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piRNAs and miRNAs can bind to mRNA of *KLOTHO* and *FGF23* genes

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Abstract: The problem of increasing life expectancy is solved with the help of many medical and social areas. It has been established that piRNAs and miRNAs can significantly modify the expression of protein-coding genes by suppressing the translation process. The aim of this work was to establish the possibility of binding piRNAs and miRNAs with mRNA of the *KLOTHO* and *FGF23* genes, which promote health and increase life expectancy through participation in key metabolic processes. We used the MirTarget program, which determines the quantitative characteristics of complementary interactions of all piRNAs and miRNAs nucleotides with mRNA of the genes. piR-44682, piR-1940042, piR-3008660, piR-3215034, piR-6885965, piR-7980636 and one miRNA (ID00756.3p-miR) binding to the mRNA of the *KLOTHO* gene were found in one cluster of binding sites (BSs). piRNA-6890096 interacted with the mRNA of *KLOTHO* gene in a fully complementary manner using only canonical nucleotides. Among 17494 human genes, target genes interacting with five piRNAs that bind to the mRNA of *KLOTHO* gene were identified. mRNA of the *AFF2, BCL2L11, CPT1A, DAZAP1, NDRG3, SKIDA1, WBP4, ZIC5, ZSWIM6* genes interacted with piR-3215034 and piR-6885965, which formed clusters of BSs located in 5'UTR, CDS and 3'UTR. The piR-576442, piR-1501557, piR-1845735, piR-2069834, and piR-3029987 had BSs in the mRNAs of the *FGF23* gene, located only in the 3'UTR. It is proposed to use piRNAs and miRNAs as regulators of the expression of *KLOTHO* and *FGF23* anti-aging genes.

Keywords: piRNA; miRNA; *KLOTHO* gene; *FGF23* gene; anti-aging gene

1. Introduction

Chronic kidney disease (CKD) is a set of symptoms resulting from a decrease in the number and function of nephrons. This leads to a violation of the excretory and endocrine activity of the kidneys. As a result, changes in the homeostasis of the internal environment occur, which manifests itself in the violation of many metabolic processes: water-electrolyte, protein, carbohydrate, lipid metabolism. There is an imbalance in the expression of the human genome. As a result, the work of all body systems is disrupted: cardiovascular, respiratory, digestive, hematopoietic and others. All these processes are based on genetically determined changes, therefore, for accurate diagnosis of diseases, it is necessary to study the key molecular genetic foundations of metabolic disorders. Recently, after sequencing of the human genome, it has become possible to establish molecular genetic relationships between various metabolic and physiological processes. Using the example of *KLOTHO* gene [1], we tried to find out what molecular mechanisms in CKD [2] may be involved in the expression of genes involved in the synthesis of proteins responsible for

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the manifestation of various diseases. It has been shown that the *KLOTHO* gene has great potential for future therapeutic purposes in both acute and chronic kidney diseases [3–5]. The *KLOTHO* gene is highly expressed in the kidney (RPKM 80.6) and placenta (RPKM 14.7). A decrease in the synthesis of this protein is observed in patients with CKD, which may underlie diabetes [6–9]. Mutations in KLOTHO protein are associated with aging and loss of bone mass and phosphorus metabolism [10, 11]. Several works are devoted to elucidating the role of *KLOTHO* in oncological diseases [12-15]. The KLOTHO protein was initially introduced as an anti-aging molecule [1]. Its deficiency significantly reduces lifespan, and its overexpression extends it and protects against various pathological phenotypes, especially renal disease. Soluble KLOTHO is an anti-aging protein mainly secreted by the kidneys [16] and has been used in diagnostics and therapy [17-22].

In recent years, the relationship between KLOTHO and FGF23 proteins has been actively studied. This association is seen in chronic kidney disease and other diseases. The KLOTHO and FGF23 proteins have been studied in renal progression, cardiovascular disorders, and mortality in CKD [23]. The effect of dietary phosphorus restriction on FGF23 and KLOTHO levels in patients with stages 1-2 CKD was studied [24]. It has been established that *KLOTHO* and *FGF23* genes regulate calcium and phosphorus metabolism [25]. The prognostic value of serum KLOTHO and FGF23 proteins was revealed in patients with multiple myeloma [26]. Research was made of the role of *KLOTHO* and *FGF23* genes in cardiovascular disorders in diabetic patients with chronic threatening limb ischemia [27]. The involvement of KLOTHO and FGF23 proteins in phosphorus homeostasis has been established [28]. Data have been obtained on the disruption of the FGF23 and KLOTHO axis in subjects with diabetes mellitus [29]. In recent years, in connection with kidney transplantation, the problem of disturbance of KLOTHO and FGF23 systems has arisen [30]. Small molecule inhibitors of FGF23 signaling have been identified using KLOTHO molecular docking [31]. A correlation has been established between a comparative analysis of FGF23 and cardiovascular diseases in individuals with chronic kidney disease, hypertensive patients, and healthy people [32]. The KLOTHO and FGF23 proteins were used as markers of calcium metabolism [33]. The role of KLOTHO and FGF23 in cardiovascular parameters in diabetic patients with chronic threatening limb ischemia was studied [34]. The FGF23 and KLOTHO proteins have been shown to play an important role in bone and vascular disease in chronic kidney disease [35]. Exercise-mediated activation of the canonical WNT signaling pathway can lead to bone formation and improved levels of the KLOTHO and FGF23 proteins [36]. The KLOTHO has been shown to act either as an obligate FGF23 co-receptor or as a soluble pleiotropic endocrine hormone. With age, human kidney function often deteriorates, and KLOTO levels decrease [20]. FGF23 is a phosphate-regulating protein that is elevated in patients with chronic kidney disease and is associated with cardiovascular mortality, the role of KLOTO has been considered [37]. *FGF23* and *KLOTHO* genes are associated with trabecular bone index but not with bone mineral density in early stages of chronic kidney disease [38].

