

Research Article

Epigenetic Regulation of Gene Activity in Epithelial Cells of Nasal Mucous Membrane in Patients with Polypous Rhinosinusitis

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Abstract

Nowadays, a wide clinico-laboratory polymorphism of "polypous rhinosinusitis" is observed. It suggests the potential role of heredity in the formation of the disease indicating the necessity of studying the role of genetic factor in the formation of various clinico-pathogenic variants of polyposis in detail.

The objective of the research was to study the degree of functional abnormalities in the epithelial cell genome of the nasal mucous membrane in patients with polypous rhinosinusitis using the cytogenetic methods.

Materials and methods. The article presents the results of cytogenetic study of 70 patients with various types of polypous rhinosinusitis (aspirin-intolerant and allergic). Hereditary predisposition to the disease was determined applying clinical and genealogical analysis.

Results. Significant differences in the quantitative characteristics of the functional state of the nasal epithelial cell genome by the criterion of chromatinization indices, the nucleolar index, the indices of the heteropyknotic X chromosome and pathologically altered nuclei were found in patients with aspirin-intolerant polypous rhinosinusitis as compared to those with allergic polypous rhinosinusitis as well as the control group. The identified changes serve as a criterion of the reduction in the activity of the transcriptional-translational processes in aspirin-intolerant polypous rhinosinusitis.

Conclusions. The studied changes in the parameters of the functional state of the epithelial cell genome in the nasal mucous membrane provided an objective confirmation of hypothesis about epigenetic nature of pathology formation.

Keywords

aspirin-intolerant polypous rhinosinusitis, epigenetic regulation

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Problem statement and analysis of the recent research

The study of the problem of polypous rhinosinusitis (PRS) including aspirin-intolerant PRS points out the fact that specific signs of the given pathology are similar to those of polygenic (formerly known as genetically determined) or multifactorial diseases: a high incidence of the disease among the population; clinical polymorphism varies from latent forms to pronounced manifestations; the features of disease inheritance do not correspond to Mendel's laws; the degree of disease severity depends on the patient's age and gender, unfavorable factors of the internal and external environment [1]. PRS is known to be characterized by a wide polymorphism of clinical manifestations, namely different variations of the clinical course, histological characteristics, the number of the affected sinuses, the presence of subclinical and atypical forms, the different frequency of recurrence and susceptibility to conventional treatments. The variability in clinical and laboratory as well as immunological parameters is observed as well [14, 26]. Such clinical diversity cannot be explained by the influence of the external factors only; it indicates that the development

of the disease may be associated with individual characteristics of a macroorganism in the determination of which the participation of genetic factors is mandatory [2, 3].

In clinical medicine, there is a number of genetically determined diseases associated with chronic PRS. They are related to a direct defect in the genes encoding certain enzymes (Kartagener syndrome, Young syndrome, ciliary dyskinesia) or their absence (mucoviscidosis) that determines their specific symptoms [4, 16, 18, 20, 22]. In the context of the studied pathology one can talk about genetically determined defect of cyclooxygenase being the key enzyme of the arachidonic acid metabolism. The role of heredity in the formation of aspirin-intolerant PRS has been proven by several studies [7, 25]. There was found the reduction in the activity of cyclooxygenase in the stromal tissue of the mucosa and polyps in such patients as well [6, 27, 28].

However, despite large cohort studies including several thousand patients, the defective gene of aspirin intolerance has not been found yet [17, 24, 29].

Molecular mechanisms of enzyme structure formation are known to be associated with the regulatory gene function [19].

The formation of protein molecules as well as the maintenance of their appropriate activity state is an integral part of proper functioning of physiological processes mediated by them. The alterations in these mechanisms result in the formation of forms which significantly differ in both structure and functional activity [9, 15, 21]. The genetic apparatus is not involved. Since the mechanisms of the aforementioned abnormalities are caused by the modification of gene expression without changes in the genome, they are called epigenetic [5, 29]. Nowadays epigenetic modification of the genome is considered as changes in gene function which are inherited but do not involve nucleotide sequence abnormalities. Epigenetic modifications of gene activity in patients with PRS have received almost no attention. From this perspective, in section 9 – “Research Needs and Search Strategies”, EPOS-2012 raised a series of issues including how to understand epigenetic regulation of the upper respiratory tract diseases [23].

