Research Article

Molecular Genetic Marker of Thyroid Tumor Formation

Kadyrova D.A[1], Mirkhamidova P[2], Shakhmurova G.A[2], Ziyamukhamedova S.A[4], Alimova R.A[5], Sattarov O.T[6]

[1] Institute of Biophysics and Biochemistry of the National University of the Republic of Uzbekistan
[2] Tashkent State Pedagogical University named after Nizami
[3] Uzbek State University of Physical Education and Sports
[4], 5 Tashkent State Agrarian University

[1]. Kadyrova D.A., d.kadyrova1949@mail.ru
[2]. Mirkhamidova P., Parida.mirkhamidova@mail.ru; https://orcid.org/0000-0002-6010-4843
[3]. Shakhmurova G.A., shga2065@yandex.ru ; https://orcid.org/0000-0001-6582-8261
[4]. Ziyamukhamedova S.A., sziyamuxamedova68@gmail.com
[5]. R.A. Alimova, alimovdt@gmail.com; https://orcid.org/0000-0001-6482-6336
[6]. Sattarov Oybek Tokhirovich Professor of the department of Faculty and Hospital Surgery No.1
Tashkent State Medical university Tashkent, Uzbekistan doctoroybek84@gmail.com, https://orcid.org/0000-0001-7908-1795

Abstract

The study of molecular mechanisms of formation of various thyroid tumors, analysis of molecular genetic markers of tumor formation for early diagnostics of these diseases is one of the urgent tasks of modern science. Most thyroid diseases, including various tumors, are caused by defective expression TG gene, i.e. disruption of complex processes regulating the synthesis of the corresponding protein. In the study of the work, the population size of individual TG polyribosomes in thyroid cells was determined in the norm and in various thyroid tumors. It was found that in papillary adenocarcinoma, the content of individual TG polyribosomes sharply decreases and is 0.07% compared to the normal thyroid gland, where the TG content in polyribosomes is 16%. One of the molecular genetic factors in the formation of thyroid tumors is a disruption of the normal suppressor function of the p27 gene. It was shown that in papillary adenocarcinoma, there is a disruption in the regulation of p27 gene expression at the transcription level. All the identified factors of tumor formation can be used as markers for the early diagnosis of various thyroid tumors.

Keywords: Molecular Genetic Markers, Tumor, Diagnostics, Thyroid Gland, Polymerase Chain Reaction, Thyroglobulins, Polyribosomes.

1. Introduction

Molecular genetic studies, which have as their main goal the clarification of the pathogenesis of the tumor process, are of great importance for the study of complex mechanisms of carcinogenesis. Until now, in contrast to the level of knowledge about tumors of other organs, the mechanism of development of malignant tumors of the thyroid gland remains incomplete, the factors influencing the transformation of these cells have not been determined. Clarification of pathogenetic intervals of development of malignant diseases, as well as issues of early diagnostics and prognosis of outcomes of various tumors of the thyroid gland is one of the most complex and sufficiently solved problems. The complexity of these problems is that the unsolved problems are the pathogenesis of tumor formation in thyroid cells and the existence of various spots of vision and diagnostics of etic pain. These problems require detailed study, especially in the conditions of our Republic, where the frequency of various tumors is high, which is associated with a deficiency (1). A comprehensive study of these issues using highly informative methods allows us to create a basis for early differential diagnostics for the prevention and treatment of these diseases. Routine diagnostic methods that exist today practically do not allow us to detect a tumor at an early stage, when the conditions for its treatment are most favorable. Namely, this is explained by the growing interest of oncologists in various molecular markers, many of which are sensitive to the use of methods used in clinical practice and often allow the manifestation of transformations of transformed cells.

1.1 Purpose of the study

The main objective of this work is to study the molecular mechanisms of formation of various thyroid tumors, analysis of molecular genetic markers of tumor formation for early diagnosis of these diseases.

2. Material and research method

A total of 20 thyroid tissue samples were used for the study: papillary adenocarcinoma (12 cases), follicular adenocarcinoma (8 cases), and blood from patients with papillary and follicular adenocarcinoma.

Isolation of high-molecular DNA from the blood of patients with various thyroid tumors using the phenol extraction method [2]. Extraction of total RNA using the SDS- phenol-chloroform method. Complementary DNA to the r27 gene is synthesized using the polymerase chain reaction (PCR) method and 0.5 μg of total RNA in the presence of reverse transcriptase with primers specific for the r27 gene: 5- CCTGGTGGATCTCAAC -3. PCR is performed in an incubation medium: 50 μl of saturated 60 мMTris-HCl (pH 8.6), 6 mM EDTA,10 мМ β- mercaptoethanol, 10 μg/ml BCA, 1 mM each of the 4 nucleotides, 2nd ed. reverse transcriptase. The PCR had a total of 55 cycles. Synthesis was carried out at 72 ^{0}C for 4 min. Subsequent cycles include key denaturation (1 min, 94 $^\circ$C), primer firing (1 min, 55 $^\circ$C), cDNA synthesis (2 min, 72 $^\circ$C). After 55 amplification cycles, the sample was lysed for 10 min at 72 $^\circ$C, then cooled, aliquots were subjected to electrophoresis on r27 gene cDNA in 1% agarose with ethidium bromide. Hybridization of total RNA with ^{3}N r27 gene cDNA was carried out at 65 $^\circ$C in 60 μl of buffer containing 0.2 M NaCl , 0,03 MTris-NS1 (rN 7.6), 0.025% Na dodecyl sulfate , 5 μl of tRNA. Continuous quantification of r27 cDNA mixed with total RNA at a concentration of 0.05 to 0.75 μg/ml. The lower part of the window was incubated with an aliquot of the Rufs nanofilter. The filters were washed with cold TXUK alcohol, and the radioactivity was counted on a Mark counter . 111"The remaining 30 μl was supplemented with S1 nuclease and incubated at 50 ^{0}C for 1 hour.

