Regulatory network of competitively interacting RNAs and effectiveness of rectal tumors radiotherapy

Regulační síť kompetitivně interagujících RNA a účinnost radioterapie u nádorů rekta

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Summary

Background: Currently, rectal tumors radiotherapy effectiveness reaches an acceptable level only in a small number of patients (they have a complete clinical response), which is associated with the formation of malignant cells radioresistance. A comprehensive study that integrates various epigenetic parameters would explain a number of molecular mechanisms of rectal tumor cells radioresistance and identify new biomarkers. In the last decade, using high-throughput sequencing, the competitively interacting RNAs regulatory network (long non-coding RNAs, miRNAs and mRNAs) has been shown. **Purpose:** The aim of the study was to analyze the features of competitively interacting RNAs regulatory network functioning in patients with rectal cancer who are radioresistant and sensitive to radiotherapy. The study was performed on 500 patients with diagnosed rectal cancer. Radiotherapy was performed on a Novalis TX linear particle accelerator according to the standard protocol (single focal dose 2.4 Gy, total focal dose 54.0 Gy). Total RNA preparations were isolated from paired biopsy fragments of tumor and non-tumor tissues of the rectum (obtained by video-colonoscopy). The relative abundance of mRNA, miRNA and lncRNA transcripts was assessed by the RT-qPCR method. Using bioinformatic analysis, the probability of potential interactions between the investigated mRNA, miRNA and lncRNA was determined. It has been shown that the effectiveness of radiotherapy depends on the level of miRNA (miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-138 -1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA--1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p) and lncRNA (XIST, HELLPAR, NEAT1, AC008124. 1, LINC01089, LINC01547, and VASH1-AS1) expression, which regulate the DNA repair system (H2AX, RBBP8) and apoptosis (BCL2). **Conclusion:** A comprehensive study of competitively interacting RNAs regulatory network and radiotherapy effectiveness of rectal tumors made it possible to establish the mechanisms of radioresistance formation and its biomarkers.

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Souhrn

Východiska: V současné době dosahuje efektivita radioterapie u nádorů rekta přijatelné úrovně pouze u malého počtu pacientů (mají kompletní klinickou odpověď), což je spojeno se vznikem radiorezistence maligních buněk. Komplexní studie, která integruje různé epigenetické parametry, by vysvětlila řadu molekulárních mechanizmů radiorezistence rektálních nádorových buněk a identifikovala seznam nových biomarkerů. V posledním desetiletí byla pomocí vysoce výkonného sekvenování prokázána existence regulační sítě kompetitivně interagujících RNA, skládající se z dlouhých nekódujících RNA, mikroRNA a mRNA. *Cíl:* Cílem studie bylo analyzovat rysy fungování regulační sítě kompetitivně interagujících RNA u pacientů s karcinomem rekta, kteří jsou radiorezistentní a citliví na radioterapii. *Materiál a metody:* Studie byla provedena na 500 pacientech s diagnózou karcinomu rekta. Radioterapie byla prováděna na lineárním urychlovači částic Novalis TX podle standardního protokolu (jednorázová fokální dávka 2,4 Gy, celková fokální dávka 54,0 Gy). Preparáty celkové RNA byly izolovány z párových bioptických fragmentů podmíněně normálních a nádorových tkání rekta (získaných videokolonoskopií). Relativní množství transkriptů mRNA, microRNA a lncRNA bylo hodnoceno metodou RT-qPCR. Bioinformatická analýza byla použita ke stanovení pravděpodobnosti potenciálních interakcí mezi zkoumanou mRNA, mikroRNA a lncRNA. Ukázalo se, že účinnost radioterapie závisí na úrovni exprese mikroRNA (miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-138 -1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p ; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p) a lncRNA (XIST, HELLPAR, NEAT1 HELLPAR, NEAT1, AC008124.1, LINC01089, LINC01547 a VASH1-AS1), které regulují systém opravy DNA (H2AX a RBBP8) a apoptózu (BCL2). *Závěr:* Komplexní studium vlastností regulační sítě kompetitivně interagujících RNA a účinnosti radioterapie u nádorů rekta umožnilo stanovit mechanizmy vzniku radiorezistence a její prediktory.

Klíčová slova

karcinom rekta – účinnost radioterapie – reparace DNA – apoptóza – síť kompetitivně interagujících RNA – genová exprese – mikro-RNA – dlouhé nekódující RNA

Introduction

In 2020, more than 339 000 deaths from rectal cancer (RC) were registered, which makes this nosology the leading one in terms of mortality among many oncological diseases [1]. Chemoradiotherapy is currently used to relieve symptoms and local control of RC. However, it is important to note that not every patient responds positively to radiotherapy (RT), which is associated with radioresistance/radiosensitivity of malignant cells. Despite the long-term improvement RT algorithms for RC, it is still difficult to predict the volume of clinical response [2]. Over the past 30 years, an impressive amount of research has been carried out to study the mechanisms of radioresistance and a significant list of its molecular markers has been proposed, none of which, however, has entered clinical practice. At present, more than ever before, it is relevant to conduct a comprehensive study that integrates various molecular parameters and allows both to explain a number of molecular mechanisms of radioresistance and to propose a list of new markers.

In the last decade, the complexity of the human genome has been identified using high-throughput RNA sequencing. The existence of a regulatory network of competing endogenous RNA (ceRNA), consisting of long non-coding RNA (lncRNA), microRNA (miRNA), and mRNA, has been shown [3].

MicroRNAs are short, non-coding RNAs that regulate gene expression by catalyzing the destruction of mRNA or by inhibiting the translation of mRNA into protein. MicroRNAs make a significant contribution to the initiation and development of various molecular events, including the initiation of oncogenesis, progression and metastasis of tumors, which makes microRNAs potential biomarkers for assessing the progression and prognosis of intestinal malignant tumors. Although miRNAs regulate the expression of genes encoding proteins, mainly through degradation or silencing of mRNA, there is growing evidence that miRNAs can interact with lncRNA, which, in turn, also regulates the expression of target genes [4]. Long noncoding RNAs (lncRNAs) are RNAs over 200 nucleotides in size that regulate the biological activity of cells in a variety of ways, including transcriptional regulation, posttranscriptional regulation, and translation regulation. lncRNAs control posttranscriptional regulation, being a component of the ceRNAs network and acting as a molecular "sponge" (sorbent) of microRNAs [5,6].

The involvement of the ceRNA regulatory network in the initiation and progression of intestinal tumors has been confirmed in a number of studies [7]. However, the role of the imbalance of the ceRNA regulatory network (lncRNA - miRNA - mRNA) in patients with rectal cancer and the formation of radioresistance remains to be elucidated [8].

The study of the regulatory network of microRNAs-long noncoding RNAs-mRNAs is of great importance both for elucidating the molecular mechanisms underlying carcinogenesis and for creating a panel of new biomarkers [4].

Therefore, the aim of the study was to analyze the features of competitively interacting RNAs (lncRNA, miRNA, mRNA) regulatory network functioning in radioresistant and radiation-sensitive patients with rectal cancer.

Materials and methods
Clinical characteristics of patients

The study included 500 patients (200 women, 300 men) with diagnosed RC (age 28–85 years, median age 62.2 ± 4) who underwent inpatient treatment in 2019–2021 at the National Medical Research Centre for Oncology. Each patient signed a voluntary informed consent to participate in the study. Permission for the study was obtained from the ethical committee of the National Medical Research Center for Oncology (protocol No. 14). The sample of histologically confirmed tumors consisted of adenocarcinomas,

Tab. 1. miRNAs identified using the TarPmiR algorithm*.

Targeting miRNA	Target gene	Interaction energy	Targeting miRNA	Target gene	Interaction energy
miRNA-1249	BCL-2	−30.1	miRNA-30b	CASP-9	−25.6
miRNA-6861	BCL-2	−29.6	miRNA-3202	H2AFX	−20.3
miRNA-8052	BCL-2	−29.3	miRNA-5195	H2AFX	−23.8
miRNA-324	BCL-2	−28.6	miRNA-130b	RBBP-8	−23.7
miRNA-6820	BCL-2	−28.1	miRNA-195	RAD50	−21.6
miRNA-4717	BCL-2	−28.0	miRNA-340	RAD50	−21.6
miRNA-3943	BCL-2	−27.8	miRNA-497	RAD50	−21.7
miRNA-557	BCL-2	−27.6	miRNA-4257	RAD50	−21.9
miRNA-4690	BCL-2	−27.3	miRNA-5187	RAD50	−22.1
miRNA-6757	BRCA-2	−25.7	miRNA-149	RAD50	−22.6
miRNA-7151	BRCA-2	−25.1	miRNA-6769a	RAD50	−22.7
miRNA-6779	CASP-9	−34.3	miRNA-5010	RAD50	−23.1
miRNA-1273h	CASP-9	−33.1	miRNA-4533	RAD50	−23.9
miRNA-6812	CASP-9	−30.6	miRNA-6856	RAD50	−23.9
miRNA-6737	CASP-9	−30.1	miRNA-138-1	RAD50	−24.2
miRNA-661	CASP-9	−29.8	miRNA-433	RAD50	−24.4
miRNA-6799	CASP-9	−29.7	miRNA-3911	RAD50	−24.4
miRNA-6893	CASP-9	−28.5	miRNA-3691	RAD50	−24.6
miRNA-6819	CASP-9	−27.0	miRNA-4786	RAD50	−25.7
miRNA-6874	CASP-9	−26.6	miRNA-6758	RAD50	−26.0
miRNA-4728	CASP-9	−26.3	miRNA-6865	RAD50	−29.0
miRNA-6808	CASP-9	−26.2	miRNA-6798	RAD50	−30.4

* Only miRNAs validated in the miRDB database with a minimum free energy of miRNA-mRNA interaction are presented.

of which 21% of tumors were classified as local (T3N0M0) and 79% of cases were those with regional lymph nodes metastases (T3N1-2M0). Remote conformal RT in combination with fluoropyrimidine chemotherapy was performed on a Novalis TX linear particle accelerator five times a week (single focal dose of 2.0 Gy to a total focal dose of 54.0 Gy) [9]. To isolate RNA, we used paired fragments of biopsy of conditionally normal and tumor rectum tissues obtained by videocolonoscopy (VCS) before RT.

