EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Experimental Approaches to Evaluating the Point of Biological System Bifurcation

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Any system transforms into a qualitatively new state if the number of its elements changes (increases or decreases in comparison with the standard checkpoint: norm, previous status of the system, *etc.*) by at least $^1/_3$.

Key Words: *biological system; transition; new status*

The search for a quantitative parameter determining the transition of a system into a qualitatively new state as soon as the quantitative changes in it surpass a critical point is one of the little studied but extremely important problems of biology and medicine. This transition received the name of "bifurcation" in synergetics, while the critical value of parameters, at which the transformation into a new status is possible, was called "bifurcation point" [1]. Threshold mechanisms are intrinsic of all processes [3], and therefore the presence of a certain "critical mass" of cells [2,5] needed for cell population transition from one functional status to a qualitatively different one seems to be a common philosophy determining the growth and development of an organism in health and disease. No direct proofs of the existence of the "threshold" determining the moment when the biological system passes to a qualitatively new level were found. But we hope it will be possible to find it if we regard a multicellular organism as a community of descendants from the same maternal cell (zygote), differing from each other only by the level of functional activity of their genome [9], as the differential activity of the genes determines the function of the cell population. In this case it is essential to know at least the criterion of cell function and the number of cells whose function has shifted from the population function before transition by the moment of this transition.

The aim of our study was to detect the moment of a biosystem transition from one to a qualitatively different state on different model systems.

MATERIALS AND METHODS

The study was carried out on two biological systems. One of them was *Asterias rubens* embryos at early developmental stages (blastula-gastrula-larva), the other were benign and malignant epithelial tumors of the large intestine (normal status→polyp→colorectal cancer) in patients treated at Cancer Research Center.

A. rubens embryos were selected because the differences in the early stages of their development can be easily and accurately identified using this model.

For example, the medium blastula is characterized by immobile dividing embryos, as no cilia are formed yet on their surface. At the late blastula stage, the embryos are mobile and rotate in the membrane due to pulsation of the cilia on the sur-

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face of blastomers. The onset of invagination ("protrusion") of archenteron (primary intestine) is clearly seen at the vegetative pole at the early gastrula stage.

At the late gastrula stage, celomic sacs are pinched from the archenteron, and at the larva stage the formation of eating embryos with oral opening torn through is over.

A total of 6-8 embryos, 100 blastomers analyzed in each of them, were used for each fixation term.

In model 2 impressions of the large intestine biopsy specimens from patients with histologically verified polyposis (n=6) and colorectal cancer (n=6) were analyzed. Control group (norm) consisted of 3 normal subjects.

The preparations were processed by standard histological methods [4]. Functional activity of cell genome [8] was evaluated by the method based on used cytophotometric analysis of cell population with the nuclei stained by Feulgen's method (staining for DNA) and with naphthol yellow S (staining for histones). Cell genome functional activity coefficient (C_{fagen}), *i.e.* histone/DNA ratio after evaluation of optical density of stained nucleus, was proposed as the criterion of cell function. Since DNA-protein complex, in which the DNA is the genetic information carrier and proteins (specifically, histones) perform the structural and regulatory functions [11], is the material basis of the genome, C_{fagen} helps to evaluate the quantitative composition of cell population in this or that status according to functional activity of their genome. The population function is analyzed by comparing the histograms of cell distribution in experiment and control (reference value) by their C_{fagen} values. The C parameter is used for evaluating the deviation of the studied population from the control (reference value). The C parameter is the sum of C_1 and C_2 parameters, where C_1 is the total percentage of experimental cells with a certain C_{fagen} value, surpassing the percentage of control cells in the control histogram, and C_2 total percentage of cells in experiment, whose C_{fagen} value has no analogs in the control.

All measurements were carried out on a Univar microspectrophotometer at λ=575 nm (quantitative evaluation of DNA) and λ=445 nm (evaluation of histone number). Statistical analysis was carried out using Student's t test (p<0.05).

RESULTS

The time course of C_{fagen} and C parameter during transition of *A. rubens* embryos from one developmental stage to another is presented in Table 1. The difference in C_{fagen} values for the early and next stages of the development are statistically significant during transition from late blastula to early gastrula and from late gastrula to early larva. Only in these cases the total C parameter is at least 33%. This fact indicates that the level of the genome functional activity during transition of *A. rubens* embryos to the next developmental stage deviates from that at the previous stage in at least $^1/_3$ of cells.