At the current stage of science development, several epigenetic phenomena including chromatin state, X-inactivation, the state of the nucleolar apparatus and pathologically altered nuclei are identified [10, 13]. Since traditional methods of clinical study do not allow identifying the depth of morpho-functional abnormalities in nasal epithelial cells in PRS, the use of the cytogenetic method is logical. Epithelial cells of the nasal mucous membrane (NMM) are the most appropriate model to study the role of genetic component in the formation of PRS [8, 12].

The objective of the research was to study the degree of functional abnormalities in the epithelial cell genome of the NMM in patients with polypous rhinosinusitis using the cytogenetic methods.

1. Materials and methods

The study included 70 patients with PRS. Hereditary predisposition to the disease was found in all the patients applying the method of clinical and genealogical analysis. Genetically predisposed patients were divided into 2 groups: Group I included 27 patients with hyper-Ig-E-dependent polyposis; Group II comprised 43 patients with aspirin-intolerant polyposis. Cytological preparations of epithelial cells of the NMM served as the study material. The preparations were made according to the methods proposed by Kovalchuk LYe, et al [11]. The results obtained when examining 30 healthy individuals were used as control ones.

The functional state of the genome (FSG) of the interphase nuclei of epithelial cells in the nasal cavity of each patient was studied using epigenetic phenomena including chromatinization indices (CI), the index of the heteropyknotic X chromosome or sex chromatin (SC), the nucleolar index (NI) and the index of pathological nuclei (PN). The FSG was assessed after the observation of 100 cells (the nuclei) of each studied patient.

2. Results and Discussion

We focused on characteristics of hereditary apparatus indicating the activity of gene expression in the NMM cells. Cell functioning is known to be caused predominantly by physical mechanisms of progressive chromatin condensation, the ratio of chromatin compaction to chromatin decompaction. Modern molecular genetic analysis of heterochromatin allows identifying evolutionarily conservative ensembles of proteins being involved in heterochromatinization - transition to inactive closed state of interphase chromatin. It is accompanied by the inhibition of gene expression. Therefore, when determining the FSG of epithelial cells in the NMM of patients with PRS the analysis of CI was primarily made – the ratio of the nuclei with predominant active euchromatin to those with predominant inert heterochromatin (Table 1).

Changes in the CI were observed in all the studied patients. In patients with hyper-Ig-E-dependent PRS, an unreliable tendency toward reduction in the CI was found; the CI was 0.83 ± 0.01 c.u. in females and 0.87 ± 0.05 c.u. in males ($p > 0.05$ in comparison with healthy individuals regardless of gender). In patients with aspirin-intolerant PRS, there was observed a reliable tendency toward reduction in the CI with more pronounced dynamics in females – 0.72 ± 0.04 ($p \leq 0.05$) and 0.73 ± 0.06 in males, respectively, ($p = 0.05$). The dynamic state of this indicator proved that it is not associated with the disruption of DNA structure; it is caused by a special chromatin state. Epigenetic mechanism which controls gene activity occurs due to changes in chromatin structure that are closely related to DNA methylation. Methylated DNA fragments may repel proteins activating the gene and, vice versa, attract other proteins being involved in tight packaging of the modified gene due to changes in chromatin structure. The latter depends on the interaction of DNA and proteins (predominantly histones) which cause the dynamics of chromatin compaction-decompaction. The mechanisms of the latter are directly related to the repression-derepression of the genes which are localized there; a particular class of human diseases caused by structural defects as well as the modification of chromatin has been singled out – “chromatin diseases”, Rett syndrome in particular. In addition, deacetylation of nucleosomal histones as well as their methylation is an essential part of gene repression. It alters chromatin structure increasing the degree of its compaction which leads to the repression of the genes localized in this chromatin fragment. Such mechanism, in our opinion, is the basis of the reduction in the CI (the reduction in the number of the nuclei with predominant euchromatin) in patients; it characterizes the degree of biological processes which are responsible for the reduction in the ability of DNA to replicate and transcribe depending on hereditary predisposition.