To evaluate the performed RT effectiveness, a combination of approaches was used: MRI, VCS and histological analysis. Histological analysis of the surgical material made it possible to classify the samples according to the Tumor Regression Grade system, the system was proposed by A.M. Mandard and collaborators [10]. It includes 5 tumor regression grades, depending on the presence of residual tumor cells and the fibrosis degree. When response evaluating, qualitative changes in tumor cells are also taken into account, such as vacuolization and/or eosinophilia of the cytoplasm, nuclear pycnosis, necrosis, as well as the degree of inflammatory infiltration, including the presence of giant cells. Efficiency assessment using the MRI and VCS approaches also had several gradations:

1. complete response (complete pathomorphosis, regression);
2. partial response (incomplete pathomorphosis, partial regression, stabilization);
3. lack of response (progression).

RNA extraction, evaluation of gene and micro-RNA expression

The tissue sample was homogenized by mechanical action method with the Trizol (QIAzol, Qiagen) addition. Total RNA isolation was performed using the RNeasy Plus Universal Kits (Qiagen) according to the manufacturer's instructions. To eliminate genomic DNA contamination, the isolated RNA was treated with DNase-1. The reaction for complementary DNA (cDNA) synthesis was carried out using the REVERTA-L kit (Interlabservice, Russia) according to the manufacturer's instructions.

Real-time qPCR (RT-qPCR) was used to assess the relative expression of 18 genes. Highly specific synthetic oligonucleotides (primers) were developed using the Primer-BLAST program and Gen-

Bank database (National Center for Biotechnology Information) (Suppl. tab. 1).

The amplification reaction was carried out in a mixture consisting of 1× PCR buffer, 0.2 mM deoxyribonucleoside triphosphates, 1.5 mM MgCl2, 0.6 mM synthetic oligonucleotides, 0.1 U/μl Taq polymerase and 15 ng complementary DNA. The mixture was incubated in a Bio-Rad CFX 96 Real-Time thermal cycler under the following temperature conditions: 94 °C for 240 seconds (sec) and then 39 cycles: 94 °C for 9 sec, 57 °C for 25 sec, and 72 °C for 45 sec. The relative expression (RE) of genetic loci was calculated using the formula $RE = E^{-\Delta\Delta Ct}$, taking into account the normalization for the reference genes (*GAPDH, ACTB, B2M*) and the mRNA level of corresponding target genes in normal tissue samples. The normalization scheme was as follows: 1 – calculation of the geometric mean C(t) of the reference genes; 2 – calculating the threshold cycle difference (ΔC(t)): C(t) target – C(t) reference; 3 – median ΔC(t) of each target gene for conditionally normal and tumor tissue; 4 – the $\Delta\Delta$C(t) calculation (the ratio $\Delta C(t)_{median\ of\ tumor\ tissue} / \Delta C(t)_{median\ of\ normal\ tissue}$); 5 – calculation of the final result $E^{-\Delta\Delta C(t)}$ [11].

The design of synthetic oligonucleotide sequences required for evaluating miRNA expression was performed using the I. Balcells algorithm. Primer-BLAST was used to design primers for long non-coding RNAs. Small non-coding RNA U6 was used as a reference to determine microRNA and lncRNA expression. To assess microRNA expression, the isolated total RNA was subjected to reverse transcription with specific RT-primers simultaneously with polyadenylation reaction [12]. The change in miRNA and lncRNA relative expression was also assessed by the RT-qPCR method.

Statistical and bioinformatic analysis
It was performed in the R language in the RStudio, version 8.10.173.987. The normality of distribution was assessed using the modified Shapiro-Wilk test (N > 50), the significance of intergroup differences was determined using the Mann-Whitney test (the Bonferroni correction), the relationships analysis of studied parameters

was performed using the Spearman correlation coefficient (r).

The hierarchical clustering and Euclidean distance parameters were used for cluster analysis. The genetic loci grouping according to their biological functions was carried out using the functional module detection algorithm. The Q-value was calculated using the one-sided Fisher's exact test (the Benjamini-Hochberg correction) [13]. Bioinformatic search for miRNAs was carried out according to the reversed TarPmiR algorithm, which is based on the random forest machine learning algorithm (combines the method of random subspaces and the Breiman bagging method), which makes it possible to predict the binding site of mRNA and miRNA [14]. To assess the participation of differentially expressed miRNAs in key cellular signaling pathways, the over-representation analysis (ORA) was performed. Statistical significance in the ORA was calculated using Fisher's exact test [15].

Analysis of miRNA and lncRNA interaction was carried out as follows:
1. HITS-CLIP, PAR-CLIP and CLASH data were extracted from the Gene Expression Omnibus database and processed using FASTX-Toolkit v0.0.13, and further analyzed using PARalyzer v1.1.
2. All coordinates of the binding sites were converted to assemblies hg19, mm9/mm10 and ce6/ce10, respectively, using the UCSC LiftOver Tool. The genomic coordinates of the conserved miRNA target sites predicted by TargetScan, miRanda/mirSVR, PITA, Pictar and RNA22 were also assembled and transformed into hg19, mm9/mm10 and ce6/ce10 assemblies using LiftOver.
3. The coordinates obtained at step 2 were compared with the previously described CLIP clusters using BEDTools [4].

Results
### Radiotherapy efficacy and genetic loci expression
Data on the transcriptional activity of 18 genetic loci in rectum tumor tissue biopsy samples were used in cluster analysis (Hierarchical Clustering & Euclidean distance parameters), which made it

possible to identify 2 clusters. In the first cluster, 85% of tumor tissue samples had increased expression of *CASP9* gene and 70% decreased expression of *BCL2, BRCA2, H2AX* and *RBBP8* genes. In the second cluster, 82% of patients have decreased expression of *CASP9* and 79% have increased expression of *BCL2, BRCA2, H2AX, RBBP8* and *RAD50*.

A comprehensive analysis of RT effectiveness results in 500 patients with RC gave the following results: in 150 RC patients, a complete response to RT was recorded; in 225 RC patients, a partial response to RT was recorded; and in 125 patients, there was no response to RT at all. At the same time, in tumor biopsy material from patients with a complete response to RT, the expression of *H2AX* and *RBBP8* was decreased 3.3 and 2.5 times (P < 0.05), and CASP9 expression was increased 4.8 times (P < 0.005) relative to these parameters in normal tissue.

In tumor biopsy material in patients with partial/no response to RT, the expression of *BCL2, BRCA2, H2AX, RBBP8* and *RAD50* was statistically significantly (P < 0.05) increased by 4.2, 3.5, 3.2, 3.4 and 3.3 times accordingly, and *CASP-9* expression was not statistically significantly different from its expression in normal tissue (Graph 1). That is, the transcriptional profile of *BCL-2, BRCA2, H2AX, RAD50, CASP9* and *RBBP8* is probably associated with the effectiveness of RT, which increases in RC-patients with *CASP-9* overexpression and hypoexpression of *H2AX* and *RBBP-8,* and vice versa, the effectiveness of RT decreases with overexpression of *BCL2, H2AX, RAD50, RBBP8* and *BRCA2* genes.