Polyribosomes were isolated from thyroid gland cysts removed during surgery for acute tumors.

Individual polyribosomal proteins with auxiliary immunosorbents, which are covalently linked complex protein antigens with insoluble basic and synthesizing antibodies.

As an insoluble main "sandwich" sorbent with a commercial ion exchanger preparation - para-aminobenzyl cellulose (PABC) (3).

The "sandwich" sorbent PABC-TG:aTG (capacity - 0.6 mg ATG and 1 mg PABC-TG) is used for the treatment of thyroglobulins (TG).

To determine the amount of polyribosomes sorbed on the PABC-TG:ATG sorbent, Kadyrova D.A. The developed method of acid hydrolysis of polyribosomes has no direct relation to the structure of RNA.

3. Results and discussion

Violation of gene expression at various levels is the cause of not only hereditary diseases, but also the cause of tumor formation. The formation of a sufficient amount of protein or its synthesis in an inactive form is inevitably accompanied by the development of the patient. With painful thyroid symptoms, the content of TG in the thyroid tissue changes greatly, changes in the structure of TG are noted. Low TG levels with various thyroid symptoms a priori, it is possible to explain the disturbances occurring at one of these stages of protein biosynthesis regulation: 1) transcription of the TG gene, 2) maturation of TG mRNA in the nucleus, 3) transport of TG mRNA into the nucleus, 4) size of the individual TG polyribosome, 5) translation of TG mRNA.

When studying the mechanism of formation of tumor-like thyroid signs, for the development of methods for early diagnostics, a separate area is interested in the question of the population size of the individual TG polyribosome, since the amount of the translated mRNA molecule determines the rate of protein synthesis.

For correct determination of the size of the population of individual polyribosomes, it is necessary, firstly, to quantitatively isolate all polyribosomal cells in native form; in-second, it will deplete the studied polyribosome fraction by individual polyribosomes; in-tertix, it carries out the measurement at a minimum level of non-specific sorption. Considering that all of them are necessary, the size of the population of individual polyribosomes of TG and fractions of total polyribosomes isolated from thyroid cells in various tumors are determined.

All pathological counting signs were subjected to histological examination. Polyribosomes isolate scar tissue in any case using the magnesium method, which can be used to obtain an optimal result. The study of the physicochemical properties of the isolated polyribosomes showed that polyribosomes can be used to determine the population size of individual TG polyribosomes and cells with thyroid signs in various tumors.

Serial incubation mixtures containing fixed polyribosomes and various "sandwich" sorbents PABC-TG:aTG were prepared for determination of TG-synthesizing polyribosomes. When determining the ratio of the population of TG-synthesizing polyribosomes and normal thyroid tissue cells, it was shown that the content of TG polyribosomes was 16%. The content of TG polyribosomes was determined in follicular adenocarcinoma. The size of the TG polyribosome population in follicular adenocarcinoma was 5.4%. The content of TG polyribosomes was determined in papillary

adenocarcinoma. Papillary adenocarcinoma is the most common form of thyroid cancer, accounting for 60-80% of malignant neoplasms. The population size of TG polyribosomes and thyroid cells in papillary adenocarcinoma is 0.07%. The absence of TG-synthesizing polyribosomes in thyroid tumors indicates that in these diseases TG mRNA is either virtually absent or is formed but is unable to be translated. Deciphering the molecular mechanisms of TG mRNA synthesis and the main protein of the thyroid gland - TG in both normal and tumor cells is important for developing methods for early diagnosis and prevention of thyroid tumor diseases.

Most painful thyroid symptoms, including various swellings, are caused by a defect in the expression of only TG, i.e., disturbances in the complex processes that regulate the synthesis of the corresponding protein.

In thyroid disease, there is a violation of the regulation of the TG gene expression at all levels, namely: neither at the translation level, nor at the transcription level, nor at the process level (maturity of molecular mRNA), nor at the level of mRNA exit from the nucleus and the origin of mRNA and polyribosomes, nor at the level of molecular DNA methylation. The relationship between DNA methylation and gene expression and the significance of this relationship in carcinogenesis was shown by Robin, who believes that gene expression in mammalian cells is controlled, in particular, by methylation of subsequent DNA at cytosine. It was shown that tumor cells have a violation of the DNA methylation process.