Materials and Methods

The nucleotide (nt) sequence of *KLOTHO*, *FGF23*, *AFF2*, *BCL2L11*, *CPT1A*, *DAZAP1*, *NDRG3*, *SKIDA1*, *WBP4*, *ZIC5* and *ZSWIM6* genes were downloaded from National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov, 2022). RPKM of genes were extracted from NCBI. The nucleotide sequences of 480 thousand piRNAs were taken from Wang et al [39]. The 3707 miRNAs were taken from article Londin et al [40], the 2567 mature miRNA sequences were taken from miRBase database (http://mirbase.org) and the 1036 miRNAs from article Backes et al [41]. The piRNA and miRNA binding sites (BSs) in mRNA were predicted using the MirTarget program [42]. This program predicts the following features of piRNA and miRNA binding to mRNA: (a) the initiation of piRNA and miRNA binding to the mRNA from the first nucleotide of the mRNA; (b) the localization of the piRNA and miRNA BSs in the 5′-untranslated region (5′UTR), coding domain sequence (CDS), and 3′-untranslated region (3′UTR) of the mRNAs; (c) the schemes of nucleotide interactions between piRNAs and miRNA with

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mRNA; (d) the free energy of the interaction between piRNAs, miRNA and the mRNA (ΔG, kJ/mol); and the ratio ΔG/ΔGm (%) is determined for each site. ΔGm equals the free energy of piRNA and miRNA binding with its fully complementary canonical nucleotide sequence. Only piRNAs and miRNA whose nucleotides interacted with mRNA using canonical (G-C and A-U) and noncanonical (G-U and A-C) nucleotides with a given ΔG value were selected from the calculated data. The MirTarget program finds hydrogen bonds between piRNAs and miRNA with mRNA according to the physicochemical characteristics of nucleotide interactions [44-48]. MirTarget differs from other programs in terms of finding piRNA BSs on mRNA in the following: it considers the interaction of piRNA and miRNA with mRNA over the entire piRNA and miRNA sequence; it considers noncanonical pairs G–U and A–C; and it calculates the free energy of the interaction of the piRNAs and miRNA with mRNA. Note that the G, A, C, and U nucleotides, which comprise the RNA structure of microorganisms, plants, and animals, interact identically under equal conditions. Therefore, the physicochemical properties of canonical and noncanonical nucleotide pairs given above do not require additional proof of the previously established physicochemical characteristics of their interaction [43-47].

Results

Of the entire piRNA database, only seven piRNAs (piR-44682, piR-1940042, piR-3008660, piR-3215034, piR-5194426, piR-6885965, piR-7980636) bound to the mRNA of the *KLOTHO* gene (Figure 1). Seven piRNAs each had one BSs and piR-6885965 had two BSs. All BSs were located with a partial overlap of nucleotides, forming a cluster of BSs from 33 nt to 71 nt, only 39 nt long. Such an arrangement of BSs piRNAs in mRNA leads to competition between them for binding to mRNA. piR-6890096 interacts with 3′UTR in a completely complementary manner (the value of ΔG/ΔGm is 100%), which, at its concentration comparable to that of mRNA, will lead to inhibition of the translation process. The *KLOTHO* gene is many times more strongly expressed in the kidneys than in other organs, and a high concentration of piRNA is required to suppress its synthesis. Of the 7310 miRNAs, only ID00756.3p-miR bound to the mRNA of the *KLOTHO* gene (Figure 1). ID00756.3p-miR can only interact with mRNA of the *KCNN2, NOTCH3,* and *ZNF592* genes. The ID00756.3p-miR was in the BSs cluster of seven piRNAs located in the CDS mRNA *KLOTHO* gene, therefore, it competed with them.

piRNA; start of BS, nt; mRNA region; ΔG,kJ/mol; ΔG/ΔGm,%; piRNA length, nt

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piR-1940042;33;CDS;-147;95;25
5'-CGCCCGCGGCCGCCGCCGCCGUCGC-3'
   |||||||||||||||||||||||||
3'-GCGGACGUCGGCGGCGGCGGCGGCG-5'
ID00756.3p-miR;34;CDS;-125;91;23
5'-GCCCGCGGCCGCCGCCGCCGUCG-3'
   |||||||||||||||||||||||
3'-CAGGCAUCAGCGGCGGCGGCGGC-5'
piR-44682;37;CDS;-149;91;26
5'-CGCGGCCGCCGCCGCCGUCGCUGUCG-3'
   |||||||||||||||||||||||||||
3'-GCGCCGGCGGCGGCGGUGGCGGCGGC-5'
piR-3215034;41;CDS;-151;91;27
5'-GCCGCCGCCGCCGUCGCUGUCGCUGCU-3'
   |||||||||||||||||||||||||||
3'-CGGCGGCGGCGGCGGCGACGACGACGG-5'
piR-6885965;42;CDS;-134;94;24
5'-CCGCCGCCGCCGUCGCUGUCGCUG-3'
   ||||||||||||||||||||||||
3'-GGCGGCGGUGGCAGCGACGACGAC-5'
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piR-7980636;44;CDS;-142;91;28
5'-GCCGCCGCCGUCGCUGUCGCUGCUGCUG-3'
   ||||||||||||||||||||||||||||
3'-UGACGGCAGCAGCGACAGCGGCGAUGAU-5'
piR-3008660;46;CDS;-134;91;26
5'-CGCCGCCGUCGCUGUCGCUGCUGCUG-3'
   ||||||||||||||||||||||||||
3'-ACGGCAGCAGCGACAGCGGCGAUGAU-5'
piR-5194426;47;CDS;-132;93;25
5'-GCCGCCGUCGCUGUCGCUGCUGCUG-3'
   |||||||||||||||||||||||||
3'-CGGCAGCAGCGACAGCGGCGAUGAU-5'
piR-6885965;48;CDS;-132;93;24
5'-CCGCCGUCGCUGUCGCUGCUGCUG-3'
   ||||||||||||||||||||||||
3'-GGCGGCGGUGGCAGCGACGACGAC-5'
piR-6890096;2890;3'UTR;-153;100;30
5'-GAUUUUGUCCAGAAGAAUUCACCGUGUGUA-3'
   ||||||||||||||||||||||||||||||
3'-CUAAAACAGGUCUUCUUAAGUGGCACACAU-5'
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Figure 1. Schemes of interaction of piRNA and miRNA with mRNA of the *KLOTHO* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green. .

Out of 8405000 piRNAs, only eight piRNAs were identified, of which piRNA-6885965 has two BSs in the mRNA of *KLOTHO* gene. piRNA-6890096 interacted with the mRNA of *KLOTHO* gene in a completely complementary manner using only canonical nucleotides. Out of 7310 miRNAs, only ID00756.3p-miR could bind to the mRNA of *KLOTHO* gene. The piRNA (except piRNA-6890096) and miRNA bound in a 39 nt region (Figure 1). Here and henceforth, piRNA BSs with overlapping nucleotide sequences will be referred to as BSs clusters. As a result of this arrangement, piRNA BSs will compete with each other for interaction with the target gene mRNA, and as a result, only one piRNA that binds mRNA more strongly than others or is in a significantly higher concentration will be bound for a longer time than other piRNAs. The formation of clusters of piRNA and miRNA BSs in mRNA is a kind of guarantee of the nonrandom association of small RNAs and their target genes.