At the same time, the number, volume and size distribution of the nucleoli serve as a stable morphological reflection of the transcription of ribosomal genes localized in the nucleolar organizers. Therefore, the next stage of the FSG assessment included the determination of the NI. Functional activity of the

Table 1. Indicators of the chromatization index of epithelial cells in the nasal mucous membrane of patients being genetically predisposed to polypous rhinosinusitis (M±m)

Indicators	Hyper-Ig-E	Aspirin	Healthy individuals	p ¹	p ²	p ³
Males	0.87±0.05	0.73±0.06	0.91±0.03	p>0.05	p>0.5	p=0.05
Females	0.83±0.01	0.72±0.04	0.89±0.02	p>0.05	p>0.05	p>0.05

Note.

p¹ – statistical significance of differences between hyper-Ig-E-dependent and aspirin-intolerant polypous rhinosinusitis;

p² – statistical significance of differences between patients with hyper-Ig-E-dependent polypous rhinosinusitis and healthy individuals;

p³ – statistical significance of differences between patients with aspirin-intolerant polypous rhinosinusitis and healthy individuals.

The difference is statistically significant at p≤0.05

nucleolar apparatus in patients with PRS was characterized by dynamic changes (Table 2).

In patients with hyper-Ig-E-dependent PRS, an insignificant tendency toward reduction in the NI was found; the NI was 9.95±0.90 in females and 11.09±0.63 in males (p>0.05 in comparison with healthy individuals regardless of gender).

The indicators of the NI reduced especially active in aspirin-intolerant PRS – to 8.98±0.46 in males and to 8.92±0.66 in females (p<0.05 in comparison with healthy individuals and patients with hyper-Ig-E-dependent PRS regardless of gender). Such result indicated the inhibition of polypeptide synthesis; it was consistent with the reduction in the amount of chromatin being accompanied by reduced gene expression as well as iRNA synthesis. It pointed out the inhibition of cellular metabolic activity.

Even in moderate activity of the transcription-translation apparatus, compensatory mechanism can reach a sufficient level if the genes necessary for cell function derepress. Therefore, the determination of facultative heterochromatin – SC is an integral part of assessing metabolic information flow in cells (Table 3). The concept of genic balance indicates that proper organism functioning requires the coordinated action of the complete set of the genes which are controlled by regulatory nucleotide sequences localized mainly in the X chromosome. Therefore, the assessment of SC indicators depends on gender.

The regulatory function of the heteropyknotic X chromosome sites in women reduced significantly as compared to the control group (28.39±0.83) - by 1.4 times (to 19.62±0.99) in hyper-Ig-E-dependent PRS and by 1.64 times (to 17.08±0.92) in aspirin-intolerant PRS (p<0.001 in both cases).

The alterations in optimal mechanisms of controlling differential gene activity were observed in the studied men as evidenced by the increase in the index of SC, i.e. the repression of regulatory loci of a single X chromosome from normal 2.76±0.13 to 4.01±0.36 in patients with hyper-Ig-E-dependent PRS (p<0.01), and to 5.82±0.51 in patients with aspirin-intolerant PRS (p<0.001). The increase in the number of cells with SC in men as well as the decrease in their number in women indicated the abnormalities in the mechanisms maintaining genic balance of individual groups of cells. It is worth mentioning that the phenomenon of two X chromosome

inactivation is lethal. Therefore, the decrease in the indicator of SC in cells of the organ affected by the pathological process in women as well as its increase in men may indicate the degree of disease severity.

It was important to determine changes in the number of PN in PRS. Changes in the nuclear membrane enhance chromatin condensation, the reduction in the activity of gene expression and, probably, slow down the release of mature iRNA to the cytoplasm. Such mechanisms cause abnormal translation and post-translational modifications of polypeptide chain.

Among men, this indicator increased by 1.23 times (to 28.18±1.21) in patients with hyper-Ig-E-dependent PRS and by 1.4 times (to 32.02±0.94) in patients with aspirin-intolerant PRS (p<0.001 in both cases) (Table 4).