miRNA expression and RT efficiency
Using our modified bioinformatics algorithm TarPmiR, 1 927 microRNAs targeting *BCL2, BRCA2, H2AX, RAD50, CASP9* and *RBBP8* were identified. Of these miRNAs, only 109 were validated in the miRDB database (Graph 2), including 44 miRNAs that form strongest complexes with the corresponding target genes (minimum free energy of interaction in a miRNA-mRNA pair): 9 miRNAs for *BCL2* gene, 2 *miRNAs* for *BRCA2*, 12 miRNAs for *CASP9*, 2 miRNAs for *H2AX*,
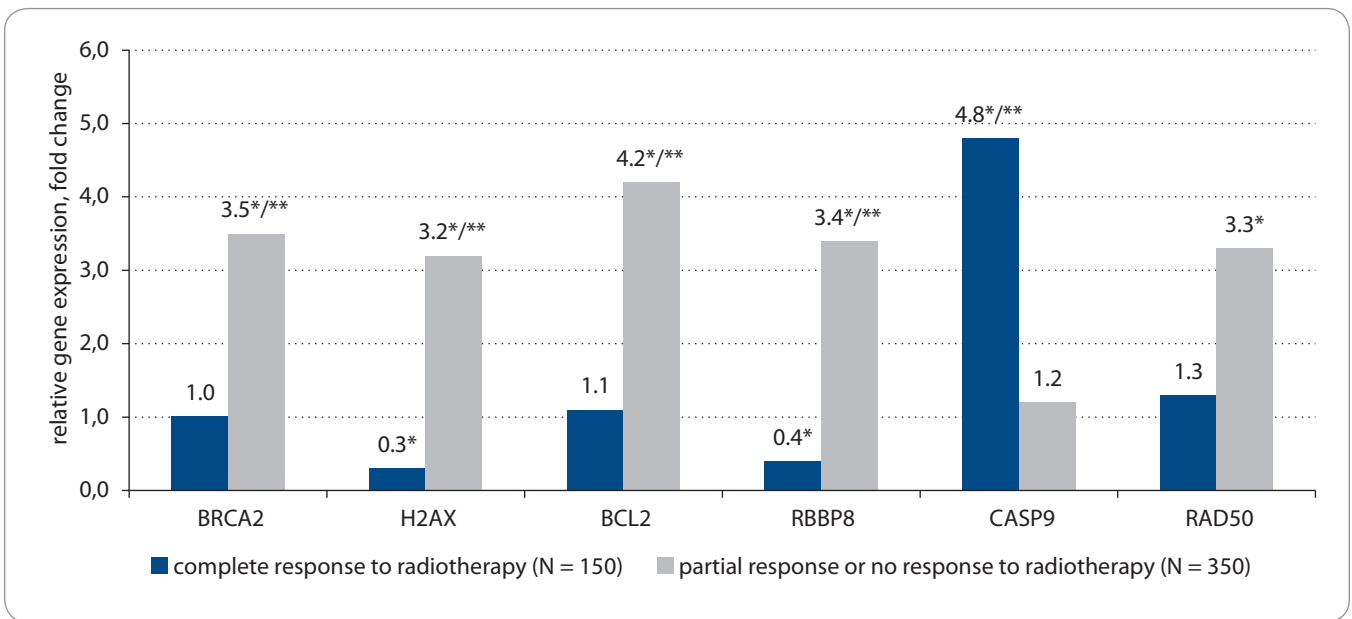
Graph 1. Tumor tissue transcriptional profile in two groups of rectal cancer patients (with a complete response to radiotherapy – Group 1, and with partial response or no response to radiotherapy – Group 2).
* statistically significant differences relative to conventionally normal rectal tissue (P < 0.05), ** statistically significant intergroup differences (P < 0.05)
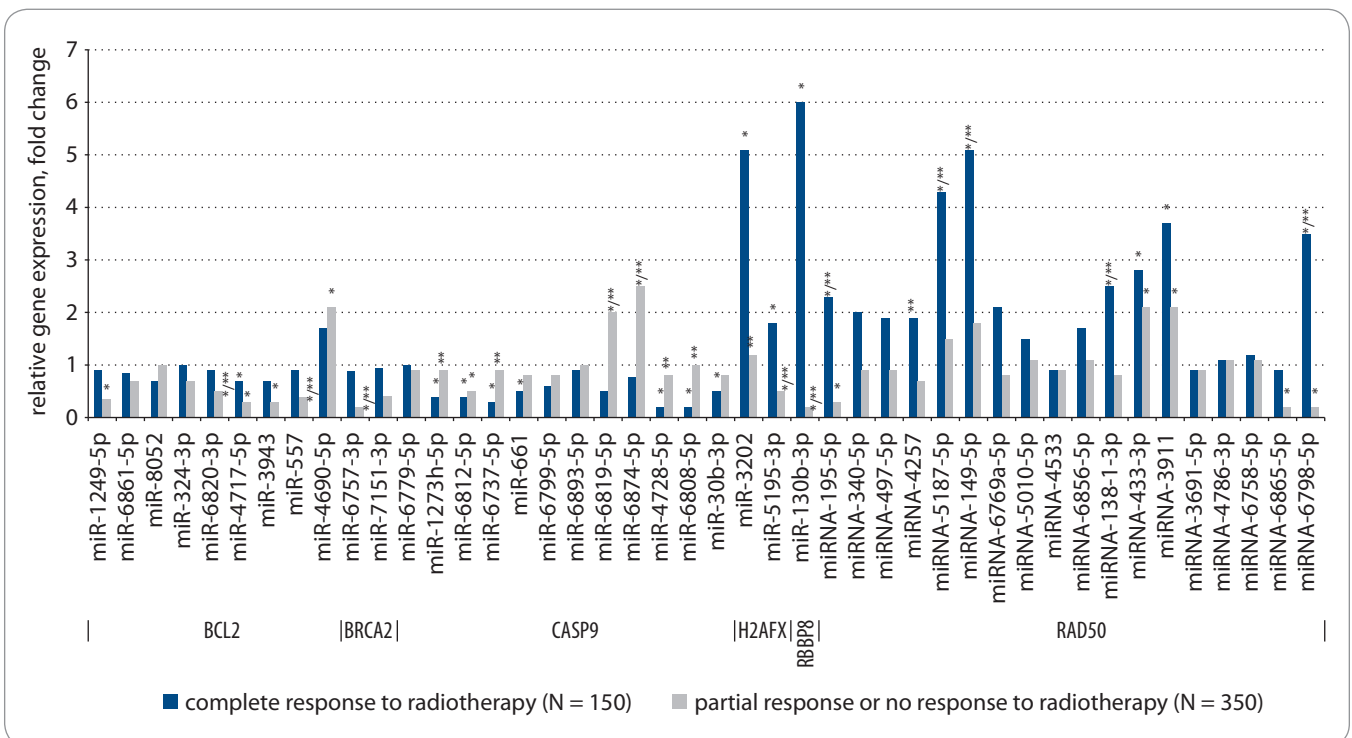
Graph 2. miRNA transcriptional profile in rectal cancer patients with complete tumor regression or its absence.
* statistically significant differences relative to normal tissue (P < 0.05), ** statistically significant intergroup differences (P < 0.05)

1 miRNA for *RBBP8* gene and 18 mRNAs for *RAD50* gene (Tab. 1).

For these 44 miRNAs, their expression in RC patients' tissues was determined.

Differential expression of a number of miRNAs, both intergroup and relative to normal tissue, was found. In the group of patients with insignificant tumor regres-

sion / lack of dynamics after RT (N = 350, low efficiency of RT), there was a statistically significant (P < 0.05) decrease in the expression of miRNA-1249-5p, miRNA-

Tab. 2. List of lncRNAs interacting with several miRNAs differentially expressed in patients with different radiotherapy efficacy.

lncRNA	Number of miRNAs interacting with lncRNA	miRNA
XIST	4	miRNA-130b-3p, miRNA-149-5p, miRNA-195-5p, miRNA-1249-3p
HELLPAR	3	miRNA-149-5p, miRNA-195-5p, miRNA-5195-3p
NEAT1	3	miRNA-130b-3p, miRNA-149-5p, miRNA-195-5p
AC005537.1	2	miRNA-149-5p, miRNA-195-5p
AC008124.1	2	miRNA-195-5p, miRNA-5195-3p
AC016717.2	2	miRNA-130b-3p, miRNA-195-5p
AC093297.2	2	miRNA-195-5p, miRNA-5195-3p
AC097634.1	2	miRNA-195-5p, miRNA-5195-3p
BX890604.1	2	miRNA-195-5p, miRNA-5195-3p
CASC9	2	miRNA-195-5p, miRNA-5195-3p
IQCH-AS1	2	miRNA-195-5p, miRNA-5195-3p
LINC00662	2	miRNA-195-5p, miRNA-5195-3p
LINC00707	2	miRNA-195-5p, miRNA-5195-3p
LINC00943	2	miRNA-149-5p, miRNA-195-5p
LINC01089	2	miRNA-130b-3p, miRNA-5195-3p
LINC01468	2	miRNA-149-5p, miRNA-5195-3p
LINC01547	2	miRNA-195-5p, miRNA-1249-3p
MALAT1	2	miRNA-149-5p, miRNA-5195-3p
MEG3	2	miRNA-195-5p, miRNA-5195-3p
MIR503HG	2	miRNA-149-5p, miRNA-195-5p
MUC20-OT1	2	miRNA-149-5p, miRNA-5195-3p
PVT1	2	miRNA-195-5p, miRNA-5195-3p
RP1-178F10.3	2	miRNA-149-5p, miRNA-195-5p
VASH1-AS1	2	miRNA-130b-3p, miRNA-149-5p

6820-3p, miRNA-4717-5p, miRNA -3943 and miRNA-557 2.9, 2.0, 3.3, 3.3 and 2.5 times, respectively, relative to normal tissue, and a decrease in the expression of miRNA-6820-3p and miRNA-557 1.8 and 2.3 times, respectively, relative to patients with complete tumor regression (N = 150, high efficiency of RT). A statistically significant ($P < 0.05$) 2.1 fold increase in miRNA-4690-5p expression relative to normal tissue was also found (Graph 2). miRNA-1249-5p, miRNA-4690-5, miRNA-6820-3p, miRNA-4717-5p, miRNA-3943 and miRNA-557 target the *BCL2* gene.

miRNA-6757 expression in tumor tissue in patients with partial/no response to RT is statistically significant ($P < 0.05$); it was 5.0 times and 4.4 times lower than normal tissue and tumor tissue of patients with complete response to therapy, respectively. *BRCA2* is the target gene of this miRNA, and its decrease should lead to an increase in this locus expression (Graph 2).