Restriction enzymes Msp-I and Hpa II recognize only one sequence (CCGG), which does not differ in the position of 5mTs and tetranucleotide. The possibility of participation in methylation and regulation of TG gene expression was investigated. TG gene methylation status in follicular and papillary adenocarcinoma. All pathological symptoms of the thyroid gland are controlled by histological examination. The 5 ^{1}- flanking region of the TG gene was analyzed, in fact, this region contains two sequences recognized by two restriction enzymes sensitive to methylation enzymes M s r 1 and Nra 11.

Secreted by thyroid cells was performed using restriction enzymes H ra11 and Msp 1 . There is an inverse correlation between the expression of the TG gene and its methylation. It was shown that the blocking of TG synthesis in papillary adenocarcinoma occurs at the transcriptional level, and two methylation-sensitive sites located in the flanking 5 ^{1} - region of the TG gene are hypermethylated in papillary adenocarcinoma.

Based on the data obtained, it is possible to exclude the possibility that transcriptional blocking is the result of a significant change either in the structural organization of the TG gene or in the regulatory 5 ^{1} regions of the gene.

When studying the relationship between TG gene expression and its methylation, it was shown that methylation-sensitive sites located in the 5 ^{1} -flanking region of the TG gene are hypermethylated in papillary adenocarcinoma, whereas they are hypermethylated in normal thyroid tissue cells. unmethylated sites are observed. And the result of the obtained results is that there is an inverse correlation between DNA methylation and TG gene expression.

One of the molecular genetic factors of tumor formation is the disruption of the normal suppressor function of the r27 gene. The r27 gene is characterized as a cell cycle regulator during malignant cell transformation. It has been shown that there is an inverse correlation between the expression of the r27 gene and the endocrine signal of the tumor during cell proliferation, including the thyroid gland. The r27 gene is located on chromosome 9, region 9r21. This gene encodes an inhibitor of cycle-dependent kinase (S dk). Defects in this gene occur at early stages of tumor formation in thyroid gland cells, which makes it possible to conduct early diagnostics of these diseases (4).

Synthesis of r27 gene cDNA is carried out by the polymerase chain reaction method (5). Complementary DNA was synthesized from 0.5 μg of total RNA using primers specific to the r27 gene and the presence of reverse transcriptase. After 35 amplification cycles, the sample was subjected to a 10-minute incubation at 72 $^{\circ}$C for final elongation, after which, at the test stage of amplification, electrophoresis was carried out with an aliquot of the reaction mixture (10 μl) and 1% agarose. Electrophoresis, ultracentrifugation, and sucrose gradient graph showed that r27 gene cDNA has a ≈sedimentation coefficient of 16 S.

3 N cDNA of the r27 gene with total RNA was performed . It was shown that the percentage of hybridization in papillary adenocarcinoma is 63%, while in the norm it is 92%. From the presented data it is evident that in papillary adenocarcinoma there is a decrease in the content of mRNA for the r27 gene by 1.46 times. Abnormal levels of mRNA of the r27 gene in the blood in papillary adenocarcinoma indicate either a violation of the regulation of the r27 gene expression, or the level of its transcription, which indicates the presence of mutational changes in the gene.

Restriction analysis of DNA isolated from patients' blood and normal blood, restriction enzyme Hind 111, EcoR 1, EcoRV , BamH 1, Fragmenty DNA Transfer and nylon filters " Biodan ", hybridization with ^{32} R cDNA for the r27 gene were performed. Restriction mapping using the restriction enzyme EcoRV in the DNA of a healthy person yielded 4 fragments containing the r27 gene sequence, while in the DNA of papillary adenocarcinoma a single fragment was detected. From the data presented, it is evident that large deletions were detected in the r27 gene in papillary adenocarcinoma.

The obtained data show the role of the r27 gene in the formation and progression of rostral thyroid tumors of various functional directions. A difference in the degree of expression of the r27 gene in papillary adenocarcinoma was established.

In this picture, activation of a number of oncogenes, inactivation of tumor suppressor genes, as well as complex losses and defects of DNA-damaged genes, pronounced disturbances and regulation of the expression of TG genes (a sharp decrease in the content of TG in individual polyribosomes, disruption of TG synthesis, structural and functional changes in nuclear chromatin, DNA hypermethylation) occur, which are molecular genetic markers of tumor formation in thyroid cells.

4. Conclusion.

1. The immunosorbent PABC-TG:ATG was obtained to determine the size of the population of individual TG polyribosomes; the achievable capacity of the obtained immunosorbent is at the level of 0.14 mg/mg.
2. Population of a certain size of individual TG polyribosomes and cytoskeletal signals and normal signals of various tumor cells. It was found that the proportion of the TG polyribosome population is: in normal thyroid tissue cells - 16%, in follicular adenocarcinoma - 5.4%, in papillary adenocarcinoma - 0.07%.
3. It has been shown that in papillary adenocarcinoma, hypermethylation of the 51- flanking region of the TG gene is observed.
4. The r27 gene cDNA is synthesized using the polymerase chain reaction method.
5. It has been shown that in papillary adenocarcinoma there is a disruption in the regulation of r27 gene expression and transcription levels.

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