ROLE OF ACE2/TMPRSS2 GENES REGULATION BY INTESTINAL microRNA ISOFORMS IN THE COVID-19 PATHOGENESIS

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Coronavirus SARS-CoV-2, the cause of the COVID-19 pandemic, enters the cell by binding the cell surface proteins: angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2). The expression of these proteins varies significantly in individual organs and tissues of the human body. One of the proteins' expression regulation mechanisms is based on the activity of the microRNA (miRNA) molecules, small non-coding RNAs, the most important function of which is the post-transcriptional negative regulation of gene expression. The study was aimed to investigate the mechanisms of the interactions between miRNA isoforms and ACE2/TMPRSS2 genes in the colon tissues known for the high level of expression of the described enzymes. The search for interactions was performed using the correlation analysis applied to the publicly available paired mRNA/miRNA sequencing data of colon tissues. Among the others, such miRNAs as miR-30c and miR-200c were identified known for their involvement in the coronavirus infection and acute respiratory distress syndrome pathogenesis. Thus, new potential mechanisms for the ACE2 and TMPRSS2 enzymes regulation were ascertained, as well as their possible functional activity in a cell infected with coronavirus.

Keywords: COVID-19, SARS-CoV-2, ACE2, TMPRSS2, miRNA, isomiR, acute respiratory distress syndrome, coronavirus

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РОЛЬ РЕГУЛЯЦИИ ГЕНОВ АПФ2/TMPRSS2 ИЗОФОРМАМИ микроРНК КИШЕЧНИКА В ПАТОГЕНЕЗЕ COVID-19

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Коронавирус SARS-CoV-2, вызвавший пандемию COVID-19, проникает в клетку, связываясь с поверхностными белками: ангиотензин-превращающим ферментом 2 (АПФ2) и сериновой протеазой 2 (TMPRSS2). Экспрессия данных белков значительно различается в отдельных органах и тканях организма человека. Одним из механизмов регуляции их экспрессии является активность молекул микроРНК — коротких некодирующих РНК, важнейшей функцией которых является посттранскрипционная негативная регуляция экспрессии генов. Целью работы было выявить механизмы взаимодействия изоформ микроРНК и генов АПФ2 / TMPRSS2 в тканях толстого кишечника, известных высоким уровнем экспрессии указанных ферментов. Поиск взаимодействий был осуществлен средствами корреляционного анализа на публично доступной выборке данных парного мРНК / микроРНК-секвенирования тканей кишечника. В числе находок оказались такие микроРНК, как miR-30c и miR-200c, известные своей ролью в патогенезе коронавирусной инфекции и острого респираторного дистресс-синдрома. Таким образом, были установлены новые потенциальные механизмы регуляции ферментов АПФ2 и TMPRSS2 и их возможная функциональная активность в клетке, инфицированной коронавирусом.

Ключевые слова: COVID-19, SARS-CoV-2, АПФ2, TMPRSS2, микроРНК, изоформа микроРНК, острый респираторный дистресс-синдром, коронавирус

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The rapid and progressive spread of the COVID-19 infection caused by the SARS-CoV-2 coronavirus deeply affected the health of hundreds of thousands of people, which became a serious challenge to healthcare systems and global economic stability. The characteristics of SARS-CoV-2, especially distinguishing the disease from influenza, are the higher infection rate combined with the increased risk of severe course and mortality, mainly due to the acute respiratory

distress syndrome (ARDS) [1]. The mechanism of the cells infection is actively studied in many laboratories. In particular, it is known that the SARS-CoV-2 viral envelope expresses the spike protein (S protein) containing the receptor binding domain with high affinity for the extracellular domain of angiotensin-converting enzyme 2 (ACE2). The further S protein cleavage by the transmembrane serine protease 2 (TMPRSS2) aimed to produce the S1 and S2 subunits is a crucial stage for membrane fusion and virus internalization by endocytosis with ACE2 in pulmonary epithelium. It is assumed that the greater virulence of SARS-CoV-2 compared to other coronaviruses can be explained by the S1 protein's significantly higher affinity for ACE2. This mechanism of the SARS-CoV-2 entering the cell leads to the loss of ACE2 on the cell surface, thereby contributing to chronic lung function impairment and severe tissue fibrosis [2].

MicroRNAs (miRNAs) are the small non-coding single stranded RNAs containing an average of 22 nucleotides. One of the most important intracellular functions of miRNA is the negative regulation of gene expression due to complementary miRNA binding with the target mRNA, leading to mRNA degradation or translational inhibition [3]. MicroRNAs are formed from the longer hairpin molecules of pre-miRNA as a result of the hairpin cleaving Drosha and Dicer enzymes' activity [4]. The cleaving site inaccuracy leads to the emergence of various microRNA isoforms that differ in several nucleotides at the ends of the molecule. It is reported that many miRNAs of canonical types are expressed much weaker than some alternative isoforms [5]. It is of key importance that different isoforms of the same miRNA may have completely different target genes. This is because the most important role in binding to the target mRNA is played by the miRNA region between the 5' nucleotides 2–7 (seed region) [6].

