ASSOCIATION OF miRNAs AND TARGET GENES AS MARKERS AT THE STROKE

Kondybayeva A.M.1, Kamenova S.U.1, Ivashchenko A.T.2, Niyazova R.E.2
1 Asfendiyarov Kazakh National Medical University, Kazakhstan
2 Al-Farabi Kazakh National University, Al-Farabi ave., Kazakhstan

The promising molecular markers for various diseases are miRNAs [1]. It was identified the involvement of miRNAs in stroke [2]. The miRNAs can influence the expression of genes involved in the development of stroke. We selected 42 genes (candidate genes) that are thought to be responsible for the stroke. It was studied the binding of the known 6266 miRNAs for identification miRNA and gene associations for use in the diagnosis of this disease.

Materials and Methods. The search of miRNA binding site in mRNA was found using the MiRTarget program which determines the beginning of the miRNA binding site in mRNA, the free energy of interaction ΔG of the entire miRNA nucleotide sequence and the degree of complementarity of the miRNA and mRNA of the site in 5'-untranslated regions (5'UTRs), coding domain sequences (CDSs), 3'-untranslated regions (3'UTRs), the schemes of nucleotide interactions between miRNA and mRNA. The miRNA nucleotide sequences were borrowed from MiRBase database and publication Londo E., et al. [3].

Results. Most of the genes involved in the development of stroke served as targets for miRNA. 12 candidate genes don’t have binding sites for 6266 miRNAs, and probably their expression is independent or weakly dependent on miRNA. The miRNA binding sites are found in 5'UTR, CDS and 3'UTR. In most candidate genes, the miRNA binding sites were randomly arranged along the length of the mRNA. However, in some genes, mRNA binding sites in mRNA were located with the overlap of nucleotide sequences (miRNA binding sites clusters), which has the biological meaning. For example, in the CDS of mRNA ADAMTS7 gene, there are four miRNA binding sites from 276 nt to 302 nt which occupy a 27 nt length. Without overlapping of nucleotide sequences, the sum of the lengths of these miRNAs is 68 nt that is 2.5 times the cluster of miRNA binding sites. If 68 nucleotides encode oligopeptides that do not participate in the function of the protein, then it is difficult to keep these nucleotides constant as they will be more mutated and will cause disturbances in the function of the protein. Therefore, the compaction of binding sites is necessary. The second consequence of site linking is the creation of a competition between miRNA for the binding site. For example, if miR-2-4697-3p binds to RISC (RNA-induced silencing complex) and the resulting miRISC (miRNA with RISC complex) complex binds to mRNA, since it has a large ΔG value of -129 kJ/mole, the other miRNAs (miR-6-18378-3p and miR-6-18428-5p) are less likely to interact with mRNA than miR-2-4697-3p, since their ΔG is -117 kJ/mole. Thus, the effect of host genes on the expression of mRNA of ADAMTS7 gene by miR-6-18378-3p and miR-6-18428-5p is decreased. In addition to the free energy binding of miRNA binding to mRNA in this region, the concentration of miRNA plays an important role. At equal interaction energy, for example, for miR-6-18378-3p and miR-6-18428-5p the advantage will be for the miRNA in a higher concentration.

In the CDS mRNA 390A5 gene, there are four miRNA binding sites and three miRNAs form a 33 nt cluster, which is half of the total length 60 nt of these miRNAs. Of the seven binding sites, six sites are located in the 5'UTR mRNA CALM1 gene and four miRNAs form a cluster from 96 nt to 129 nt with a length 34 nt which consists of binding sites for miR-9-20317-3p, mir-13-32613-3p, mir-17-39416-3p and mir-19-43342-3p. The sum of the lengths of these miRNAs is 92 nt which is almost three times the length of the cluster. The 3'UTR miRNA of the CARD8 gene contains nine miRNA binding sites that are located without overlapping nucleotide sequences. In the 3'UTR mRNA CD40LG gene, there are 16 miR-574-5p binding sites, beginning at 24 CA-dinucleotides. These polysites are a kind of clusters of miRNA binding sites. The functional role of mononucleotides, dinucleotides and trinucleotides recurring in the 3'UTR is unknown. However, we established [4, 5], as in this case, that these repeats are binding sites for different miRNAs. The mir-574-5p with a length of 23 nt binds to 24 CA-dinucleotides with a value of -113 kJ/mole and at 93% of the complementarity of miRNA nucleotides with mRNA. The miRNA of ERRF11, FLI45453, HP31, HTRA1, ITGA2, PPAP2B, RAI1, SH2B3, SORT1, TMEM163 genes contain clusters of three miRNA binding sites. miRNA of genes which contain clusters comprising five or more miRNA binding sites are of particular interest. The 5'UTR, CDS, 3'UTR of mRNA HTRA1 gene bind to 21 miRNAs. At 5'UTR from 85 nt to 125 nt, there are 13 miRNA binding sites with a total binding site length of 460 nt which is 11 times the length of the cluster. Note that the average free energy of interaction of these miRNAs with miRNA is -132 kJ/mole. Another cluster of miRNA binding sites is detected in the 5'UTR mRNA MMP2 gene, which contains 11 miRNA binding sites with an average ΔG of -123 kJ/mole. The largest cluster of binding sites is found in the CDS mRNA NOTCH3 gene, which contains 36 sites with -127 kJ/mole and a total length of 836 nt. The cluster length is 88 nt which is 9.5 times less than the total length of 36 sites.