These results were confirmed in studies of the cell genome function in benign and malignant forms of epithelial tumors of the large intestine. Comparative analysis of the functional activity of cell genome showed that deviation of a cell population from the reference (normal) value is associated with a statistically significant change of the C_{fagen} value in at least $^1/_3$ of cells in the population (Table 2). The differences between C_{fagen} and its normal value were statistically negligible in patients with polyps, the C parameter not surpassing 17.7%. It means that in this case the population did not acquire a qualitatively new status (in other words, more than 80%

TABLE 1. Time Course of C_{fagen} and C Parameter during Transition of *A. rubens* Embryos from One Developmental Stage to Another

Group of comparison	C_{fagen}	C, %
Median blastula — late blastula	1.55 (1.49-1.61)	20
	1.59 (1.53-1.65)	
Late blastula — early gastrula	1.59 (1.53-1.65)	33
	1.85* (1.77-1.93)	
Early gastrula — late gastrula	1.85 (1.77-1.93)	29
	1.95 (1.85-2.05)	
Late gastrula — early larva	1.95 (1.85-2.05)	34
	2.15* (2.01-2.29)	

Note. *Significant in comparison with the previous developmental stage. Here and in Table 2: threshold values of the parameter are shown in parentheses.

TABLE 2. Changes in C_{fagen} and C Parameter in Colorectal Polyps and Cancer

Group of comparison	C_{fagen}	C, %
Norm — polyp	1.64 (1.59-1.69)	17.7
	1.61 (1.55-1.67)	
Norm — cancer	1.64 (1.59-1.69)	41.7
	2.08 (2.01-2.15)	
Polyp — cancer	1.61 (1.55-1.67)	33.0
	2.08* (2.01-2.15)	

Note. *Significant difference compared to previous group of comparison.

cells were close to cells in normal tissue by their function).

The study of cell population in patients with colorectal cancer showed that the biological system (cell population) acquired a qualitatively new status: norm→cancer or polyp→cancer, the transition being associated with changes in the genome function in 41.7% cells for the former case and in 33.0% cells for the latter. These facts indicate that the neoplastic process is caused by transition of the cell population from one qualitative status into another one on condition that $^1/_3$ cells in it are functionally changed.

Our results are confirmed by published data. Study of pathophysiology of the nervous system [6] showed that severe myasthenia associated with disorders in nervous mechanisms of motor regulation is caused by a decrease in the number of acetylcholine receptors (to $^1/_3$ of their normal content) on the membrane of the skeletal muscle fiber terminal plates. The decrease in the number of acetylcholine receptors leads to a decrease in the terminal plate potential, which can drop below the threshold level for stimulation of muscle fiber. The increase in the number of these fibers determines rapid muscular fatigue in myasthenia.

In children with acute lymphoblastic leukemia in remission, the changes in the qualitative composition of bone marrow cell population precede the onset of a relapse [9]. Study of patient' bone marrow cell population by the morphological method (by the percentage of blast cells) showed that the content of cells deviating from the norm by the functional activity of cell genome surpassed 33% (C parameter) 3-13 months before clinical manifestation of a relapse. If the C parameter was below 33%, the patient was in remission during the entire period of observation (4 years).

The results of studies on experimental models of growing tumors [10] also indicate that empirically found 33% value is the measure of transition of a system from one qualitative status to another. An additional proof of this hypothesis are the data obtained in the sphere of molecular biology and physical chemistry of polymers. Studies of DNA structure [12] showed that conformation changes in natural DNA depend on the content of guanine-cytosine (GC) pairs and humidity. At high (95%) humidity only the B form exists, which transforms into A form in low humidity, with an obligatory additional condition (the content of GC pairs should be higher than 30%). Study of physicochemical characteristics of the cholesteric type liquid crystal polymers showed that the cholesteric mesophase structure of copolymers depends on the concentration of cholesteric components [7]. Introduction of few components in the nematic polymer, inducing a spiral supramolecular structure, does not modulate the pattern of location of the lateral mesogenic groups (rigid fragments in the polymer structure). However increase in the content of components by more than 30% leads to alteration of the mesogenic groups packing. Roentgenograms of copolymers containing more than 30% components show the emergence of reflexes in the minor diffusion angles, indicating the formation of lamellar elements (package of components as a lamellar structure). These data are extremely interesting in light of modern concepts on the polymeric structure of DNA as a liquid crystal [7], as they are associated with a possible scheme of formation of the DNA supramolecular structure.

Hence, any system transforms into a qualitatively new state if the content of the elements constituting it changes by some parameters by at least $^1/_3$ in comparison with the standard checkpoint (norm, previous status of the system, *etc.*).

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