The results obtained give hope for the possibility of specific regulation of *KLOTHO* gene expression using piRNAs and miRNAs. However, given that some miRNAs and piRNAs can bind to mRNAs of several or even hundreds of human genes [48-51], it is necessary to identify human genes that may be affected by piRNAs and miRNAs that act on mRNAs of *KLOTHO* gene. That is, it is necessary to identify the possible side effect of these piRNA and miRNA on the expression of all human genes if they are used as therapeutic drugs. To this end, we studied the possible binding of ID00756.3p-miR and nine piRNAs to 17484 human genes.

The largest number of target genes was found for piR-3215034 and piR-6885965. In the mRNA of the *AFF2* gene, for each of these piRNAs, Eleven and ten BSs were found, respectively, located in one cluster (Figure 2). The beginning of all BSs were located in the 5'UTR through three nucleotides. The free energy of piR-3215034 binding to the mRNA of the *AFF2* gene varied from -155 kJ/mol to 161 kJ/mol, and the ΔG/ΔGm value varied from 94% to 97%. During the interaction of piR-3215034 with mRNA, canonical nucleotide pairs were involved in the last two BSs, except for two C-A bonds. These results indicate a high efficiency of piR-3215034 influence on *AFF2* gene expression. piR-6885965 is 24 nt long and, therefore, the free energy of interaction with the mRNA of the *AFF2* gene was lower. Since the BSs of these piRNAs are in the same cluster, piR-3215034 has the advantage of binding. However, at a significantly higher concentration of piR-6885965, it will have an advantage in binding over piR-3215034. Therefore, when determining the effectiveness of the action of piRNAs on translation, one should take into account the free energy of their interaction with mRNA and the concentration of competing piRNAs.

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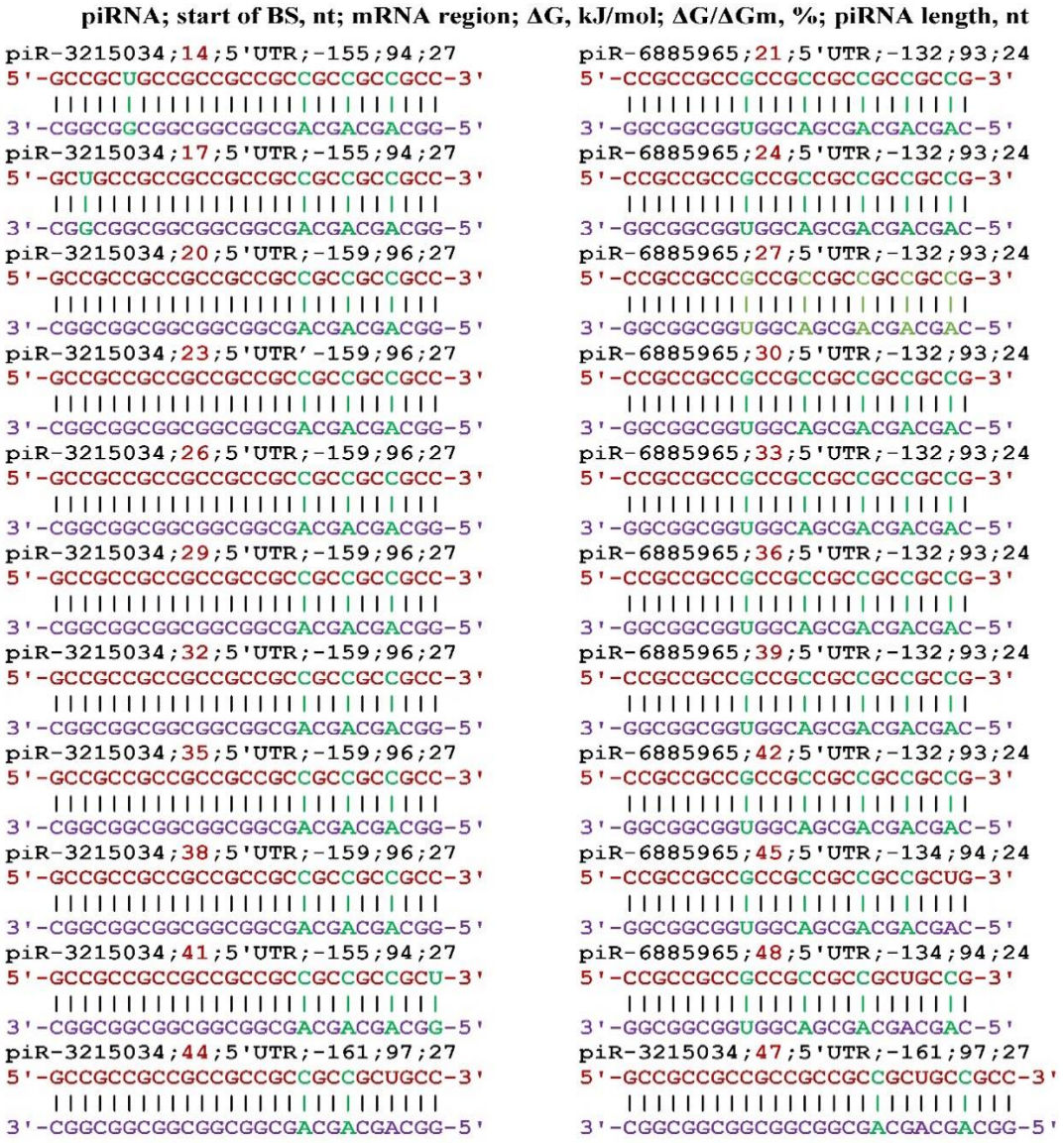

Figure 2. Schemes of interaction of piR-3215034 and piR-6885965 with the mRNA *AFF2* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

Since the *AFF2* and *KLOTHO* genes are targets for piR-3215034 and piR-6885965, it is necessary to compare the possible effect of piRNAs on these genes. It should be noted that the *AFF2* gene is involved in the development of squamous cell carcinoma [52], sinonasal tract cancer [53], thoracic carcinoma [54], carcinomas of head and neck [55], and in renal cell carcinoma [56, 57]. Therefore, by suppressing the oncogenesis caused by the *AFF2* gene with piR-3215034 and piR-6885965, the expression of *KLOTHO* gene will be simultaneously suppressed.

Several publications have established the involvement of the *BCL2L11* gene in oncogenesis [58–61]. For piR-3215034, 12 BSs were identified, which form a cluster of BSs from 55 nt to 114 nt, 60 nt long (Figure 3). piR-6885965 also binds in this BSs cluster, but with a ΔG/ΔGm value of less than 90%, which is below the selection criterion for significant piRNAs.