It was also found that the expression of miRNA-1273h, miR-6812-5p, miRNA-6737, miRNA-661, miRNA-4728, miRNA-30b and miRNA-6808 was statistically significantly ($P < 0.05$) decreased 2.5, 2.5, 3.3, 2.0, 5.0, 2.0 and 5.0 times, respectively, in tumor tissue of patients with a complete response to therapy relative

to normal tissue. An increase in expression of miRNA-1273h 2.3 times ($P < 0.05$), miRNA-4728 4.0 times ($P < 0.05$), miRNA-6819 4.0 times ($P < 0.05$), miRNA-6737 3.0 times ($P < 0.05$), miRNA-6874 3.3 times ($P < 0.05$) and miRNA-6808-5p 5.0 times ($P < 0.005$) was found in tumor tissue of RC patients with a partial response to therapy relative to tumor tissue in patients with a complete response to therapy. The expression of miRNA-6812 was reduced in tumor tissue of patients with complete and partial response to therapy 2.5 ($P < 0.05$) and 2.0 ($P < 0.005$) times, respectively, relative to normal rectal tissue.

In RC patients with a complete response to RT, statistically significant ($P < 0.05$) changes in the miRNA-5195 and miRNA-3202 expression were revealed. Thus, in tumor tissue, the expression of miRNA-3202 was 5.1 times higher than in normal tissue; and 4.3 times higher than in tumor tissue in RC patients with partial tumor response/no response to RT. Expression of miRNA-5195 was 1.8 times higher than in normal tissue, and 3.6 times higher than expression in tumor tissue in RC with partial/no response to RT (Graph 2). These miRNAs target mRNA of the histone protein H2AX.

In the course of the study, it was found that in tumor tissue of RC-patients with a complete response to therapy, miRNA-130b expression level was increased 6.0 times ($P < 0.05$) relative to normal tissue. At the same time, in RC patients with a partial response to therapy in tumor tissue, the expression level of this miRNA was decreased 5.0 times ($P < 0.05$).

Accordingly, in patients of the first group (complete response to therapy), the expression of miRNA-130b is 30.0 times higher ($P < 0.0005$) compared to expression in second group patients (partial response to RT), which may contribute to a decrease *RBBP-8* gene transcriptional activity (regulates cell proliferation) in patients with a complete response to RT and increase its expression in RC-patients with a partial response to RT (Graph 2).

Out of 18 miRNAs targeting the *RAD50* gene, only 8 have aberrant expression in rectal tumor tissue (miRNA-195-5p,

miRNA-5187-5p, miRNA-149-5p, miRNA-138-1-3p, miRNA-433- 3p, miRNA-3911, miRNA-6865-5p, miRNA-6798-5p). Thus, the expression of miRNA-195-5p relative to normal rectal tissue is 2.3 (P < 0.05) times higher in patients with complete tumor regression and 3.3 times (P < 0.05) lower in patients with incomplete tumor regression. At the same time, the differences in miRNA-195-5p expression between these groups are 7.7-fold (P < 0.05) (Graph 2). The expression of miRNA-5187-5p and miRNA-149-5p is increased 4.3 and 5.1 times (P < 0.05) in tumor tissue in patients with a complete response to therapy relative to normal tissue. The difference between 2 groups of patients in the expression of these microRNAs is 2.9 and 2.8 times (P < 0.05), respectively. There is also a statistically significant (P < 0.05) 2.7-fold difference in the expression of miRNA-4257 between these groups of patients. The expression of miRNA-138-1-3p, miRNA-433-3p, and miRNA-3911 is statistically significantly (P < 0.05) increased in patients with complete regression relative to expression in normal tissue by 2.5, 2.8 and 3.7 times, respectively. The expression of miRNA-6798-5p relative to normal rectal tissue is 3.5 (P < 0.05) times higher in patients with complete tumor regression and 5.0 times (P <0.05) lower in patients with incomplete tumor regression. At the same time, the differences in miRNA-6798-5p expression between these groups are 17.5 times (P < 0.005) (Graph 2). The expression of miRNA-6865-5p is reduced 5.0 times (P < 0.05) in tumor tissue in patients with incomplete tumor regression relative to normal rectal tissue.

The over-representation analysis (ORA) (Suppl. tab. 2) was performed for differentially expressed miRNAs in tumor tissues of two groups of RC patients. The ORA results show that miRNAs differentially expressed in two groups of patients, in addition to regulating the transcriptional activity of *BCL2, CASP9, BRCA2, RAD50, H2AX* and *RBBP8* genes, are involved in the control of key tumor cells survival and proliferation signaling pathway (Suppl. tab. 2). Thus, rectal tumor tissue sensitivity, which determines the effectiveness of RT, is also
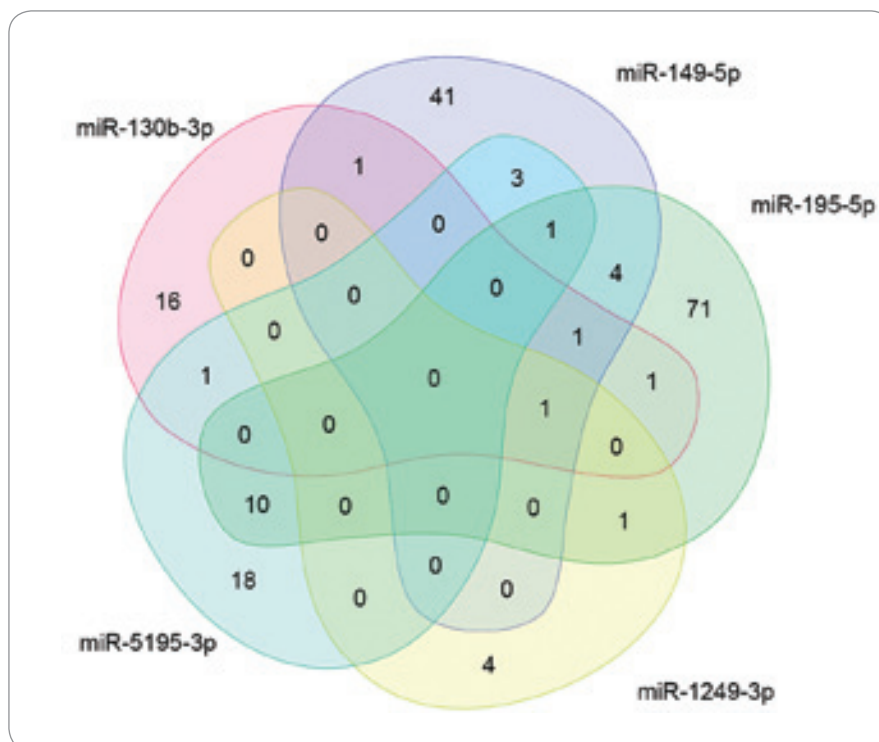

Fig. 1. Euler-Venn diagram of overlapping interactions of 5 miRNAs and 202 lncRNA.

associated with the differential expression of miRNA-195, miRNA-4257, miRNA-5187, miRNA-149, miRNA-138, miRNA-6798, miRNA-6820, miRNA-557, miRNA-6757, miRNA-1249, miRNA-1273h, miRNA-6737, miRNA-6819, miRNA-6874, miRNA-4728, miRNA-6808, miRNA-3202, miRNA-5195-3p and miRNA-130b.

Mathematical prediction of potential interactions probability between miRNAs and long noncoding RNAs

The use of bioinformatic algorithms made it possible to identify for 5 (miR-130b, miR-149, miR-195, miR-1249, miR-5195) of 44 miRNAs interactions with 202 long noncoding RNAs (Suppl. tab. 3).

For miR-130b interactions with 21 lncRNAs were predicted, for miR-149 with 90 lncRNAs, for miR-1249 with 6 lncRNAs, and for miR-5195 with 33 lnc-RNAs. From this list of long noncoding RNAs 24 interacted with several miRNAs differentially expressed in patients with different RT efficacy (Tab. 2, Fig. 1).

The next stage of the work was the analysis of the representation of 24 lncRNA transcripts in biological samples of two RC patients' groups.

Features of lncRNA expression and radiotherapy effectiveness

Analysis of long non-coding RNA transcripts relative abundance showed differences in this indicator for two groups of patients with different responses to RT.

Thus, in patients with a complete response to RT, a statistically significant (P < 0.05) decrease in the representation of lncRNA *XIST, HELLPAR, NEAT1, AC008124.1* and *AC016717.2* transcripts was found by 2.5, 3.3, 2.0, 2.5 and 5.0 times, respectively, relative to normal tissue and in patients with partial/no response to RT a statistically significant (P < 0.05) increase in the representation of lncRNA *XIST, HELLPAR, NEAT1, AC008124.1, LINC01089, LINC01547* and *VASH1-AS1* transcripts was found in 2.9, 3.9, 3.5, 3.7, 2.6, 4.7 and 1.9 times, respectively, relative to normal tissue.

At the same time, statistically significant differences (P < 0.05) in the representation levels of lncRNA XIST, HELLPAR, NEAT1, AC008124.1, AC016717.2, LINC01089, LINC01547 and VASH1-AS1 transcripts, i.e. 7.3, 13.0, 7.0, 9.3, 5.0, 3.1, 4.8 and 2.2 times, respectively, were found between two patients' groups
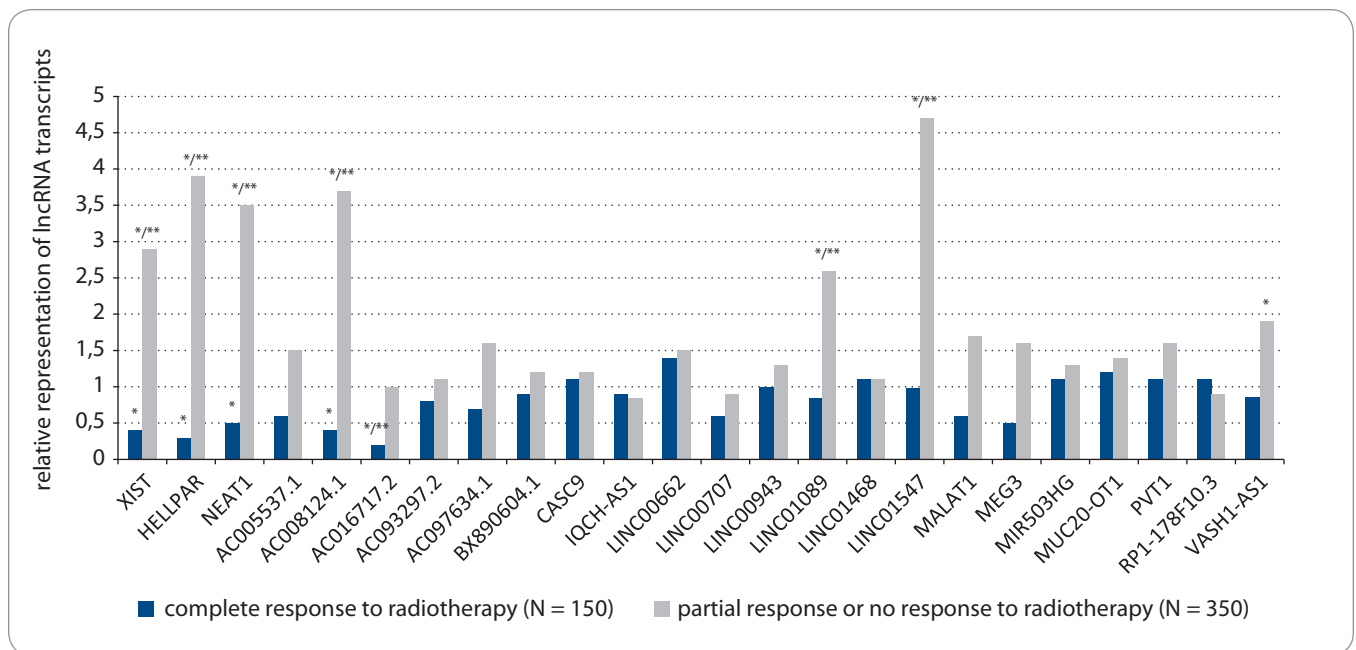
Graph 3. Relative representation (relative to normal tissue) of long noncoding RNAs transcripts in rectum tumor tissue of two groups of patients with complete or partial response/non-response to radiotherapy.
* statistically significant differences relative to normal tissue (P < 0.05), ** statistically significant intergroup differences (P < 0.05).

(Graph 3). Thus, the data obtained on the representation of long noncoding RNA transcripts will make it possible to model the network of molecular interactions underlying the radioresistance of tumor cells.

Discussion

The completed research stages allowed us to collect the necessary information on all components of competitively interacting endogenous RNA (ceRNA) regulatory network, including long non-coding RNA (lncRNA), miRNA and mRNA (gene expression).

A relationship was established between the transcriptional profiles of *BCL2, BRCA2, H2AX, RAD50, CASP9* and *RBBP8* genes and RT effectiveness. Thus, overexpression of *BCL2, H2AX, RAD50, RBBP8* and *BRCA2* genes is associated with low efficacy of RT, and overexpression of *CASP-9* and, at the same time, hypoexpression of *H2AX* and *RBBP8* was associated with high efficacy of RT. The use of modern bioinformatics algorithms made it possible to identify miRNAs targeting these genetic loci. These miRNAs expression in the tissues of cancer patients was evaluated. Thus, the target gene of miR-NAs miRNA-1273h, miRNA-4728, miRNA-

6819, miRNA-6737, miRNA-6874 and miRNA-6808-5p is CASP9; therefore, their high level of expression should contribute to a decrease in the level of mRNA of the initiator caspase-9, which is necessary to trigger the process of apoptosis. miRNAs targeting *BCL2* gene in the tumor tissue of patients with low RT efficiency are predominantly hypoexpressive, both relative to normal tissue and relative to tumor tissue of patients with a complete response to RT. Accordingly, a decrease in the expression of these miRNAs (miRNA-1249-5p, miRNA-4690-5, miRNA-6820-3p, miRNA-4717-5p, miRNA-3943, miRNA-557) can lead to the overexpression of *BCL-2* genetic locus, the product of which inhibits caspases and apoptosis [16]. Moreover, the overexpression of miRNA-3202/miRNA-5195, targeting mRNA histone protein H2AX, which triggers the process of changing chromatin conformation upon DNA damage under the influence of radiation [17], can promote *H2AX* hypoexpression, and vice versa.

miRNAs have many targets [18]; therefore, an over-representation analysis was carried out for differentially expressed miRNAs in tumor tissues of two groups of RC-patients, which made it possible to reveal their participation in a va-

riety of cellular signaling pathways, including regulation of the cell cycle and apoptosis.

Comprehensive analysis of these data, combined with the data of mathematical prediction of interaction miRNA and lncRNA and their validation by PCR, allowed us to form a model of the regulatory network of competitively interacting RNAs under RT for rectal tumors (Fig. 2).

According to this model, a decrease in the representation of lncRNA XIST, HELLPAR, NEAT1, AC008124.1 and AC016717.2 transcripts in patients with a complete response to RT leads to a decrease in the efficiency of interaction between lncRNA and targeting miR-NAs (miRNA-130b-3p, miRNA-149-5p, miRNA-195-5p, miRNA-1249-3p, miRNA-5195-3p). Disruption of the lncRNA-miRNA complex formation leads to an increase in the amount of free miRNA miRNA-130b-3p, miRNA-149-5p, miRNA-195-5p, miRNA-5195-3p and their effective interaction with target genes (mRNA). Accordingly, the transcripts representation of miRNA target *RBBP8* genes (miRNA-130b-3p, miRNA-149-5p, miRNA-195-5p) and *H2AX* (miR-5195-3p) decreases, and, consequently, the DNA repair efficiency decreases.
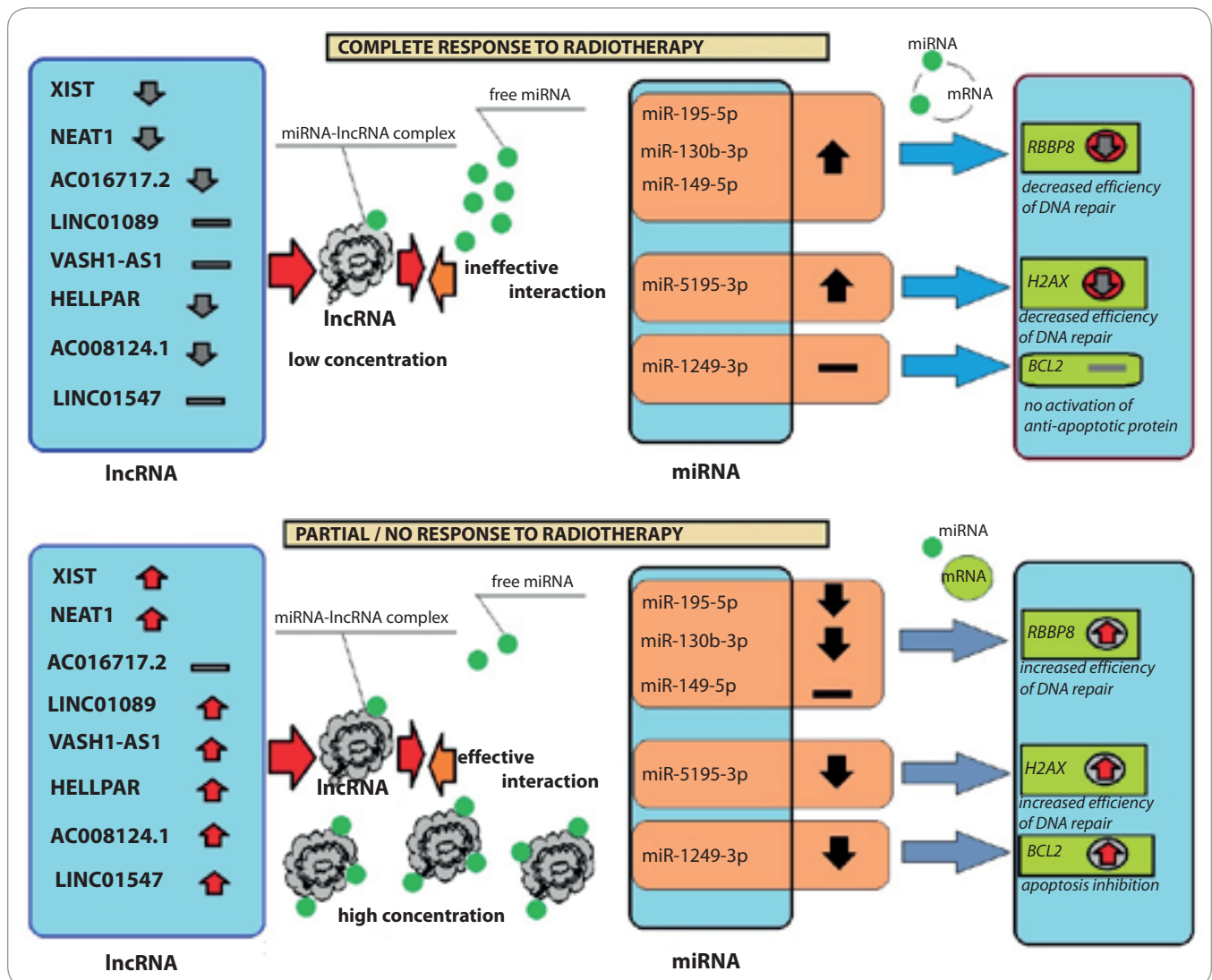
Fig. 2. Features of competitively interacting RNAs regulatory network in patients with different responses to radiotherapy.

The opposite effect is observed in patients with partial response to RT. An increase in the representation of lncRNA XIST, HELLPAR, NEAT1, AC008124.1, LINC01089, LINC01547 and VASH1-AS1 transcripts in these patients leads to an increase in the efficiency of interaction between lncRNA and miRNA (miRNA-130b-3p, miRNA-149-5p, miRNA-195-5p, miRNA-1249-3p, miRNA-5195-3p). The formation of the lncRNA-miRNA complex leads to a decrease in the amount of free miRNAs miRNA-130b-3p, miRNA-149-5p, miRNA-195-5p and miRNA-5195-3p and a decrease in their interaction with target genes (mRNA).

Accordingly, the abundance of miRNA target genes transcripts *RBBP8* (miRNA-130b-3p, miRNA-149-5p, miRNA--195-5p) and *H2AX* (miRNA-5195-3p) increases, and, consequently, an increase in the efficiency of DNA repair and radioresistance formation (Fig. 2).

Conclusion

Thus, our comprehensive study of competitively interacting RNAs regulatory network features and rectal tumors RT effectiveness made it possible to establish both the mechanisms of radioresistance formation and its predictors. RT efficiency for RC depends on the miRNA expression level (miRNA-195-5p, miRNA-4257, miRNA-5187-5p, miRNA-149--5p, miRNA-138-1-3p, miRNA-6798-5p, miRNA-6819-5p, miRNA-4728-5p, miRNA-1249-5p, miRNA-557, miRNA-1273h-5p, miRNA-6737-5p, miRNA-6808-5p, miRNA-3202, miRNA-5195-3p, miRNA-130b-3p) and lncRNA (XIST, HELLPAR, NEAT1, AC008124.1, LINC01089, LINC01547 and VASH1-AS1), providing effective regulation of the DNA repair system (*H2AX* and *RBBP8*) and apoptosis (*BCL2*).

References

1. Sung H, Ferlay J, Siegel RL et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71(3): 209–249. doi: 10.3322/caac.21 660.

2. Feeney G, Sehgal R, Sheehan M et al. Neoadjuvant radiotherapy for rectal cancer management. World J Gastroenterol 2019; 25(33): 4850–4869. doi: 10.3748/wjg.v25.i33.4850.

3. Wang L, Cho KB, Li Y et al. Long noncoding RNA (lncRNA)-mediated competing endogenous RNA networks provide novel potential biomarkers and therapeutic targets for colorectal cancer. Int J Mol Sci 2019; 20(22): 5758. doi: 10.3390/ijms20225758.

4. Novikova IA, Timoshkina NN, Kutilin DS. Differential microRNA expression in tumor and normal colon tissues. Yakut Med J 2020; 4(72): 74–82.

5. Cao C, Zhang T, Zhang D et al. The long noncoding RNA, SNHG6-003, functions as a competing endogenous RNA to promote the progression of hepatocellular carcinoma. Oncogene 2017; 36(8): 1112–1122. doi: 10.1038/onc.2016.278.

6. Bailerley OA, Gareev IF, Pavlov VN et al. Exosomal long noncoding RNAs as biomarkers and therapeutic targets in cancer. Creative Surg Oncol 2019; 9(4): 297–304. doi: 10.24060/2076-3093-2019-9-4-297-304.

7. Liu Q, Deng J, Wei X et al. Integrated analysis of competing endogenous RNA networks revealing five prognostic biomarkers associated with colorectal cancer. J Cell Biochem 2019; 120(7): 11256. doi: 10.1002/jcb.28403.

8. Tang F, Lu Z, Wang J et al. Competitive endogenous RNA (ceRNA) regulation network of lncRNAs, miRNAs, and mRNAs in Wilms tumour. BMC Med Genomics 2019; 12(1): 194. doi: 10.1186/s12920-019-0644-y.

9. Babaei M, Jansen L, Balavarca Y et al. Neoadjuvant therapy in rectal cancer patients with clinical stage II to III across European countries: variations and outcomes. Clin Colorectal Cancer 2018; 17(1): e129–e142. doi: 10.1016/j.clcc.2017.09.002.

10. Mandard AM, Dalibard F, Mandard JC et al. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. Cancer 1994; 73(11): 2680–2686. doi: 10.1002/1097-0142(19940601)73:11<2680::aid-cncr2820731105>3.0.co;2-c.

11. Kutilin DS. Regulation of gene expression of cancer/testis antigens in colorectal cancer patients. Mol Biol 2020; 54(4): 580–595. doi: 10.31857/S0026898420040096.

12. Balcells I, Cirera S, Busk PK. Specific and sensitive quantitative RT-PCR of miRNAs with DNA primers. BMC Biotechnol 2011; 11(1): 70. doi: 10.1186/1472-6750-11-70.

13. Krishnan A, Zhang R, Yao V et al. Genome-wide prediction and functional characterization of the genetic basis of autism spectrum disorder. Nat Neurosci 2016; 19(11): 1454–1462. doi: 10.1038/nn.4353.

14. Ding J, Li X, Hu H. TarPmiR: a new approach for microRNA target site prediction. Bioinformatics 2016; 32(18): 2768–2775. doi: 10.1093/bioinformatics/btw318.

15. Backes C, Khaleeq QT, Meese E et al. miEAA: microRNA enrichment analysis and annotation. Nucleic Acids Res 2016; 44(W1): W110–116. doi: 10.1093/nar/gkw345.

16. Urbańska K, Orzechowski A. Unappreciated role of LDHA and LDHB to control apoptosis and autophagy in tumor cells. Int J Mol Sci 2019; 20(9): 2085. doi: 10.3390/ijms20092085.

17. Scully R, Xie A. Double strand break repair functions of histone H2AX. Mutat Res 2013; 750(1–2): 5–14. doi: 10.1016/j.mrfmmm.2013.07.007.

18. Abdelsattar ZM, Wong SL, Regenbogen SE et al. Colorectal cancer outcomes and treatment patterns in patients too young for average-risk screening. Cancer 2016; 122(6): 929–934. doi: 10.1002/cncr.29716.

For Supplementary tables, see the online version of the article at www.linkos.cz

Supplementary table 1. Synthetic oligonucleotides for gene expression determination.

No.	Primers	Primer sequences	No.	Primers	Primer sequences
1	BRCA1-(F)	ACC TGT CTC CAC AAA GTG TCT	19	BAX-(F)	GGG ACG AAC TGG ACA GTA ACA
2	BRCA1-(R)	ACA CTG TGA AGG CCC TTT TA	20	BAX-(R)	GCT GCC ACT CGG AAA AAG AC
3	BRCA2-(F)	AGT TGG CTG ATG GTG GAT GA	21	P53-(F)	TTG GAA CTC AAG GAT GCC CA
4	BRCA2-(R)	GGA TCC ACA CCT GGA GTG CC	22	P53-(R)	CGG GAG GTA GAC TGA CCC T
5	RBBP8-(F)	ACC GAG GAT TTG GCA CTC TG	23	MDM2-(F)	TAG GAG ATTT GTT TGG CGT GC
6	RBBP8-(R)	TCC GAG ATT GCC TCG GGA TT	24	MDM2-(R)	CCT GCT GAT TGA CTA CTA CCA A
7	H2AX-(F)	AGG CCT CCC AGG AGT ACT AA	25	CASP3-(F)	CTG GAA TAT CCC TGG ACA ACA GT
8	H2AX-(R)	CTG AAG CGG CTC AGC TCT TT	26	CASP3-(R)	TCG ACA TCT GTA CCA GAC CGA
9	KU70-(F)	ACG TAG AGG GCG TTG ATT GG	27	CASP8-(F)	CTG AAG CAA ACA GCC AGT GC
10	KU70-(R)	TGG CTA CTG CTC ACT TTG GCC	28	CASP8-(R)	GAC CTC AAT TCT GAT CTG CTC AC
11	RAD50-(F)	GCG TGC GGA GTT TTG GAA TAG	29	CASP9-(F)	TGA GAC CCT GGA CGA CAT CT
12	RAD50-(R)	TTG AGC AAC CTT GGG ATC GT	30	CASP9-(R)	TCC CTT TCA CCG AAA CAG CA
13	RAP80-(F)	GAG TGA GCA GGA AGC TAG GG	31	ACTB-(F)	AAC CGC GAG AAG ATG ACC C
14	RAP80(R)	AGA AGG CCG GCA ACT ATT CA	32	ACTB-(R)	AGC ACA GCC TGG TAG CAA C