To use miRNA as markers, the associations of miRNA and mRNA of their target genes which interact with a value greater than -125 kJ/mole are proposed. There are 18 such associations of miRNA and mRNA. In addition, it is recommended to control the expression of CD40LG, HTRA1, MMP2, NOTCH3, SORT1, ZDHHC22 and ZFHX3 genes which have binding clusters in mRNA for ten or more miRNAs.

References:
According to the World Health Organization, traumatic brain injury (TBI) is currently the third most common cause of overall mortality. However, the status and role of the immune system in the formation of clinical manifestations, possible complications in victims with TBI is still a poorly studied problem. Goal. Evaluation of clinical and immunological data in patients with traumatic brain injury of various severity in the acute period of the disease.

Materials and methods. Three groups of patients were identified: patients with mild TBI (n = 23), with moderate to severe TBI (n = 23), and control group, relatively healthy individuals (n = 40).

Main survey methods:
- Anamnesis;
- Assessment of the physical status of patients;
- Assessment of the neurological status of patients;
- Neuropsychological examination
- Application of structural methods of neuroimaging
- Immunological examination: clinical blood test, determination of concentration in immunoglobulins in blood serum, level of T- and NK-cell differentiation, analysis of subpopulations of regulatory T cells, analysis of T-helper subpopulations, analysis of B cell subpopulations, determination of cytokine level (PV, PT, chemokines) in the cerebrospinal fluid.

Results. During the research, it was found that in patients with craniocerebral trauma of mild severity, the number of cells with the phenotype CD3+CD4+ (Th2, Th1), naive Th (Th17/Th22, Th1/Th17, DP Th17, Th17/Th22), CM Th (Th1/Th17, Th17/Th22), (p <0,05) increased in comparison with the parameters in the control group. Also, in patients of this group there was a decrease in the number of memory cells. Among them, CM Th cells (CXC5-CXCR3-CR6-CR4, Th17/Th22, Th17/Th17), EM Th (Th17/Th22, CXC5-CXCR3 + CCR6-CR4+) significantly decreased (p<0.05).

When conducting a comparative analysis of the indicators of the immune status, we found that in patients with craniocerebral trauma of mild severity, the number of cells with the phenotype CD3 + CD4+ (Th2), naive Th (Th17/Th22, Th1/Th17, DP Th17, Th17/Th22), CM Th (Th1/Th17, Th17/Th22), (p <0,05) increased in comparison with the parameters in the control group. Also, in patients of this group there was a decrease in the number of memory cells. Among them, CM Th cells (CXC5-CXCR3-CR6-CR4, Th17/Th22, Th17/Th17), EM Th (Th17/Th22, CXC5-CXCR3 + CCR6-CR4+) significantly decreased (p<0.05).

When conducting a comparative analysis of the indicators of the immune status, we found that in patients with craniocerebral trauma of mild severity, the number of cells with the phenotype CD3 + CD4+ (Th2), naive Th (Th17/Th22, Th1/Th17, DP Th17, Th17/Th22), CM Th (Th1/Th17, Th17/Th22), (p <0,05) increased in comparison with the parameters in the control group. Also, in patients of this group there was a decrease in the number of memory cells. Among them, CM Th cells (CXC5-CXCR3-CR6-CR4, Th17/Th22, Th17/Th17), EM Th (Th17/Th22, CXC5-CXCR3 + CCR6-CR4+) significantly decreased (p<0.05).

Prospects. The results of the study contribute to the construction of an optimal plan for therapeutic and diagnostic work aimed at timely early detection of complications and consequences of brain trauma. Based on the obtained research materials, algorithms for predicting intracranial purulent-inflammatory complications, disorders of liquor dynamics , as well as the risk of the formation of certain neurological syndromes at the end of the acute period of TBI will be developed.

References: