DOI: 10.29256/v.02.02.2018.escbm67

MICRORNAs - MOLECULAR MARKERS FOR DETECTING CANCER IN THYROID NODULES

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Thyroid nodules are one of the most common pathology of thyroid gland. The prevalence of thyroid nodules varies depending on age of the patients and methods of detection, such as palpation or ultrasonography (US). It varies from 2–6% (palpation) to 19–68% (US), and 8–65% (autopsy) [1]. Despite morbidity level only about 5-10% of such nodules are malignant [2]. Fine needle aspiration cytology of the thyroid nodule is currently the primary diagnostic tool for determining the nature of a thyroid nodule. It is considered as the gold standard for diagnostics of thyroid cancer but in 30 % of cases cytological conclusion is uncertain. Cytological research is not enough to differentiate benign and malignant tumors. The need to improve the effectiveness of fine needle aspiration biopsy findings led to the search for new diagnostic biomarkers and the creation of diagnostic panels on their basis for their application in the diagnostics of uncertain nodules. Determination of molecular markers in the thyroid aspirate will allow to differentiate benign and malignant tumors more accurately at the preoperative stage and to reduce number of inappropriate surgery. Micro(mi)RNAs are endogenous, single-stranded, non-coding RNAs (~ 22 nucleotides) that regulate gene expression by direct degrading of mRNA or suppressing of the post-transcriptional protein translation by binding to the 3' untranslated region (3' -UTR) of the respective target mRNAs. The miRNAs have been reported to regulate 30% of the human genome, and are involved in many cellular processes such as cell proliferation, apoptosis, and development [3]. MicroRNA (miR) expression signatures are proposed to be able to differentiate thyroid cancer from benign thyroid lesions. We selected nine miRs (miR-221, −146b, −187, −199b, −205, −183, −551, −375, -7) to examine the potential use of miRs to supplement diagnostic cytology in cases designated as follicular adenoma (suspicion of follicular carcinoma) (FTC)) and benign nodules.

Materials and Methods. This study was approved by the ethics committee of the Institute of Molecular Biology and Biophysics, Siberian Branch of the Russian Academy of Medical Sciences. MiR expression was measured in thyroid fine needle aspiration (FNA) specimens by quantitative polymerase chain reaction. Gene expression analyses were performed in a training sample set (n=79) to obtain a classification rule to predict FNA cases as benign or malignant. The patients were divided into two groups: benign nodules (n=63) and follicular adenoma (suspicion of follicular carcinoma) (n=16).

Results. A comparative analysis of the expression levels of miRNAs-146b, -221, -375, -205, -187, -199b, -551 -183,-7 between the subgroups of follicular adenoma (suspicion of follicular carcinoma) and thyroid subgroup of benign nodules showed a statistically significant increase of expression level of miRNA-146b 8-fold ($p<0.05$), miRNA-221 in 3-fold ($p<0.05$), miRNA-375 4-fold ($p<0.05$), miRNA-199b 3-fold ($p<0.05$), miRNA-183 4-fold ($p<0.05$) in the subgroup of tumors with a diagnosis of follicular adenoma (suspicion of follicular carcinoma) thyroid.

Conclusion. Strong data were collected regarding significance of the determination of molecular markers (miRNA-146b, -221, -375, -205,- 199b, -183) in the thyroid aspirate for differentiation between benign and malignant tumors.

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Key words: thyroid, thyroid nodules, molecular markers, FNA, MicroRNA

Accepted for printing on 24 Sept 2018

DOI: 10.29256/v.02.02.2018.escbm68

DINAMICS OF CYTOCHROME C, BAX AND BCL-2 EXPRESSION IN EXPERIMENTAL DIABETIC CARDIOMYOPATHY

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The most important determinants of myocardial remodeling include cell death [1]. Two ways of cardiomyocytes death – necrosis and apoptosis were identified. Apoptosis is of greater interest due to its possible pharmacological correction. Proteins regulate apoptosis at the level of mitochondria,Bax is proapoptoticandBcl-2–antiapoptotic

protein. The ratio of these proteins (Bcl-2/Bax), called as a cell survival index, indicates whether the cell will survive or die by apoptosis [1-3]. Another potential apoptogenic factor is cytochrome C. The aim of the study was to determine the immunohistochemical expression of Bax, Bcl-2 cytochrome C proteins in the dynamics of diabetic cardiomyopathy in an experimental study.