15	RNF168(F)	GCC AGT TCG TCT GCT CAG TA	33	GAPDH-(F)	GTC AAG GCT GAG AAC GGG AA
16	RNF168(R)	CTG CCG CCA CCT TGC TTA T	34	GAPDH-(R)	TCG CCC CAC TTG ATT TT GGA
17	BCL2(F)	GGA TCC AGG ATA ACG GAG GC	35	B2M-(F)	AGA TGA GTA TGC CTG CCG TG
18	BCL2(R)	GAA ATC AAA CAG AGG CCG CA	36	B2M-(R)	CCA TGATGC TGC TTA CAT GTC TC

Supplementary table 2. Differential expressed miRNA representation in the signaling pathways.

Signaling pathway / process	P-value	miRNA
ECM-receptor interaction	5.53e-6	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
notch signaling pathway	1.87e-5	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
protein digestion and absorption	2.98e-4	miRNA-195-5p; miRNA-4257; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
glutathione metabolism	7.10e-4	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-4728-5p; miRNA-557; miRNA-1273h-5p; miRNA-6808-5p
oxidative phosphorylation	9.45e-4	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
RIG-I-like receptor signaling pathway	0.001412	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-130b-3p

phosphatidylinositol signaling system	0.0021463	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-130b-3p
apoptosis - multiple species	0.0023771	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-130b-3p
phagosome	0.0023569	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
signaling pathways regulating pluripotency of stem cells	0.0028218	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
estrogen signaling pathway	0.0037057	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
inflammatory mediator regulation of TRP channels	0.0040433	miRNA-195-5p; miRNA-5187-5p; miRNA-149-5p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-130b-3p
pyrimidine metabolism	0.0044015	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-130b-3p
inositol phosphate metabolism	0.0046988	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-130b-3p
necroptosis	0.0049473	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
cell cycle	0.0061424	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p

ErbB signaling pathway	0.0 059 231	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
parathyroid hormone synthesis, secretion and action	0.0 064 135	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
ribosome biogenesis in eukaryotes	0.0 057 282	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-138-1-3p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
VEGF signaling pathway	0.0 077 348	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-130b-3p
C-type lectin receptor signaling pathway	0.0 081 841	miRNA-195-5p; miRNA-4257; miRNA-5187-5p; miRNA-149-5p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-557; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-130b-3p
phospholipase D signaling pathway	0.0 088 879	miRNA-195-5p; miRNA-4257; miRNA-149-5p; miRNA-138-1-3p; miRNA-6798-5p; miRNA-6819-5p; miRNA-4728-5p; miRNA-1249-5p; miRNA-1273h-5p; miRNA-6737-5p; miRNA-6808-5p; miRNA-3202; miRNA-5195-3p; miRNA-130b-3p
nitrogen metabolism	0.0 097 981	miRNA-195-5p; miRNA-5187-5p; miRNA-149-5p; miRNA-4728-5p; miRNA-1273h-5p; miRNA-6808-5p

ECM – extracellular matrix, RIG – retinoic acid-inducible gene, TRP – transient receptor potential, VEGF – vascular endothelial growth factor

Supplementary table 3. List of long non-coding RNAs and miRNAs interacting with them.

miRNAs -	lncRNA target	miRNA sequence	Alignment	Long non-coding RNA sequence
miR-130b-3p	LINC01087	uacGGGAAAGUAGUAACGUGAc	:| | | : ||||||||	uauUCGUCUGGACAUUGCACUu
	AC016717.2	uaCGGGAAAGUAGUAACGUGAc	|:|:| |:||| |||||||	agGUCUUGUUAUC-UUGCACUa
	AC109588.1	uacgggaaagUAGUAACGUGAc	| | |||||||	uacuuaauaaAACCUUGCACUc
	AC124016.2	uacgggAAAGUAGUAACGUGAc	|||:| : ||||||	auagggUUUUAAU-UUGCACUg
	LINC01091	uacgggaaAGUAGUAACGUGAc	| :|| ||||||	gccuaggcUGGUC-UUGCACUc
	AL591030.1	uacgggaAAGUAGUAACGUGAc	| :||: ||||||	gacagaaUAUAUU-UUGCACUa
	AP003469.4	uacgggaaaguagUAACGUGAc	||||||||	uauucagccuggaAUUGCACUa
	AL845472.1	uaCGGGA--AAGUAGUAACGUGAc	|||:| ||| :||||||	ugGCCUUCAUCCCUAGUUGCACUa
	PCAT5	uacGGGAAAGUAGU--AACGUGAc	|||| ::||| |||||||	ucuCCCUGCUGUCACUUUGCACUu
	LINC00839	uacGGGA--AA-GUAGUAACGUGAc	|||| || | | |||||||	uccCCCUGAUUCCCACUUUGCACUu
	NEAT1	uacGGGAAAGUAGUAACGUGAc	|: || ::|| |||||||	ggaCUGUUCUGUCCUUGCACUg
	LINC01089	uacGGGAAAGUAGUAACGUGAc	:|| | |||||||||	gcuUCCAACCUCCAUUGCACUg
	AL355338.1	uacgggaAAGUAGUAACGUGAc	| || |||||||||	cugagacUGCACCAUUGCACUc
	VASH1-AS1	uacgggaaAGUA--GUAACGUGAc	|||| |||||||||	agccgagaUCAUGCCAUUGCACUc
	AC142472.1	uaCGGGAAAGUAGU---AACGUGAc	| | || || || |||||||	cuGACAUUCCAGCACGUUUGCACUg
	LINC01476	uacGGGAAAGUAGU-AACGUGAc	|| | ||| | ||||||	aucCCAUGACAUGAUUUGCACUc
	LINC00667	uacGGGAAAGUAGUAACGUGAc	|:|| | : ||||||	aauCUCUAU-GCCUUUGCACUg
	ILF3-AS1	uacgggAAAGUAGUAACGUGAc	|| | |||||||||	ccgagaUUGCUCCAUUGCACUc
	CTD-3093M3.1	uacgggaaaguaguAACGUGAc	|||||||	uauuuguaaaaaauUUGCACUu
	LINC02575	uacgggaaaGUAGUAACGUGAc	|| | |||||||	acucaauaaCA-CUUUGCACUc
	XIST	uacgggaAAGUAGU-AACGUGAc	||| ||| ||||||	uaaacaaUUC-UCAGUUGCACUu
miR-149-5p	AL645608.1	ccCUCACUUCUGUGC-CUCGGUCu	|: ||:||:|: | |||||||	cgGGAUGGAGGCGGGAGAGCCAGg

LINC01356	cccucacuucUGUGCCUCGGUCu	‖ ‖‖‖‖‖‖‖	uuuaauacccACCAGGAGCCAGu
BLACAT1	cccUCACUUCUGUGCCUCGGUCu	│ ‖‖ :│ ‖‖‖‖‖‖‖	agcACUGA--GCUGGGAGCCAGg
AC007878.1	cccucACUUCUGUGCCUCGGUCu	‖:: │ ‖ ‖‖‖‖‖‖	cagccUGGGCA-ACAGAGCCAGg
AC009237.14	cccucacUUCUGUGCCUCGGUCu	‖‖:│ ‖‖‖‖‖‖‖	gaagcacAAGGCUGAGAGCCAGu
MIR4435-2HG	cccUCACUUCUG--UG-CCUCGGUCu	‖‖│ :│ │: ‖‖‖‖‖‖‖	uccAGUAAUUGCUAAUGGGAGCCAGu
AC079922.2	cccUCAC-UUCUGUGCCUCGGUCu	‖‖‖ : ‖ : ‖‖‖‖‖‖‖	ugcAGUGUGUCACCUUGAGCCAGu
AC097468.3	ccCUCACUUCUGUGCCUCGGUCu	‖ ‖‖ │ ‖‖‖‖‖‖‖	auGAAUGACCCAAAGGAGCCAGg
AC109779.1	ccCUCACUUCUGUGC---CUCGGUCu	│: ‖ │::│:│ ‖‖‖‖‖‖‖	guGGCUGCUGGUAUGCAUGAGCCAGc
AC131235.4	cccucACUUCUGUGC---CUCGGUCu	‖ │::│:│ ‖‖‖‖‖‖‖	uuauaUGCUGGUAUGCCAGAGCCAGa
MUC20-OT1	cccucACUUCUGUGCCUCGGUCu	│ ::│::│ ‖‖‖‖‖‖‖	uguuuUAGGGGUA-GGAGCCAGg
AC024132.1	cccUC-ACUUCUGUGCCUCGGUCu	‖ ‖:│ : ‖‖‖‖‖‖‖	cucAGCUGGACCAGAAGAGCCAGa
SNHG8	cccuCACUU--CUGU--GCCUCGGUCu	│ ‖‖ │ ‖ : ‖‖‖‖‖‖‖	ccccGAGAACCGUCAGUUUGAGCCAGa
SCAMP1-AS1	cccUC-ACUUC--UGUGCCUCGGUCu	‖ ‖‖‖‖ ‖‖ ‖‖‖‖‖‖‖	cuuAGAUGAAGCUACAGGGAGCCAGa
LINC01554	cccucacuucuguGCCUCGGUCu	│ ‖‖‖‖‖‖‖	cgcagcuucacucCUGAGCCAGc
LINC01848	ccCUCACUUCUGUGCCUCGGUCu	‖‖ │:│ │:‖‖‖‖‖‖	uuGAG-GGAGCAAUGGAGCCAGc
AC094104.1	cccuCACUUCUGUGCCUCGGUCu	‖:::‖ │ ‖‖‖‖‖	gaacGUGGGGA-AGUGAGCCAGg
C5orf66-AS1	cccuCACUUCUGUGCCUCGGUCu	‖ ‖ │ ‖‖‖‖‖	aaccGUGCAGUGCCAGAGCCAGa
AL390955.2	cccucACUUCUGUGCCUCGGUCu	‖:‖:│ : ‖‖‖‖‖	cucccUGGAGG-A--GAGCCAGc
AC092171.3	ccCUCACUUCUGUGCCUCGGUCu	‖‖ │:‖:│ ‖‖‖‖‖‖‖‖	ggGAGCGGAGGC-CGGAGCCAGc
AC005537.1	ccCUCA-CUUCUGUGCCUCGGUCu	│ ‖ │:│ ‖ ‖ ‖‖‖‖‖‖‖	ggGCGUAGGACCCUCCGAGCCAGg

