ISSN 1607-6729, Doklady Biochemistry and Biophysics, 2020, Vol. 493, pp. 208–210. © Pleiades Publishing, Ltd., 2020.
Russian Text © The Author(s), 2020, published in Doklady Rossiiskoi Akademii Nauk. Nauki o Zhizni, 2020, Vol. 493, pp. 400–403.

BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Differences in the Drosha and Dicer Cleavage Profiles in Colorectal Cancer and Normal Colon Tissue Samples

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Presented by Academician S.I. Kolesnikov

Received March 24, 2020; revised March 31, 2020; accepted March 31, 2020

Abstract—Human colorectal adenocarcinoma cell line Caco-2 is often used as a model of healthy intestinal epithelium, in particular, in miRNA studies. The work of the enzymes Drosha and Dicer is an integral part of the process of miRNA formation. Inaccuracies in the work of these enzymes lead to a change in the nucleotide sequences of miRNAs with the formation of new isoforms, which, in turn, can change intracellular regulatory mechanisms. In the framework of this study, it was shown that the quantitative estimates of inaccuracies in Drosha and Dicer activity significantly differ between the specimens of normal colon tissue and malignant colorectal tumors.

Keywords: Drosha, Dicer, colorectal cancer, miRNA, microRNA, microRNA isoform, isomiR
DOI: 10.1134/S1607672920040122

Cell models of the intestinal barrier in vitro make it possible to study various biological aspects, including the barrier, transport, and secretory functions of the intestinal epithelium, the interaction with the microbiome, and cell biology features under normal and pathological conditions. The use of immortalized tumor cell lines as models of human organs (e.g., human colorectal human adenocarcinoma Caco-2 cells as a model of the intestinal barrier [1]) allows obtaining well reproducible standardized results, excluding the contribution of many factors in a normal living organism that can barely be taken into account and reducing the variability inherent in the primary cell cultures [2]. The addition of extracellular matrix and the circulation of the nutrient medium in microfluidic devices additionally make the microenvironment of barrier models more similar to real conditions [3]. However, when studying the biological processes in such models, the possible differences between the normal and tumor cells should always be borne in mind.

Currently, the role of miRNAs in the regulation of the intestinal barrier is being actively studied. MicroRNAs are short RNA molecules approximately 22 nt long that play the key role in RNA interference [4]. Their most important function is the posttranscriptional suppression of the expression of target genes due to complementary binding of miRNA to the 3'-untranslated region of the target mRNA, which blocks further translation and/or degradation of mRNA [5].

The process of miRNA formation includes several stages. At the first stage, as a result of transcription, a miRNA molecule with a hairpin structure is formed. At the next stage of maturation, the intranuclear enzyme Drosha binds to pri-miRNA and cuts the unpaired ends of the molecule to form pre-miRNA [6]. Finally, after transporting the pre-miRNA to the cytoplasm, the Dicer enzyme cuts the loop of the pre-miRNA hairpin, as a result of which two miRNA molecules are formed [7] (Fig. 1). The inaccuracy of the work of these two enzymes leads to the formation of the so-called miRNA isoforms (isomiRs)—molecules that differ from the canonical miRNA by 1–3 nucleotide bases at the ends of the sequence [8]. The importance of taking these molecules into account is that a noncanonical isoform of miRNA may often prevail in the cell [9] and lead to a significant change in the list of target genes of miRNA [10, 11].

In this study, the described effects were investigated by analyzing the isomiR profile data of healthy

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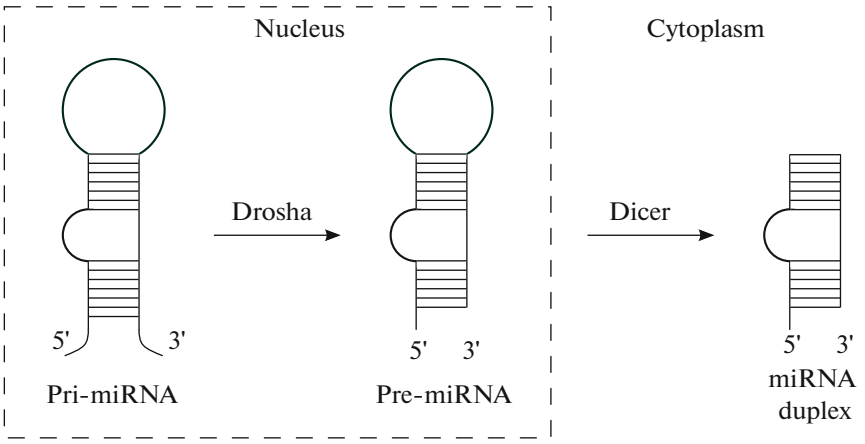

Fig. 1. miRNA maturation stages.

and tumor colon tissues. For this purpose, we used the results of miRNA analysis by the new-generation sequencing in 16 biological samples from the project The Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD) [12]. Samples were obtained from healthy and cancer colon tissues (two samples from each of eight patients). The miRNA sequencing results were obtained in the form of a miRNA isoform expression table containing information on the number of reads corresponding to each isoform (the quantities were normalized per million of aligned reads).

The weakly expressed isomiRs (namely, the isoforms whose average expression was less than 100 reads per million aligned reads) were excluded from the analysis. To compare the expression profiles of isomiRs between the healthy and pathological tissues, an agglomerative hierarchical clustering of samples was performed using the Ward method [13] on the basis of the data for the most clearly expressed isoforms (with average expression of more than 1000 reads per million aligned reads). Two major clusters of specimens were completely consistent with the clinical characteristics of the tissues.

To study the role of inaccuracies in the work of Drosha and Dicer enzymes in the healthy and tumor tissues, we compiled tables containing data on the total number of reads per million aligned reads for each sample for each value of deviation from the canonical miRNA form. The shifts from the 5'-end of the 5'-chain of miRNAs and from the 3'-end of the 3'-chain of miRNAs were attributed to the inaccuracies in the work of Drosha, and the shifts from the 3'-end of the 5'-chain and from the 5'-end of 3'-chain were attributed to the inaccuracies in the work of Dicer. The constructed tables were divided into two parts corresponding to the specimens of the healthy tissues and tumors. Then, the values in each of the tables were normalized to bring the data to the percentage scale.

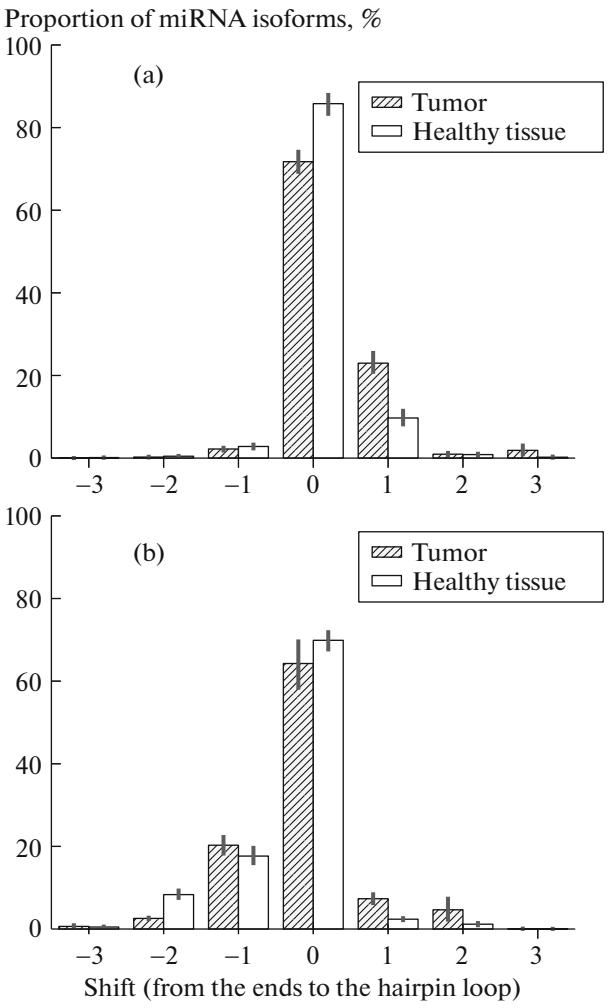

Fig. 2. Quantitative estimates of inaccuracies in (a) Drosha enzyme and (b) Dicer enzyme activity in the healthy and tumor tissues. The bars at the ends of the columns show the standard deviation.

The obtained data are shown in Figs. 2a and 2b (shift values are shown in the direction from the ends to the hairpin loop).

It can be seen that, on the whole, the intranuclear treatment of miRNA with the Drosha enzyme proceeds with a lower percentage of errors as compared to the cytoplasmic treatment with the Dicer enzyme. The fact that the inaccuracies in the Drosha work are shifted towards the hairpin loop, whereas the inaccuracies in the Dicer work are shifted towards the hairpin ends is also of interest. Thus, the work of both enzymes is aimed mainly at shortening the miRNA molecule. The comparison of data for the healthy and tumor tissue samples using the Mann–Whitney *U* test showed a significant change in frequencies in the variants of work of Drosha and Dicer. For example, the differences in the activity of Drosha at shift values of 0, 1, and 3 (p-values 4.69×10^{-4}, 4.69×10^{-4}, and 2.69×10^{-3}, respectively) and Dicer at shift values of –2, 1, and 2 (p-values were 4.69×10^{-4}, 4.69×10^{-4}, and 6.79×10^{-3}, respectively) were statistically significant.

Thus, the process of formation of miRNA isoforms, associated with the activity of Drosha and Dicer proteins, significantly differs between the healthy and tumor colon tissues. These data should be taken into account when studying the role of miRNAs in the regulation of the functional activity of healthy intestinal epithelium, including the vesicular transport [14, 15], using human colorectal adenocarcinoma cells. In choosing and developing the miRNA detection methods, as well as in analyzing the target genes of these miRNAs and changes in their expression, it is recommended to take into account the set of the existing miRNA isoforms.

FUNDING

The study was supported by the Russian Foundation for Basic Research (project no. 20-34-70092).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

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Translated by M. Batrukova