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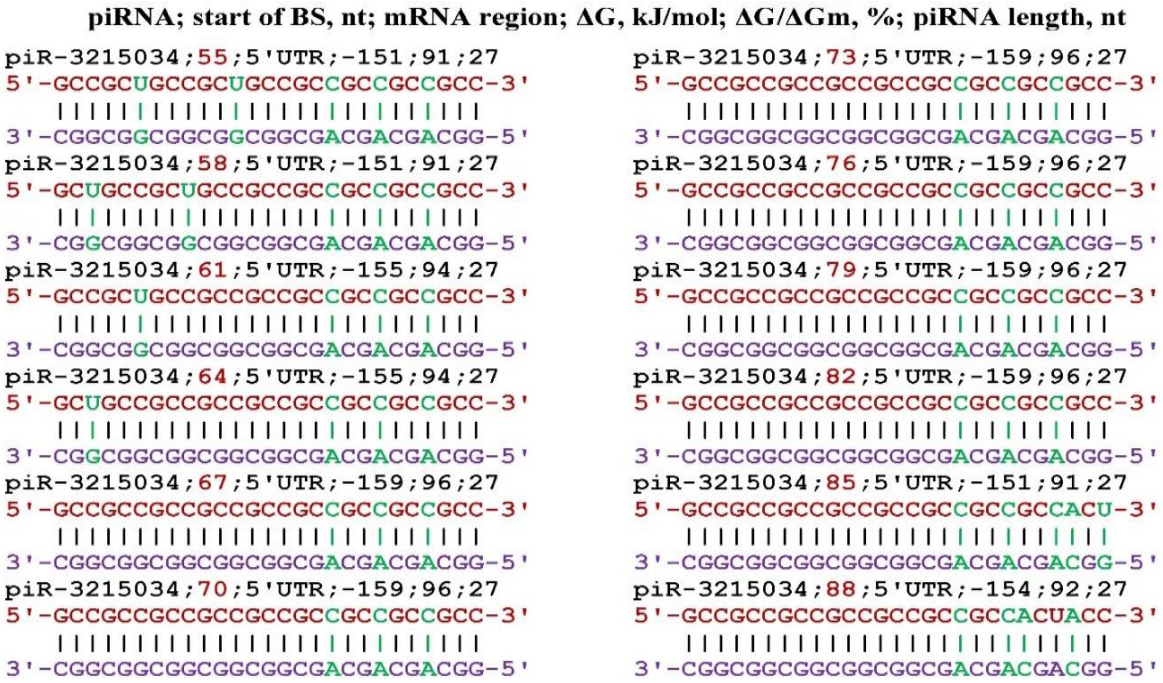

Figure 3. Schemes of interaction of piR-3215034 with the mRNA of the *BCL2L11* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

The *CPT1A* gene is involved in fatty acid metabolism and manifests its effect during oncogenesis, diabetes, and cardiomyopathy [62–67]. Figure 4 shows the interaction schemes of piR-3215034 and piR-6885965 with mRNA of *CPT1A* gene, which show that their BSs are located in the same cluster from 99 nt to 137 nt. piR-3215034 interacts with the mRNA of *CPT1A* gene with a ΔG/ΔGm value of 99%, i.e., almost canonical base pairs are formed.

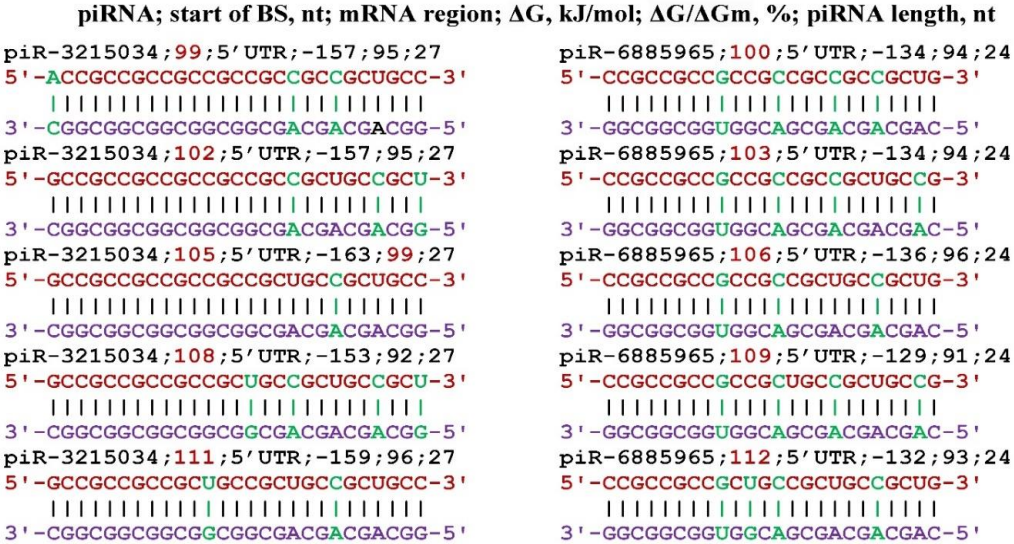

Figure 4. Interaction schemes of piR-3215034 and piR-6885965 with mRNA of the *CPT1A* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

High expression of the *DAZAP1* gene is observed in hepatocarcinoma and can serve as a prognostic marker of the disease. Knockdown of *DAZAP1* small interfering RNA markedly inhibited proliferation, migration, and invasion of hepatocarcinoma cells [68, 69]. piR-3215034 and piR-6885965 can repress *DAZAP1* mRNA translation (Figure 5). The

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results obtained indicate that these piRNAs can significantly influence the expression of the *DAZAP1* gene (Figure 5). It should be noted that both piRNAs bind to the mRNA of gene in the same cluster of BSs located in the 5'UTR, i.e. they can stop protein synthesis before the translation process. The *DAZAP1* gene is highly expressed in testis (RPKM 19.6), appendix (RPKM 11.8).

