ONCOLOGY

Change in the Ploidy of Prostatic Epitheliocyte Nuclei during Carcinogenesis

G. G. Avtandilov and L. V. Gundorova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 1, pp. 105-107, January, 2005
Original article submitted October 20, 2003

Comparative computer-assisted microspectrophotometric analysis of histological preparations showed that the mean nuclear ploidy increased 1.3 times in glandular hyperplasia and 2-fold in adenocarcinoma of 3 progressive degrees of dysdifferentiation. Proliferative activity of cells also increased from stage to stage. The data visualize cell nuclei ploidy and facilitate objective differential diagnostic decision making.

Key Words: *prostate; ploidy; carcinogenesis*

Increasing incidence of malignant prostate neoplasms necessitates improvement of diagnosis of stages of carcinogenesis associated with imbalance between proliferation and differentiation processes. Apart from benign adenoma and prostatic cancer, prostatic tumors include of low- and high-degree intraepithelial neoplasia (PIN-I and PIN-II) [8]. However, there is no uniform definition of tumor degree, which necessitates additional specific studies with oncomarkers [4] and evaluation of DNA content in interphase cell nuclei [1-3]. DNA is exponentially accumulated in cell nuclei during malignant transformation [5]. Qualitative evaluation of the process by histograms of nuclear ploidy distribution in tumor cell nuclei is also important for the diagnosis of carcinogenesis stages,

Four types of histograms are distinguishes according to classification [8]: type I — peak in the "diploid region" (1.5-2.5 s); the content of nuclei with ploidy higher than 2.5 s should not exceed 10% of all studied nuclei; type II — peak in the "tetraploid region" (3.5-4.5 s) and peak in the "diplod region". These two peaks should contain almost 90% of all examined nuclei, the ploidy of remaining nuclei >4.5 s; type III — the same as the previous, but with predominance of the tetraploid region in the distribution and <5% nuclei with ploidy >4.5 s; and type IV — is characterized by the presence of aneuploid nuclei, *i. e.* the ploidy of more than 5% nuclei surpasses 4.5 s.

According to another classification [11], tumor histograms are subdivided into 3 types: D type with half of nuclei in the diploid and tetraploid regions of the histogram (modal class is the diploid one); T type, the same as D type, but with the tetraploid region as the modal class; and A type with more than half of the nuclei beyond the diploid and tetraploid regions.

These classifications are not sufficiently substantiated from the viewpoint of morphological statistical analysis, because they neglect the mean values of the entire sampling of the objects [2].

We studied changes in the prostatic epitheliocyte nuclear ploidy at different stages of carcinogenesis.

MATERIALS AND METHODS

Histological study of biopsy specimens and surgery material of prostates from 73 patients was followed by computer-assisted microspectrophotometric analysis of tumor cell nuclei stained by the method of Feulgen on histological sections (8 μ) [10]. Comparative microspectrophotometry was applied [1-3].

Russian Medical Academy of Postgradual Education, Moscow. ***Address for correspondence:*** avtand@e-mail.ru. G. G. Avtandilov

0007-4888/05/1391-0091

Cell nuclei (n=1332) were measured on a Imager-CG PC image analyzer (with Avtan-San software version for ploidometry). The device included Axioscop microscope (Carl Zeiss) with plan objective 40×/0.65, 570 nm photofilter, gray scale TV camera, and PC. Integral brightness of the nuclei (magnification 400) reflecting their DNA content was measured. In order to define "tissue ploidy standard", the mean integral brightness of minor lymphocyte nuclei in histological sections was evaluated, which corresponded to double chromosome set (2 s) [6]. The data on the nuclear ploidy of the examined prostatic tumor cells were obtained automatically by dividing the integral brightness of their nuclei by half of the standard. The results of measurements were presented on the monitor and histograms by pseudocolors by the degree of nuclear ploidy [3].

The measurements were carried out in the following sites of the preparation: with normal structure of the gland and with signs of epithelial hyperplasia, with symptoms of slight dysplasia (PIN-I), with signs of PIN-II, in sites of infiltrating prostatic adenocarcinomas with different degree of dysdifferentiation.

Proliferative activity of cell nuclei was evaluated by the excess of the mean nuclear ploidy above the diploid value [3].

The data were processed statistically. Arithmetic means (M), weighed arithmetic means (DNA accumulation index; $\bar{x}$), mean square deviations (σ), sample errors (m), values at the interface of transition into the neighboring diagnostic groups (at 0.5 probability, σ=0.67) were used. The differences were considered significant at 0.95 confidence level.

