Epidermocyte Nuclear-Nucleolar System in Atopic Dermatitis and Lichen Planus

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 9, pp. 352-354, September, 2007
Original article submitted July 2, 2007

Study of the epidermocyte nuclear-nucleolar system in atopic dermatitis and lichen planus by computer morphometry of preparations stained with 50% $AgNO_3$ showed an increase in their morphometric parameters, reflecting hyperregeneratory processes.

Key Words: *atopic dermatitis; lichen planus; epidermocytes; nuclear-nucleolar system; computer morphometry*

The status of the nucleolar system is an adequate indicator of the protein-producing function of cells. In some cell populations, activity of the nucleolar system reflects activity of proliferative processes [5,6].

Hyperregeneratory reaction is characteristic of skin areas involved in pathological processes in atopic dermatitis (AtD and lichen planus (LP) [4]. The intensity of hyperregenerative process is usually evaluated by ^{3}H-thymidine autoradiography or by immunohistochemical methods (Ki-67, PCNA antigen). Computer morphometry opens new vistas for morphological characterization of cell populations.

We studied the nuclear-nucleolar system in epidermocytes involved in hyperregeneratory processes in AtD and LP.

MATERIALS AND METHODS

Biopsy specimens of 7 patients with AtD and 7 patients with LP were studied. Biopsy specimens from 7 patients without skin diseases served as the control. After 24-h fixation in 10% neutral formalin, the specimens were embedded in paraffin and 6-μ sections were made on a Leuca microtome. Deparaffinized sections were stained with 50% $AgNO_3$ [3]. Morphometric parameters of the cell nuclear—nucleolar system were measured using Mekos computer image analysis system. About 50 nuclei of the basal and prickle-cell layers were measured in each case.

The results were statistically processed using Statistica 5.0 software. The significance of differences between the means was evaluated using Student's t test. The most significant of the 15 morphometric signs were determined by multifactorial analysis by distinguishing the main components at their significance of >0.7. K-means clustering algorithm was also used. Mathematical simulation was carried out on the basis of the main components using the discriminant analysis [1,2].

RESULTS

Cell nuclei in the germinative zone (basal and parabasal layers) of stratified squamous epithelium looked enlarged in AtD in comparison with the control. The nuclei in this zone were also visually enlarged. A similar picture was observed in the germinal zone of stratified squamous epithelium in LP.

Multifactorial analysis showed that the number of nucleoli, nuclear area, summary area of the nucleoli, and the nucleolar/nuclear ratio were the main components among the 15 studied morphometric parameters of the cellular nucleolar-nuclear system

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0007-4888/07/1443-0376

TABLE 1. Morphometric Parameters of the Nuclear-Nucleolar Systems in Cells of the Basal and Prickle-Cell Layers of Stratified Squamous Epithelium in Controls and Patients with AtD and LP

Group	Layers	Number of nucleoli	Area of nuclei, μ²	Area of nucleoli, μ²	Nucleolar/ nuclear ratio
Control	Basal layer	1.44±0.12	30.43±0.96	4.57±0.54	0.151±0.016
	Prickle-cell layer	1.61±0.30	42.20±8.54	5.15±1.32	0.123±0.014
AtD	Basal layer	1.93±0.32*	44.00±4.56*	5.71±0.92*	0.130±0.013*
	Prickle-cell layer	1.56±0.34	61.58±16.07*	8.20±2.42*	0.136±0.022
LP	Basal layer	1.89±0.35*	44.19±4.23*	5.74±0.98*	0.131±0.014*
	Prickle-cell layer	1.61±0.31	62.16±11.12*	8.18±2.22*	0.130±0.022

Note. *$p<0.05$ compared to the control.

in the basal and prickle-cell layers, their sum surpassed 81.68% dispersion of all morphometric signs. Therefore these parameters were used for statistical analysis.