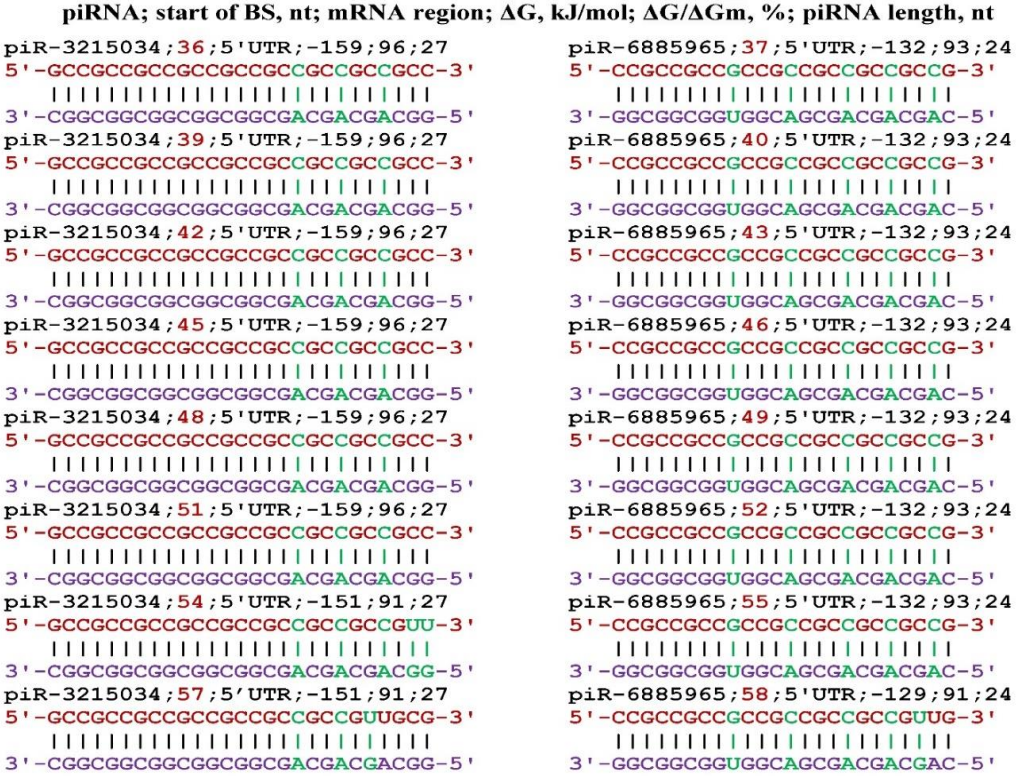

Figure 5. Schemes of interaction of piR-3215034 and piR-6885965 with mRNA of the *DAZAP1* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

A number of publications have shown the involvement of the *NDRG3* gene in the development of oncogenesis, and in most cases, increased expression is observed in cancer of various organs [70-76]. Therefore, it is important to know whether piR-3215034 and piR-6885965 can suppress the expression of the *NDRG3* gene. The results shown in Figure 6 indicate that *NDRG3* gene expression can be downregulated by these piRNAs.

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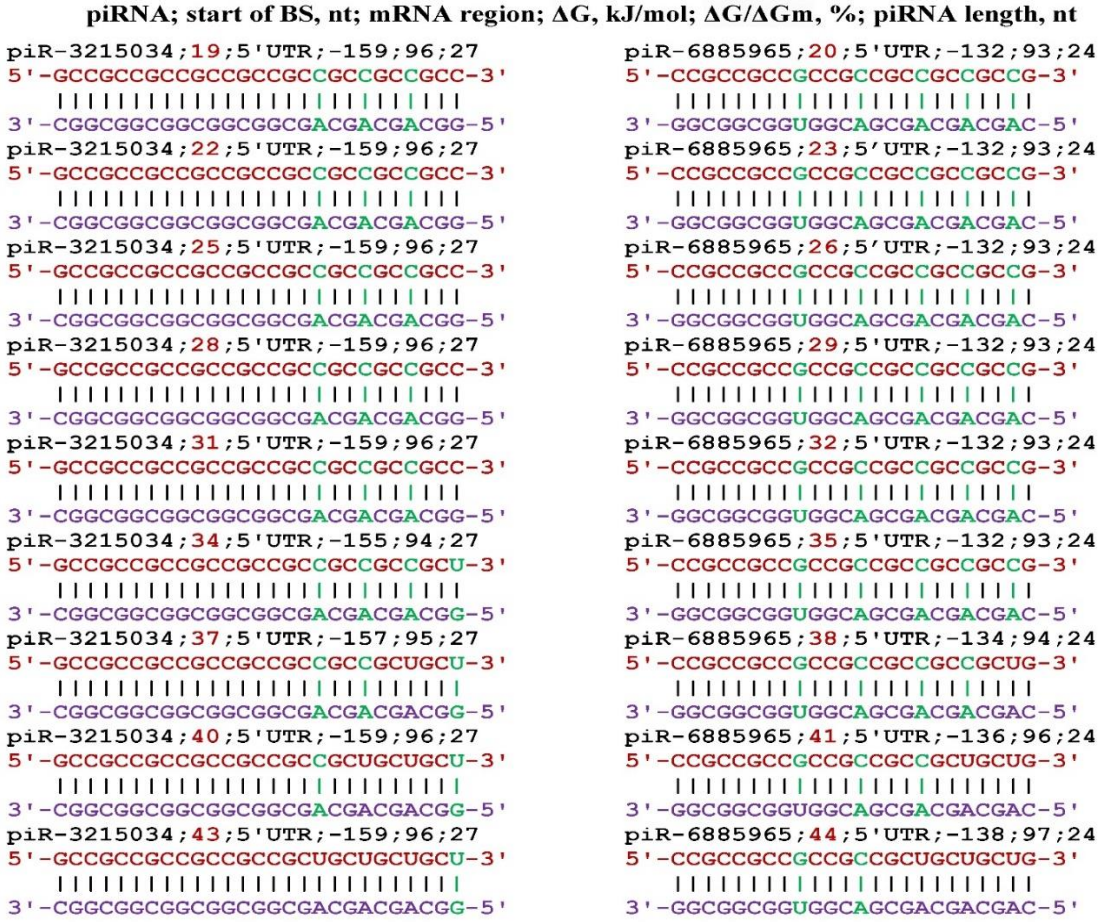

Figure 6. Schemes of interaction of piR-3215034 and piR-6885965 with mRNA of the *NDRG3* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

The *RHOT1* gene is involved in the modification of the development of breast cancer [77], pancreatic cancer [78, 79], non-small cell lung cancer [80], hepatocellular carcinoma [81], and the risk and occurrence of Parkinson's disease [82]. Figure 7 shows the interaction schemes of piR-3215034 and piR-6885965 with mRNA of the *RHOT1* gene. Both piRNAs have eight BSs in the 5'UTR of the mRNA of the *RHOT1* gene located in the same BSs cluster from the first nucleotide to 46 nt. The next BS was located after three nucleotides, and the free energy of interaction was the same in each of the sites for both piRNAs. Therefore, the value of ΔG/ΔGm was the same for each piRNA.

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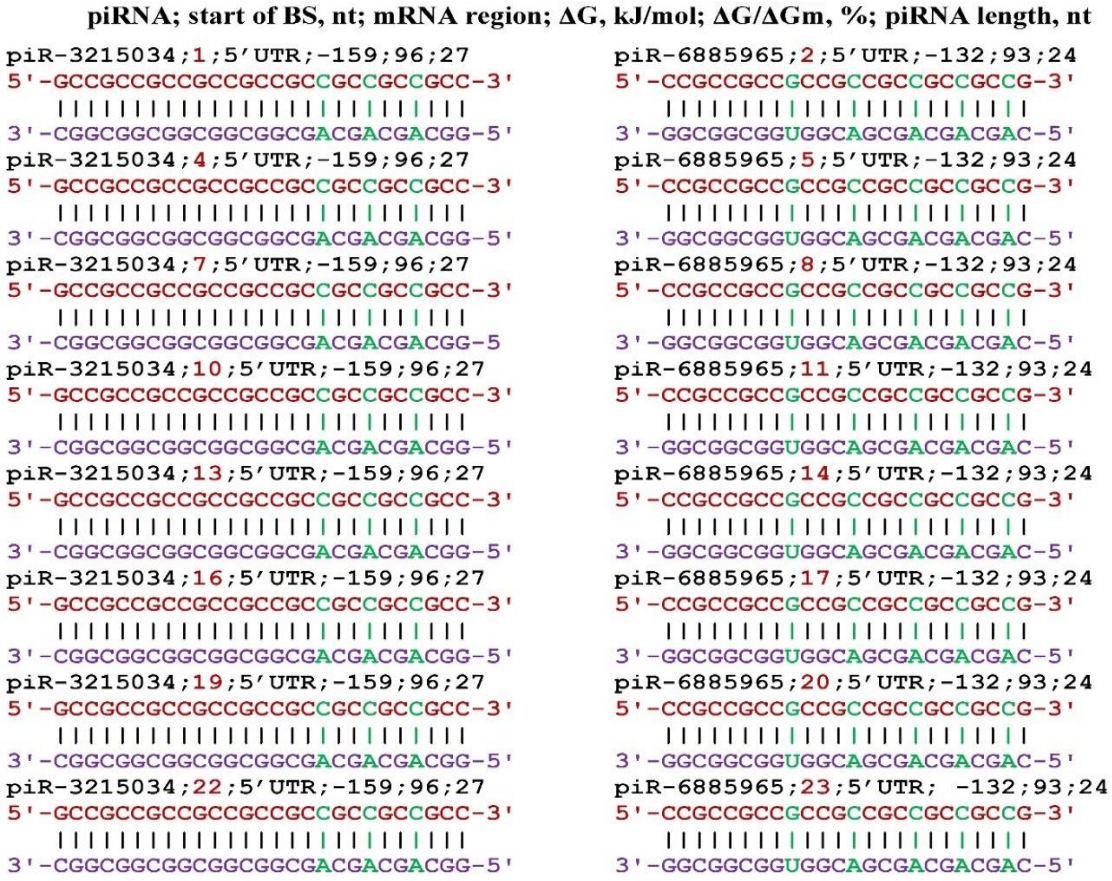

Figure 7. Interaction schemes of piR-3215034 and piR-6885965 with mRNA of the *RHOT1* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

SKIDA1 was significantly overexpressed in all molecular subgroups, except for only two subgroups of acute myeloid leukemia. In validation analyses, *SKIDA1* was associated with a higher sensitivity and specificity in acute myeloid leukemia. We highlight that *SKIDA1* is one of the promising markers, which has consistent overexpression among several types of acute leukemia. *SKIDA1* identified gene in breast cancer [83]. For piR-3215034, 12 BSs were identified in the mRNA of the *SKIDA1* gene (Figure 8). At two sites, piR-3215034 interacts with mRNA almost as complementary as possible, since the ΔG/ΔGm value is 99%. Cluster of piR-3215034 BSs with 61 nt in length guarantees the binding of two of these 27 nt piRNAs at once.

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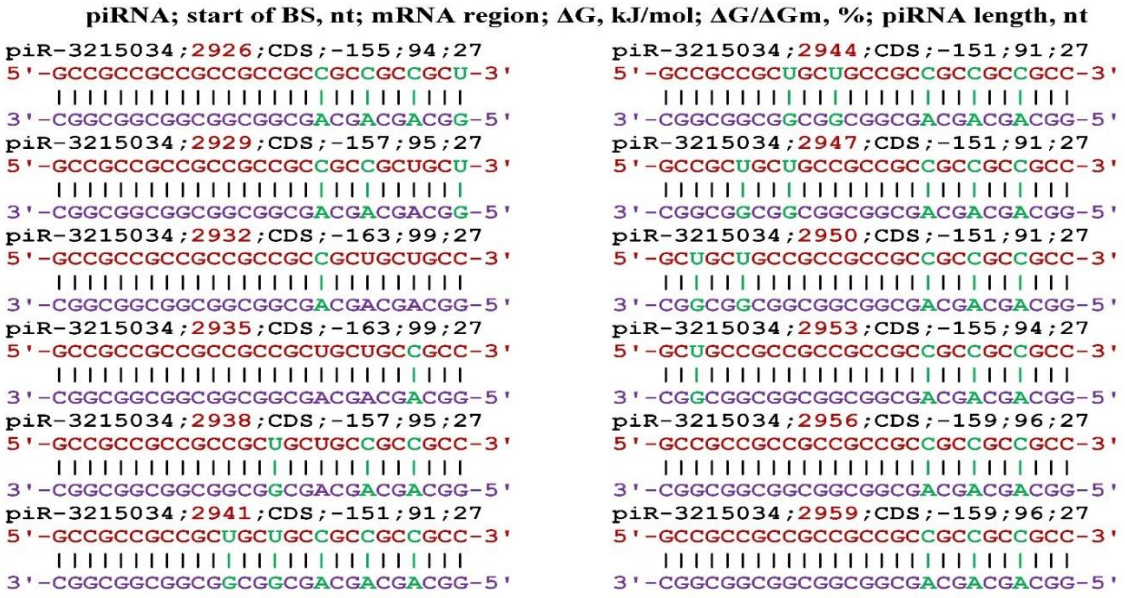

Figure 8. Schemes of interaction of piR-3215034 with the mRNA of the *SKIDA1* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

The *WBP4* gene (synonymous name *FBP21*) is involved in splicing and therefore affects the maturation of the mRNA of many genes involved in metabolism [84-86]. Eight piR-3215034 and six piR-6885965 binds to the mRNA of the *WBP4* gene (Figure 9). At position 94 nt, the 5'UTR of piR-3215034 binds only via canonical base pairs. piR-6885965 binds at the 94 nt position, also with a high ΔG/ΔGm value of 97%.

