to the doxorubicin activity. The part of the tumor cell surface containing one BMN can be also the potential marker for the determination of the sensitivity to the doxorubicin action as well.

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