Materials and Methods. Diabetic cardiomyopathy was modeled by intraperitoneal injection of streptozotocin at a dose of 60mg/kg. Four groups were in experiment. Group 1 (early stage of diabetic cardiomyopathy): animals were removed in three weeks after streptozotocin injection. Group 2 (intermediate stage of diabetic cardiomyopathy): animals were removed in five weeks. Group 3 (late stage of diabetic cardiomyopathy): animals were removed in eight weeks. The control group (4) consisted of 5 intact rats. Immunohistochemical study was performed using monoclonal antibodies Bax (1:300, Abcam) and Bcl-2 (1:300, Thermo Scientific), cytochrome C (RTU, Neomarkers). Using the morphometric program AperioImageScope carried out the calculation of the percentage positivity of expression (PPE) of the studied markers. Statistical analysis was carried out using the standard statistical software package STATISTICA 7.1.

Results. In group 1 (n=15) the PPE of Bax varied from 33.4% to 38.4%, the average value was 34.4%. The PPE of Bcl-2 ranged from 9.4% to 17.3%, the average value was 14.2%. The PPE of cytochrome C varied from 5.9% to 7.4%, the average value was 6.8%. In group 2 (n=15) the PPE of Bax ranged from 16.8% to 40.9%, the average value was 31.9%. The PPE of Bcl-2 varied from 45.3% to 72.4%, the average value was 65.9%. The PPE of cytochrome C ranged from 6.1% to 17%, the average value was 12.8%. In group 3 (n=15) the PPE of Bax varied from 30.1% to 39.9%, the average value was 36.9%. The PPE of Bcl-2 ranged from 58.2% to 74.6%, the average value was 68.5%. The PPE of cytochrome C varied from 24.7% to 30.3%, the average value was 26.1%. In the control group (n=5), the average value of PPE of the analyzed markers was 5.9%, 56.0% and 3.07% (for Bax, Bcl-2 and cytochrome C, respectively). The cell survival index (CSI) was 0.38 (0.36; 0.43) in the first group. CSI was 2.24 (1.61; 2.70) and 1.87 (1.60; 2.04), respectively in 2 and 3 groups.The cell survival index was 9.97 (6.65; 11.25) in the control group. In group 1the PPE of Bcl-2 was a significant decreased and the PPE of Bax – increased. In 2 and 3 groups PPE of Bax was also statistically significant increased, but PPE of Bcl-2 was not statistically significant different to compare to control group. Statistical analysis revealed a significant change of PPE of Bax (H=12.95, p=0.0048) and Bcl-2 (H=13.85, p=0.003) in the studied groups. As diabetic cardiomyopathy progressed, there was a significant increase the level of PPE of cytochrome C in the cytoplasm of cardiomyocytes compared to the control group and in the intergroup analysis (H=17.10, p=0.0007). The correlation analysis established a positive relationship between the expression of Bax and cytochrome C (r=0.6, p=0.017).

Conclusion. The study revealed a change in the expression of proteins regulating cell apoptosis. Experimental modeling of diabetic cardiomyopathy showed an increase in the expression of markers of anti-and proapoptotic proteins in cardiomyocytes with a change in the cell survival index, which indicated the activation of the apoptotic system due to glycaemia. The low survival index at the early stage of the experiment indicated increased damage to the heart muscle cells and apoptosis in glycaemia. The increase in the survival index at the intermediate station indicated the formation of adaptive processes to glycaemia. Repeated decrease in the survival index of cells in the later stages of the experiment may indicate the depletion of adaptation to the damaging effects of glucose in the dynamics of diabetic cardiomyopathy. The positive correlation between Bax and cytochrome C expression indicates the increasing role of the proapoptic system in the progression of diabetic cardiomyopathy in the experiment.

Prospects for further research. The obtained data can serve as a basis for correcting the treatment of cardiomyopathy in patients with diabetes mellitus.

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Keywords: cytochrome C, Bax, Bcl-2, diabetic cardiomyopathy.

Accepted for printing on 24 Sept 2018

DOI: 10.29256/v.02.02.2018.escbm69

MALONDIALDEHYDE VARIATIONS IN EXPERIMENTAL MYOCARDIAL INFARCTION

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Acute myocardial infarction remains the main clinical model of ischemia/reperfusion injury associated with oxidative stress [1]. The unbalanced production of reactive oxygen species (ROS) accelerates the peroxidation of lipids and generates a large variety of the toxic lipid peroxidation products, including malondialdehyde (MDA) [2]. Reacting with Lys residues, MDA induces the protein modifications (intramolecular and/or intermolecular cross-linking), and alters the cellular responses [3]. The experimental studies performed recently *in vitro* have suggested the utility to assess MDA as a biomarker of oxidative stress in the myocardium damage caused by ischemia/reperfusion [4]. The aim of our research was to evaluate the variations of MDA content in the homogenate in experimental