The Law of Stage-by-Stage Development of Human Tumors, Derived from the Results of Ploidometric Studies

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The ploidy of 56,000 epithelial cell nuclei was studied at different stages of carcinogenesis by Imager-CG computer image analyzer. The linear relationship between carcinogenesis stages and quantitative parameters (indexes of mean ploidy of cell nuclei) at a certain stage was detected and mathematically described. A step-by-step increase of DNA content in cell nuclei by one unit of ploidy leads to emergence of new biological properties, measured by the number of units surpassing the normal cell nuclear ploidy values, which was called "the malignancy code".

Key Words: *law; tumors; stage-by-stage carcinogenesis; ploidometry; morphological diagnosis*

Classification of tumors based on the three main stages of tumor process development (benign, borderline, and malignant) is essential for clinicians. However, despite wide use of modern methods for histological and cytological diagnosis of cancer and precancer of various organs, the incidence of erroneous conclusions and ambiguous interpretation of the same preparations by different specialists remains rather high [5,6,8,9]. For example, repeated examinations of patients with the diagnosis of "atypical endometrial hyperplasia" revealed well-differentiated endometrial cancer in 8% cases [6], though timely therapeutic intervention depended on the morphological diagnosis.

The importance of this problem necessitates the development of more objective and accurate methods for the differential diagnosis of carcinogenesis stages. It is known that the tumor consists of several cell clones differing by the DNA content in cell nuclei, in other words, by their ploidy value. This "clonal constitution" of tumor tissue is stable, thus determining certain duration of each stage of tumor process development.

It is known that changes in the volume and quality of the genetic material in cell nuclei is the initial and integral sign of tumor development [1,4]. The appearance of clones presented by cells with aneuploid and polyploid nuclei is characteristic of malignant tumors.

Computer-aided microscopy offers the possibility of using the results of quantitative analysis of changes in the content of genetic material in the nuclei (ploidometry method) [4,10] for improving the quality and reproducibility of morphological diagnoses. Published data on DNA content in tumor cell nuclei [10] have not yet been subjected to mathematical simulation for detecting the regularities in the development of the entire carcinogenesis process.

The aim of the present study was mathematical description and simulation of the dynamics of tumor development on the basis of ploidometric data with the aim of detecting the regularities of carcinogenesis.

MATERIALS AND METHODS

The study was carried out on tumors obtained from 418 cancer patients. Using histological and cytological preparations stained by Feulgen's method, the nuclear DNA content was studied in more than 56,000 cells of tumors of the cervix uteri and corpus uteri, mammary, prostatic, and thyroid glands, kidneys, and skin using

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the Imager-CG image analyzer and special ploidometric software [4].

The content of DNA in cell nuclei was expressed in ploidy units (c).

Statistical differences between the studied groups were considered significant only at the 0.95 level of correct conclusion.

RESULTS

The results of statistical processing of measurements of cell nuclear ploidy in analyzed tumors are summed up in Table 1.

The starting point of analysis of the material was evaluation of the content of genetic material in the cell nuclei of normal tissue. The mean DNA level in the germinal zone reached 2.4c. A 0.4 excess of the diploid level indicates a synthetic process and reflects physiological regeneration of tissue.

The following characterization of carcinogenesis can be derived with consideration for the errors in the sampling of cell nuclei in the typical germinal zones of tumors.

The cell nuclei ploidy of hyperplastic tissue and benign tumors did not surpass 3.4c.

The next stage of carcinogenesis is the appearance of the so-called borderline tumors with cell nuclei ploidy of up to 4.4c, after which invasive (growing into adjacent tissues) malignant tumors develop (with nuclear ploidy of 4.5c and higher, with pronounced aneuploidy).

The dedifferentiation of carcinomas from stage I to stage IV augments according to the same pattern. Changes in the malignant tumor structure and cell composition with its progress give grounds to histologically diagnose the tumors as well-, moderately, poorly, and undifferentiated, with the mean nuclear ploidy of these tumors increasing in the following order: 5.4, 6.4, 7.4, 7.4c and more, respectively.

Proliferative activity of tumor cells evaluated by the proposed method is an indirect sign of tumor growth rate and can be obtained without expensive immunohistocytological studies of the preparations.

The rate of tumor cell multiplication increases 6-fold in benign tumors, 11-fold in borderline formations, and 18-fold in malignant tumors in comparison with normal initial tissue. The ratio of growth rates during three stages of tumor progress is 1-2-3.

Based on the mean group parameters, the tumor progress can be theoretically presented as a summary histogram (Fig. 1).

These features of carcinogenesis can be mathematically described by a functional linear relationship between the stage (time of development) and qualitative sign — parametrical characteristics of the mean ploidy of nuclei in the cells predominating during a certain stage of tumor development:

$$y=N+xc,$$

where y is the mean nuclear ploidy in cells at a certain stage of carcinogenesis, N is the mean index of nuclear ploidy in cells of initial normal tissue (2.4c), c is

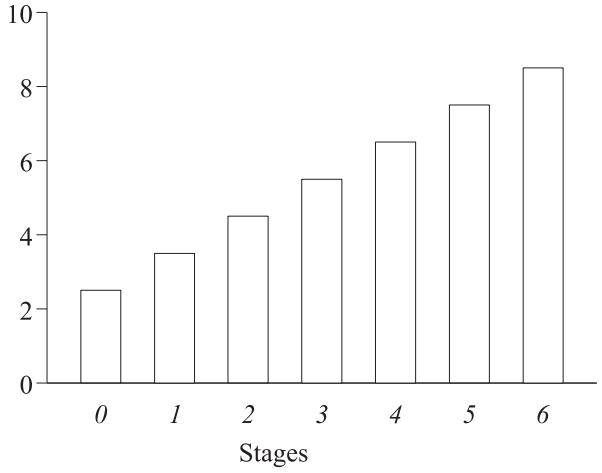

Fig. 1. Theoretical model of step-by-step development of carcinogenesis stages with consideration for the mean content of DNA in cell nuclei. Ordinate: mean ploidy of each carcinogenesis stage and degrees of carcinoma dedifferentiation. *0*: normal tissue; *1*: hyperplasia; *2*: intratissue neoplasm (*in situ* cancer); *3*) carcinomas with 1st degree dedifferentiation; *4*: with 2nd degree; *5*: with 3rd degree; *6*: with 4th-degree dedifferentiation.

TABLE 1. Mean Group Characteristics of Tumor Cell Ploidy at Different Stages of Carcinogenesis for 8 Tumor Types

Diagnosis	Parameter		Multiplicity of proliferative activity increment
	ploidy ($M\pm m$)	proliferative activity	
Normal value	2.20±0.04	0.2	—
Hyperplasia, benign tumors	3.20±0.04	1.2	6
Intraepithelial neoplasm (precancer)	4.2±0.1	2.2	11
Invasive carcinoma	5.6±0.2	3.6	18

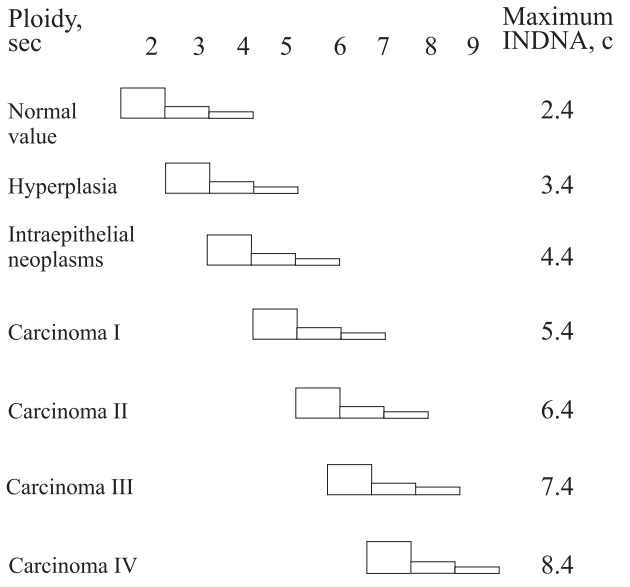

Fig. 2. Theoretical model of step-by-step development of carcinogenesis stages, demonstrating the changes in cell proportions and increase in the total content of DNA (DNA index) in cell nuclei at different stages of tumor development.

ploidy unit, and *x* is the number of the carcinogenesis stage (1-6) with a certain predominating cell clone.

We see from this formula, which can be called the mathematical model of carcinogenesis, that step-by-step development of subsequent tumor stages with new biological properties is characterized by an increase in the mean parameters of tumor cells by one unit of nuclear ploidy. Cell proportions maintaining the status of a certain stage of tumor development have been determined.