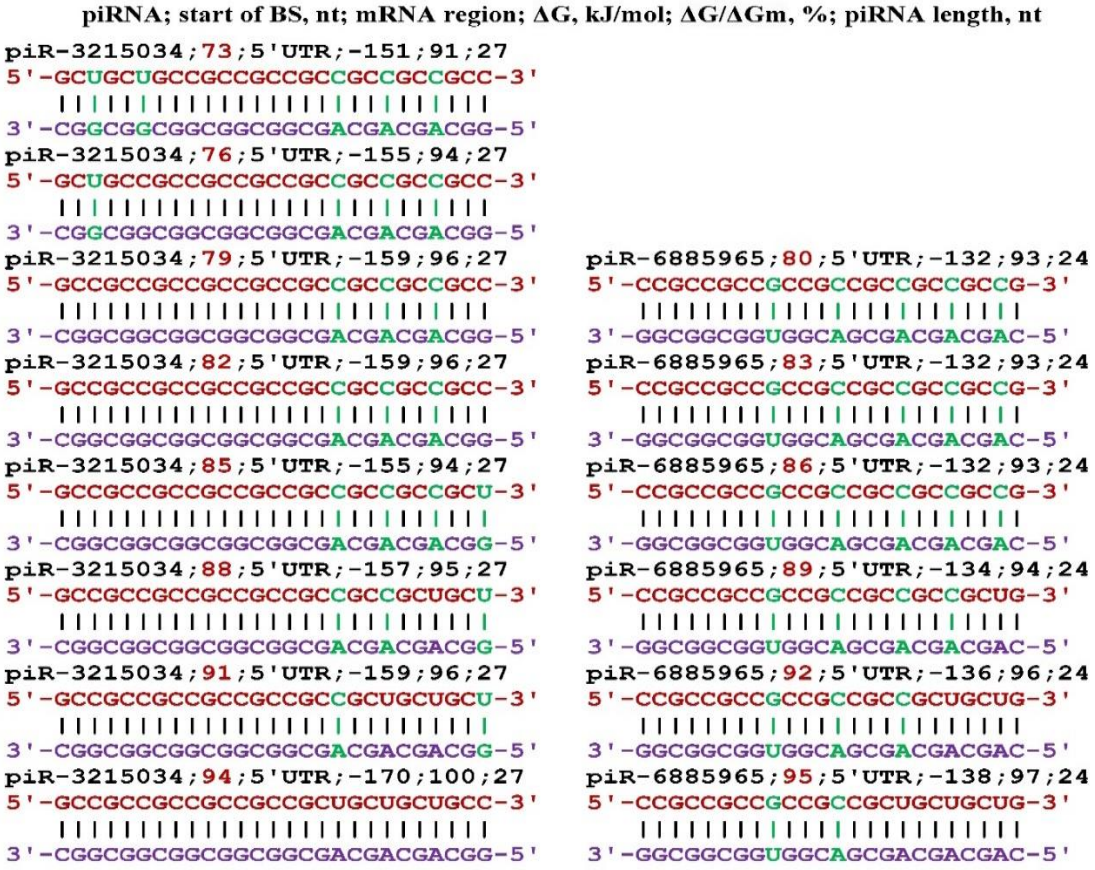

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Figure 9. Schemes of the interaction of piR-3215034 and piR-6885965 with mRNA of the *WBP4* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

The *ZIC5* gene acts as a transcriptional repressor. Increased expression of this gene is observed in various types of human cancer and may contribute to cancer progression [87-90]. Figure 10 shows the interaction schemes of piR-3215034 and piR-6885965 with mRNA of the *ZIC5* gene, which show a high degree of influence of piR-3215034 on translation. At four positions, the ΔG value is -196 kJ/mol and the ΔG/ΔGm ratio is 96% of the maximum value. For piR-6885965, there are only four BSs in the cluster with a ΔG/ΔGm value greater than 90%.

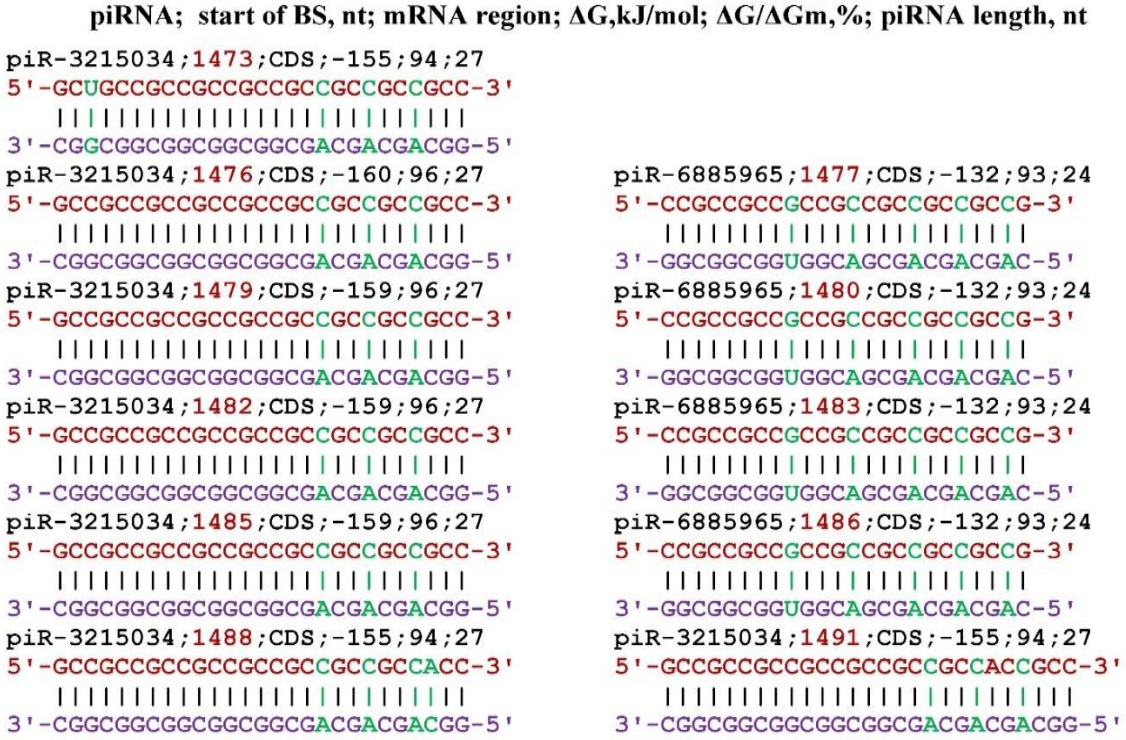

Figure 10. Schemes of interaction of piR-3215034 and piR-6885965 with the mRNA of the *ZIC5* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

The transcription factor encoded by the *ZSWIM6* gene is synthesized in the brain and can affect the expression of a number of genes. Mutations in this gene lead to malformations of the brain [91-93]. Figure 11 shows the binding schemes of piR-3215034 and piR-6885965 to the mRNA of the *ZSWIM6* gene. piR-3215034 had seven BSs forming a cluster and piR-6885965 had only three BSs in the same cluster. Transcription factors are difficult to study because the product of their activity can be diverse and difficult to control. The importance of their biological role is undoubted.