Similar tendency was observed among women. The number of cells with PN increased significantly (p<0.001) to 31.01±0.64 in female patients with hyper-Ig-E-dependent PRS and to 34.48±1.15 in women with aspirin-intolerant PRS.

Thus, a combined determination of all the parameters of the FSG of the interphase nuclei in the NMM cells serves as an objective criterion of the reduction in the activity of the transcriptional-translational processes in patients being genetically predisposed to PRS and especially those with aspirin-intolerant PRS. The quantitative changes in the FSG manifested themselves as the reduction in CI, the NI, the index of the heteropyknotic X chromosome (in women) and the increase in the number of morphologically altered nuclei and SC (in men). The aforementioned changes were the reflection of transcriptional abnormalities, i.e. epigenetic changes in nasal epithelial cells of patients with aspirin-intolerant PRS.

3. Conclusions

- A statistically significant difference in the quantitative changes in the degree of heterochromatization, the detection rate of the heteropyknotic X chromosome, the activity of the nucleolar apparatus and the number of pathologically altered nuclei was found in patients with aspirin-intolerant PRS as compared to healthy individuals and those with hyper-Ig-E-dependent PRS.
- The determination of changes in the parameters of the

Table 2. Indicators of the nucleolar index of epithelial cells in the nasal mucous membrane of patients being genetically predisposed to polypous rhinosinusitis (M±m)

Indicators	Hyper-Ig-E	Aspirin	Healthy individuals	p ¹	p ²	p ³
Males	11.09±0.63	8.98±0.46	12.65±1.01	p<0.05	p>0.05	p<0.05
Females	9.95±0.90	8.92±0.66	10.26±0.63	p<0.05	p>0.05	p<0.05

Note.

p¹ – statistical significance of differences between hyper-Ig-E-dependent and aspirin-intolerant polypous rhinosinusitis;

p² – statistical significance of differences between patients with hyper-Ig-E-dependent polypous rhinosinusitis and healthy individuals;

p³ – statistical significance of differences between patients with aspirin-intolerant polypous rhinosinusitis and healthy individuals.

The difference is statistically significant at p≤0.05

Table 3. Indicators of sex chromatin of epithelial cells in the nasal mucous membrane of patients being genetically predisposed to polypous rhinosinusitis (M±m)

Indicators	Hyper-Ig-E	Aspirin	Healthy individuals	p ¹	p ²	p ³
Males	4.01±0.36	5.82±0.51	2.76±0.13	p<0.01	p<0.01	p<0.001
Females	19.62±0.99	17.08±0.92	28.39±0.83	p>0.05	p<0.001	p<0.001

Note.

p¹ – statistical significance of differences between hyper-Ig-E-dependent and aspirin-intolerant polypous rhinosinusitis;

p² – statistical significance of differences between patients with hyper-Ig-E-dependent polypous rhinosinusitis and healthy individuals;

p³ – statistical significance of differences between patients with aspirin-intolerant polypous rhinosinusitis and healthy individuals.

The difference is statistically significant at p≤0.05

Table 4. Indicators of pathological nuclei of epithelial cells in the nasal mucous membrane of patients being genetically predisposed to polypous rhinosinusitis (M±m)

Indicators	Hyper-Ig-E	Aspirin	Healthy individuals	p ¹	p ²	p ³
Males	28.18±1.21	32.02±0.94	22.78±0.61	p>0.05	p<0.001	p<0.001
Females	31.01±0.64	34.48±1.15	23.12±0.49	p<0.01	p<0.001	p<0.001

Note.

p¹ – statistical significance of differences between hyper-Ig-E-dependent and aspirin-intolerant polypous rhinosinusitis;

p² – statistical significance of differences between patients with hyper-Ig-E-dependent polypous rhinosinusitis and healthy individuals;

p³ – statistical significance of differences between patients with aspirin-intolerant polypous rhinosinusitis and healthy individuals.

The difference is statistically significant at p≤0.05

FSG of the interphase nuclei in the NMM cells provides an objective confirmation of the involvement of epigenetic mechanisms in the regulation of the transcriptional-translational process activity as well as the formation of pathology in patients with aspirin-intolerant PRS.

4. Prospects for further research

The study of changes in the functional state of the genome under the influence of treatment is promising.

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