It is reported that the functional impairment of miRNAs and their isoforms is associated with a large number of pathological conditions, including cancer, neurological and cardiovascular diseases [7]. A large number of papers is devoted to the study of the role of miRNAs in the pathogenesis of viral infections: some of them are aimed to study the therapeutic potential of the direct miRNA interaction with the virus [8], and the others are aimed to investigate the potential interactions of miRNAs and proteins playing a key role in the viral vital processes [9]. However, the ACE2 and TMPRSS2 expression regulation by miRNA in subjects with COVID-19 remains poorly understood. The study was aimed to reveal the mechanisms of the interactions between miRNA isoforms and ACE2/TMPRSS2 genes in the colon tissues known for the high level of expression of the described enzymes.

METHODS

To search for miRNA isoforms interacting with ACE2 and TMPRSS2 enzymes, we performed the integrated analysis of the paired mRNA and miRNA expression in the normal colon tissues' sample (the enzyme is most intensively expressed in the colon tissues). The tissue selection was also due to the fact that the gut models were often used for *in vitro* studies of viruses [10, 11]. The available for public access samples from The Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD) collection were used. The sample analysis was carried out by the next-generation mRNA and miRNA sequencing [12]. The data were expression matrices of thousands of mRNA and miRNA isoforms in eight samples, the unit of expression was the binary logarithm of the corresponding transcript number of reads normalized to the upper quartile of the overall distribution (FPKM-UQ). To search for potential regulatory interactions between miRNA isoforms and TMPRSS2 the Spearman correlation coefficients between the expressions of 25% of the most highly expressed isoforms with the expressions of the corresponding mRNA were calculated, with subsequent filtering in accordance with the p-value (significance level 0.05).

RESULTS

The ACE2 and TMPRSS2 expression at the mRNA level turned out to be very high: TMPRSS2 was in the list of the most highly expressed genes (1%), and the ACE2 expression was between the 93rd and 94th percentiles, which was fully consistent with published data [13] (see Figure). Correlation analysis allowed us to detect the miR-21 miRNA demonstrating a significant negative correlation with the ACE2 gene expression, as well as the following miRNA families regulating TMPRSS2: let-7a/let-7d, miR-30a, miR-30c, miR-127, miR-194, miR-200c, miR-361 and miR-423. The let-7a miRNA was represented by the hsa-let-7a-5p isoform, which differed from the canonic type by adenine added at the 5' end of the molecule. The miR-194 was represented by the hsa-miR-194-3p isoform, which lacked the 5' first nucleotide. The absence of the corresponding miRNA canonical forms in the list indicates the importance of taking into account the profiles of all miRNA isoforms, not just canonical isoforms.

DISCUSSION

Some of the discovered miRNAs have already been detected during the virological studies. Thus, it was shown that the miR-30c expression in the lungs of the mouse changed significantly upon infection with SARS-CoV virus [14], which made it possible to put forward a hypothesis about the involvement of that miRNA in the development of a disease caused by the virus. The miR-200c miRNA is also of great interest. In 2017, a paper was published reporting that miR-200c miRNA played a key role in the virus-induced ARDS pathogenesis [15]. The researchers found out that the H5N1 avian influenza virus promoted the miR-200c expression, the target of which was the ACE2 receptor. Moreover, the viral proteins were detected responsible for promoting the miRNA expression. The discovery of the interaction possibility between the described miRNA and

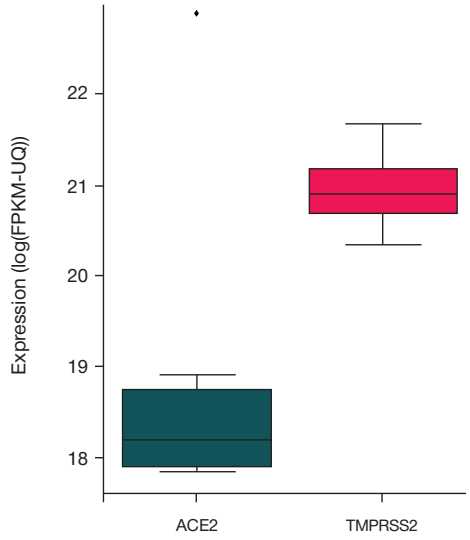

Fig. ACE2 and TMPRSS2 expression distribution in the colon tissues. Borders of the boxes correspond to the lower and upper quartiles, vertical segment inside the box represents the median value. Black rhombus shows outlier

the TMPRSS2 enzyme emphasizes the need for studying the role of miR-200c in the COVID-19 pathogenesis.

CONCLUSION

The results obtained indicate the presence of numerous regulatory interactions between miRNA isoforms and ACE2/TMPRSS2 enzymes. Such information is extremely important due to the key role of enzymes in the mechanism of cell infection with SARS-CoV-2 coronavirus. Further research is needed for refining and experimental validation of the findings. In particular, it is possible to discover new treatment options based on the ACE2 and TMPRSS2 expression regulation via microRNAs.

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