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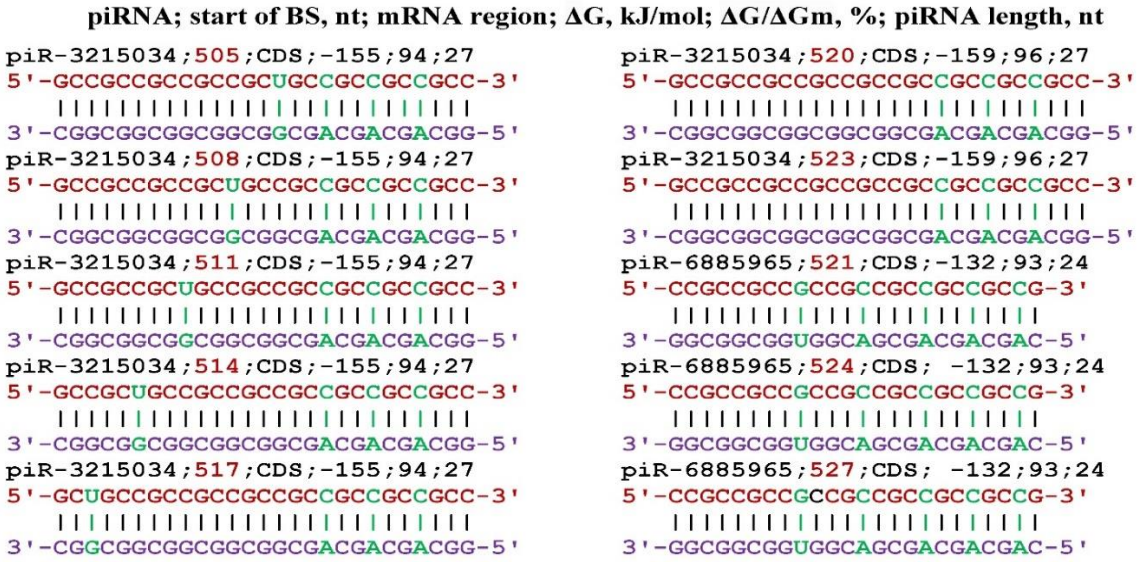

Figure 11. Schemes of interaction of piR-3215034 and piR-6885965 with the mRNA of the *ZSWIM6* gene. Note: The mRNA nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green.

The *FGF23* gene is a member of a large family of fibroblast growth factors [94], which is most associated with the *KLOTHO* gene in anti-aging processes [95]. A number of publications have examined the relationship of *FGF23* and *KLOTHO* genes in physiological processes and in various diseases [96-104]. In this regard, we studied the possible effect of piRNA on the expression of the *FGF23* gene. Only piR-576442, piR-1501557, piR-1845735, piR-2069834, piR-3029987 could bind to the mRNA of the *FGF23* gene, the interaction schemes of which are shown in Figure 12. All BSs of these piRNAs were located in the 3'UTR at a considerable distance from each other, i.e., BSs did not form BS clusters.

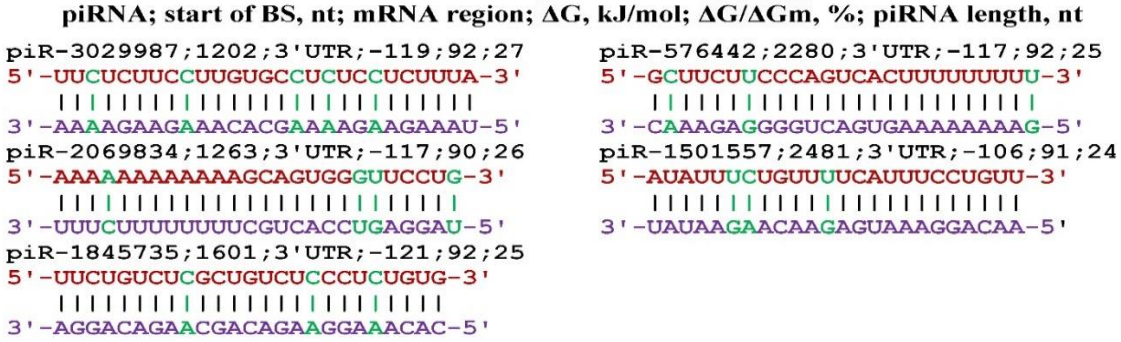

Figure 12. Schemes of piRNA interaction with the mRNA of the *FGF23* gene. Note: The mRNA of nucleotides are highlighted in red. The piRNA nucleotides that form canonical pairs with gRNA are highlighted in violet and noncanonical pairs are highlighted in green. .

Discussion

The piRNA groups that repress *KLOTHO* expression can repress the expression of oncogenes. Therefore, if the concentration of these piRNAs is reduced, then life expectancy will increase, but this will increase the likelihood of oncogenesis. Therefore, in order to increase lifespan, it is necessary to reduce the concentration of only piRNA, which suppresses the expression of *KLOTHO* gene with high selectivity. Some authors call KLOTHO protein a hormone, others an anti-inflammatory agent. The latter function has reason to be, because the composition of KLOTHO protein has an increased content of phenylalanine compared to conventional proteins and is comparable to antioxidant proteins.

Groups of piRNAs that inhibit *KLOTHO* expression can inhibit the expression of oncogenes. Consequently, if the concentration of these piRNAs is reduced, longevity may increase due to increased KLOTHO protein synthesis, but this will increase the likelihood

of oncogenesis and other diseases. Common piRNAs for several genes represent a pool of gene expression regulators and maintain the expression homeostasis of these target genes. A change in the expression of any of these genes will cause a redistribution of the degree of influence of the piRNAs on other genes. Therefore, to increase longevity it is necessary to reduce the concentration of only piRNA-6890096 which suppresses the expression of the *KLOTHO* gene with high selectivity. Some publications have identified an anti-inflammatory effect of the KLOTHO protein. The latter function has reason to be because the KLOTHO protein has an increased phenylalanine content (6%) compared to the phenylalanine content of conventional proteins and comparable to antioxidant proteins.

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