The number of nucleoli, nuclear area, and summary area of the nucleoli increased in the basal layer nuclei in AtD, while the nucleolar/nuclear ratio decreased. All parameters differed significantly from the control ($p<0.05$). In LP, the number of nucleoli in the basal layer nuclei, nuclear area, and nucleolar area were also significantly higher than the corresponding parameters in the control group ($p<0.05$), while the nucleolar/nuclear ratio in the basal layer was significantly below the control. No appreciable differences in the morphometric parameters of the nuclear-nucleolar system of the basal layer cells of the stratified squamous epithelium in AtD and LP were detected.

The area of nuclei in prickle-cell layer and the summary area of the nucleoli in AtD and LP were significantly higher than in the control ($p<0.05$). The number of nucleoli and the nucleolar/nuclear ratio did not differ from the control (Table 1). No appreciable differences in the morphometric parameters of the nuclear-nucleolar system of the prickle layer cells in the stratified squamous epithelium in AtD and LP were revealed either.

Mathematical simulation on the basis of discriminant analysis showed that correct summary classification of morphometric images of all three studied clinical morphological forms was 71.43%, the intact stratified squamous epithelium of controls was recognized with 100% reliability. Stratified squamous epithelium of the skin in AtD and LP were differentiated by discriminant analysis far worse.

Computer database of the values measured in 1012 nucleolar-nuclear systems of the basal layer cells in all patients by the cluster and discriminant analyses was distributed into 5 groups. The number of groups was chosen empirically, so that it was sufficiently high, but the discrimination of values of the nuclear/nucleolar parameters of cells was not very fractional (the values in the neighboring groups differing from each other significantly). The first cell discrimination was carried out on the basis of K-means clustering of the summary nucleolar area, and its results were then used for discriminant division of cells by 4 morphometric signs. Each group represented a totality of cells of certain morphotype with close values of morphometric parameters. Analysis of morphometric parameters showed that the areas of nuclei and nucleoli differed negligibly for morphotypes 1-3. The areas of nuclei and nucleoli were significantly larger in the nuclei of morphotypes 4 and 5 (Table 2). The index of correct summary classification of morphotypes by discriminant analysis was 96.24%.

TABLE 2. Morphometric Parameters of the Nucleolar-Nuclear System of Cells of Different Morphotypes in the Basal Layer Epithelium of the Skin

Morphotype	Number of nucleoli	Area of nuclei, μ²	Area of nucleoli, μ²	Nucleolar/nuclear ratio
1	1.27	30.36	2.55	0.089
2	1.56	34.00	4.19	0.132
3	1.91	40.96	6.16	0.164
4	2.44	53.88	8.79	0.169
5	2.75	58.16	11.54	0.202

TABLE 3. Percentage of Nuclei of Different Morphotypes in Basal Layer of Stratified Squamous Epithelium of the Skin in Controls and Patients with AtD and LP

Group	Morphotype				
	1	2	3	4	5
Control	30.36	38.52	21.17	7.91	2.04
AtD	19.74	30.74	23.30	18.45	7.77
LP	17.04	33.76	24.76	17.04	7.40

The nuclei of morphotypes 1-5 were detected in the basal layer in the control group. The percentage of morphotype 4 nuclei was 7.91% and morphotype 5 nuclei 2.04%. In AtD the percentage of morphotype 4 nuclei was 18.45% and morphotype 5 nuclei 7.77%. In LP the percentage of morphotype 4 nuclei in the basal layer was 17.04%, of morphotype 5 7.40% (Table 3).

Analysis of correlations of the morphometric values of the nuclear-nucleolar system measured in this study and previous reports on proliferative activity [4] (according to Ki-67) showed high positive correlation. Pearson's coefficient of correlation between the summary area of the nucleoli and proliferative activity index was 0.78, between the percentage of morphotypes 4 and 5 nuclei 0.87-0.88.

It seems that activation of the nucleolar system in AtD and LP can serve as the indicator of hyperregeneratory reaction.

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