Normal tissue contains 70% cells with diploid nuclei, 20% with triploid, and 10% cells with tetraploid nuclei. Stepped histogram is retained for a certain period, after which the cells with nuclei, containing greater content of DNA, multiply. These cells become the predominant clone and determine the emergence of new properties in tumor tissue.

For example, cell proportion described as the normal can shift under certain conditions towards the predominance of cells with triploid nuclei, which will manifest by tissue hyperplasia. However, at this stage the cell nuclei in the growing tissue retain the normal genome and the tumor is benign. During subsequent stages of carcinogenesis the quantitative proportions between the cells are retained, but cells with higher ploidy, with genetic aberrations start to predominate at each stage, which determines the changes in the summary indicator of genetic material quantity: DNA accumulation index ((NDNA; Fig. 2).

With tumor progress, cells with tetraploid nuclei start predominating in the tumor and the mean index of nuclear ploidy reaches the level of 4.4c, which indicates borderline noninvasive status of tissue (precancer). Further increase in the mean ploidy of cell nuclei above 4.5c characterizes all the subsequent invasive stages of tumor development.

The other part of our study consisted in comparison of the data of the suggested theoretical model with the results of actual histocytological observations of tumor development. The increment in the content of genetic material in tumor cell nuclei, presented in the theoretical model, coincided with the data of DNA measurements in cells nuclei of the studied tumors in all cases [1,3,7]. The absence of statistical differences between theoretically expected values and the results of analysis of biopsy and operation material confirmed the correctness of the suggested theoretical model of precancer and cancer development (Table 2).

Hence, the parameters of theoretical mathematical model correspond to experimental results of genetic material measurements in cell nuclei at different stages of carcinogenesis, which validated the law of step-by-step stages of carcinogenesis development in humans (Diploma No. 300 of January 27, 2006).

According to the regularity of step-by-step staged development of tumors of human organs and tissues (Avtandilov regularity), a step-by-step increase in the DNA content of tumor cell nuclei by one ploidy unit leads to emergence of new biological properties, measured by the number of units surpassing the normal cell nuclear ploidy value ("malignancy code") [1,3]. The "malignancy code" equal to one unit indicates a benign tumor, code 2 indicates a borderline (precancer) status, while codes 3-6 indicate a malignant process with progressive reduction of malignant tumor cell differentiation (four cancer stages) [2].

The assumptions of this regularity and the ploidometric diagnostic method are used for the differential

TABLE 2. Stage-by-Stage Carcinogenesis: Ploidometry Data [2]

Stage and degree of process	Ploidy values, c
Normal tissue	<2.4
Hyperplastic tissue, benign tumors	2.5-3.4
Borderline preinvasive tumors: high degree of intraepithelial neoplasia, *in situ* cancer	3.5-4.4
Malignant tumors, with augmenting degree of cell disdifferentiation:	>4.5
I degree	4.5-5.4
II degree	5.5-6.4
III degree	6.5-7.4
IV degree	>7.5

diagnosis between noninvasive *in situ* cancer and well-differentiated carcinoma.

In order to improve the efficiency of histocytological studies, in future the assumptions of the regularity should be used for not only differential diagnosis of cancer/precancer of different location, but also for objective expert evaluation of the quality of morphological conclusions, for planning surgical and therapeutic interventions in cancer patients.

REFERENCES

1. G. G. Avtandilov, *Vestn. Rossiisk. Acad. Estestv. Nauk*, **5**, No. 3, 65-69 (2005).
2. G. G. Avtandilov, *Radioelectronics in Medicine* [in Russian], Moscow (2005), pp. 9-10.
3. G. G. Avtandilov, *Ploidometry Methods for the Diagnosis of Tumor Development Stages. Methodological Recommendations* [in Russian], Moscow (2005).
4. G. G. Avtandilov, *Diagnostic Medical Ploidometry* [in Russian], Moscow (2006).
5. D. I. Golovin, *Errors and Difficulties in Histological Diagnosis of Tumors* [in Russian], Leningrad (1982).
6. N. M. Khmel'nitskaya, E. L. Neishtadt, and Z. K. Khalimdzhanov, *Arkh. Patol.*, No. 6, 39-42 (2006).
7. G. G. Avtandilov, *Virchows Arch.*, **448**, No. 5, 702 (2006).
8. E. Foucar and M. K. Foucar, *Error in Anatomical Pathology*, Washington (2001).
9. L. L. Leape, *JAMA*, **272**, No. 23, 1851-1857 (1994).
10. R. G. Steinbeck, *Acta Oncol.*, **36**, 3-10 (1997).