RESULTS

Half of the mean value of integral brightness of 396 minor lymphocytes in prostatic sections was takes for standard tissue ploidy (1 s). Variability of measurements did not surpass 2%.

The mean ploidy of typical cell clones in benign tumors differed significantly from that of malignant tumors (Table 1). The mean normal epitheliocyte nuclear ploidy increased 1.07 times in hyperplasia, 1.32 in PIN-1, 1.53 in PIN-II, and in adenocarcinomas of three progressive degrees of dysdifferentiation 1.92, 1.96, and 2.4 times, respectively, in comparison with the mean nuclear ploidy of normal epithelium.

When the mean weighed ploidy values of interphase nuclei (DNA accumulation indexes) were compared, these differences were more expressed and /for the same groups were 1.35, 1.57, 1.71, 2.21, 2.32, and 2.46, respectively.

Proliferative activity of cells increased with malignant degeneration of prostatic epithelium (Table 1).

TABLE 1. Diagnostic Parameters of Ploidy of the Main Clone of Prostatic Epitheliocytes at Different Stages of Carcinogenesis

Histological diagnosis	n	Mean nuclear ploidy, s			DNA accumulation index			Proliferative activity of cell nuclei
		$M\pm m$	M-0.67 σ	M+0.67 σ	$\bar{x}\pm M$	M-0.67 σ	M+0.67 σ	
Normal tissue	124	2.80±0.05	2.4	3.2	2.80±0.05	2.4	3.2	0.8
Hyperplasia	480	3.00±0.04	2.4	3.6	3.80±0.05	3.4	4.2	1.0
PIN-I	122	3.70±0.09	3.1	4.2	4.4±0.1	3.7	5.1	1.7
PIN-II	153	4.30±0.12	3.4	5.2	4.8±0.1	3.8	5.8	2.3
Adenocarcinoma								
well-differentiated	123	5.40±0.01	4.6	6.2	6.20±0.12	5.3	7.1	3.5
moderately differentiated	307	5.50±0.08	4.6	6.2	6.50±0.09	5.5	7.5	3.5
poorly differentiated	147	6.7±0.1	5.9	7.5	6.9±0.1	6.1	7.7	4.7

On the basis of the analysis of histogram of cell nuclei distribution by ploidy, we proposed the following classification of histograms: type I — predominance of the paradiploid nuclei modal class (1.5-2.5 s) — normal physiological regeneration of epithelial cells; histogram shift to the right till 2.5-2.9 s ploidy; type II — benign tumors with predominance of nuclei with 3.0-3.4 s ploidy, characterizing PIN-I; type III — borderline states with predominance of the histogram region 4.0-4.4 s, reflecting PIN-II; type IV — malignant tumors (carcinomas of different dysdifferentiation degree), regions with values above 4.5-4.9 s; pronounced aneuploidy.

Additional results of histological studies given by PC ploidometry visualize the cell nuclei ploidy and help to make objectively based decisions for differential diagnosis of developmental stages of prostatic tumors and tumors of other location.

REFERENCES

1. G. G. Avtandilov, *Problems in the Pathogenesis and Pathoanatomical Diagnosis in the Morphometry Aspects* [in Russian], Moscow (1984).
2. G. G. Avtandilov, *Computer-Aided Microtelephotometry in Diagnostic Histocytopathology* [in Russian], Moscow (1996).
3. G. G. Avtandilov, L. V. Gundorova, and K. B. Saniev, *Urologiya*, No. 3, 8-11 (2002).
4. A. S. Belokhvostov and A. G. Rumyantsev, *Oncomarkers. Molecular Genetic, Immunochemical, Biochemical Analyses* [in Russian], Moscow (2003).
5. *Regularity of Exponential Accumulation of DNA in the Nuclei of Stem Population Cells in Dysplasias and Malignant Tissue Growth in Humans* [in Russian], Diploma No. 68, *Nauchnye Otkrytiya*, Moscow, St. Petersburg (2000), pp. 64-65.
6. *Molecular Clinical Diagnosis. Methods*, Eds. S. Hurrington *et al.* [in Russian], Moscow (1999).
7. *WHO Classification of Prostatic Tumors*. Geneva (1993).
8. G. Auer, T. Caspersson, and A. Wallgren, *Analyt. Quant. Cytol.*, **3**, 161-165 (1980).
9. D. G. Bostwick and M. K. Brawer, *Cancer*, **59**, 788-794 (1987).
10. R. G. Steinbeck, *Mitotic Failure and Genome Stability in Benign, Premalignant, and Malignant Human Tissues*, Stockholm (1998).
11. A. Zettenberg and P. L. Esposti, *Scand. J. Urol. Nephrol.* **55**, 53-58 (1980).