AC078846.1	cccucacUUCUGUG--CCUCGGUCu	`   |||:::|` `|||||||`	accaaaaAAGGUGCCUUGAGCCAGa
AL158152.1	cccUCACU-UCUG--UGCCUCGGUCu	`	||| ||:: |:`   ` |||||||`	uucAUUGAUAGGUUGAUUGAGCCAGa
AL365199.1	cccucaCUUCUGU-GCCUCGGUCu	`    |: |::|` `:|||||||`	acccaaGGUGGUAUUGGAGCCAGg
LINC01468	cccucaCUUCUGUGC---CUCGGUCu	`    |||||:: |` `|||||||`	aaacugGAAGAUGAGGAAGAGCCAGc
OLMALINC	cccucacuucUGUGCCUCGGUCu	`	:|`   `|||||||`	gcuccucgccAGGCUGAGCCAGg
C10orf91	cccucacuucugugcCUCGGUCu	` ` `|||||||`	cacaggcggcuggaaGAGCCAGu
NEAT1	cccUCACUUC-UGUG-C-CUCGGUCu	` || ||| ||	|`   ` |||||||`	aaaAGGAAAGUACCCUGAGAGCCAGu
MALAT1	ccCUCACUUCUGUGCCUCGGUCu	`|| :||	|||||||`	agGAAAAGAGUCCAGGAGCCAGu
AC012531.1	cccucacuucuguGCCUCGGUCu	`         |` `|||||||`	uccucgucgcuauCUGAGCCAGg
AC023794.6	cccucacuucuGUGCCUCGGUCu	`       |||` `|||||||`	ccaccccugucCACAGAGCCAGg
HELLPAR	cccUCACUU-CUGUGCCUCGGUCu	`   ||| || ||::` `   |||||||`	aaaAGUUAAUGAUGGUGAGCCAGg
AC125603.2	cccucacuucuguGCCUCGGUCu	`         |` `|||||||`	ugcagcuucacucCUGAGCCAGc
LINC00943	ccCUCACUUCUGUGCCUCGGUCu	`|: |||  :|| |||||||`	caGGCUGA--GCAAGGAGCCAGu
LINC01257	cccUCACUUCUGUGCCUCGGUCu	`:||| :|:: :|` `  |||||||`	cgcGGUGCGGGAGCAGAGCCAGc
AF111169.1	cccucaCUUCUG-UGCCUCGGUCu	`    ||| || :` `|||||||`	ggggcaGAACACUGGGGAGCCAGg
VASH1-AS1	ccCU-CACUUCUGU-GCCUCGGUCu	`|| || : :|| |` `   |||||||`	caGAUGUUGCUGCAGCUGAGCCAGa
LINC02288	cccuCACUUCUGUGCCUCGGUCu	`|| |||	|||||||`	cccuGUCUAGA-A-GGAGCCAGu

	DRAIC	ccCUCAC- UUCUGUGCCUCGGUCu	‖‖ | : |:‖| ‖‖‖‖‖	cuGAGAGCGCGGCAC-GAGCCAGa
	LINC02188	cccUCACUUCUGUGCCUCGGUCu	:‖| ‖| : ‖‖‖‖‖	gaaGGUGUAGA-GGAGAGCCAGg
	RP1-178F10.3	cccucacuucuGUGCCUCGGUCu	‖:‖‖‖‖‖	aucgaggucauCAUGGAGCCAGc
	AC068512.1	cccucacuUCUGUGCCUCGGUCu	‖ : :‖‖‖‖‖	ggcccuacAGCUUUGGAGCCAGg
	AC006504.5	cccUCACUUC- UGUGCCUCGGUCu	‖ | | :: | ‖‖‖‖‖	uccAGGCAUGUGUCCUGAGCCAGg
	AL121894.2	cccucACUUCUGUGCCUCGGUCu	‖‖:| ‖ ‖‖‖‖‖	ccccaUGAGGCCAGUGAGCCAGa
	LINC00895	cccucaCUUCUG-UGCCUCGGUCu	‖:|:| : ‖‖‖‖‖	uuguuaGAGGGCUGAAGAGCCAGc
	OGFRP1	ccCUCACUUCUGUGC- CUCGGUCu	‖ | : | | | ‖‖‖‖‖	guGACUUGUGCGAAGUGAGCCAGg
	MIRLET7BHG	cccUCACUUC- UGUGCCUCGGUCu	| ‖:| ‖ ‖‖‖‖‖	gaaACUGAGGCCCAGAGAGCCAGg
	XIST	cccucacuUCUGUGCCUCGGUCu	‖ | : ‖‖‖‖‖	ccugggccAGUC-UUGAGCCAGc
	FTX	cccucaCUUCUGUGCCUCGGUCu	‖‖ : | ‖‖‖‖‖	ugcgcaGAACUUCCCGAGCCAGc
	LINC00630	cccucacuucugugcCUCGGUCu	‖‖‖‖‖	ugucugucaccuuaaGAGCCAGu
	DANT2	cccucacuucuguGCCUCGGUCu	| ‖‖‖‖‖	uauaaacgcucucCUGAGCCAGu
	MIR503HG	cccucacuucuGUGCCUCGGUCu	| :‖‖‖‖‖	guacgguucccCCUGGAGCCAGa
miR-195-5p	AL645608.3	cgGUUAUAAAGAC---ACGACGAu	| | | ‖‖ ‖‖‖‖‖	ccCCAGAGCUCUGCCCUGCUGCUg
	AL031281.2	cggUUAUA-AAGACACGACGAu	| ‖| | ‖ ‖‖‖‖‖	gugAUUAUCUCCUCUGCUGCUa
	AL691459.1	cgguuauaaagacACGACGAu	‖‖‖‖‖	uuaggaaagagaaUGCUGCUa
	RP6-206I17.2	cgGUUAUAAAGACACGACGAu	‖‖‖ ‖| | ‖‖‖‖‖	ccCAAUUUUAC--UGCUGCUg
	RASAL2-AS1	cgguuauaaAGACACGACGAu	‖| ‖‖‖‖‖	cgguccgggUCUCUGCUGCUc

MIR29B2CHG	cgguuauaaagacACGACGAu	|||||||	acugcagaggaacUGCUGCUc
LINC01703	cgguuauaaagacACGACGAu	|||||||	cgugauuagagauUGCUGCUg
AC108488.1	cgguuauaaagacACGACGAu	|||||||	gcuuugugaagaaUGCUGCUg
AC010969.2	cggUUAUAAAGACACGACGAu	::| || || ||||||	uggGGUCUUGCUAUGCUGCUc
AC104794.2	cgguuauaaaGACACGACGAu	|| |||||||	gcuaugcacgCU-UGCUGCUa
AC073046.1	cgguuauAAAGAC-----ACGACGAu	||||| |||||||	gaucccuUUUCUGAGGGCUGCUGCUg
AC019069.1	cgGUUAUAAAGACACGACGAu	| :|| || ||||||	gcCUGUA-GUCCCUGCUGCUc
TEX41	cgguuauaaagacACGACGAu	|||||||	agaugcauaaaauUGCUGCUu
LINC02478	cgguuauAAAGACACGACGAu	||||| |||||||	uuccuccUUUCU-UGCUGCUa
AC016717.2	cgGUUAUAAAGACACGACGAu	||||| ||| |||||||	agCAAUAGUUCAGUGCUGCUc
AC097634.1	cggUUAUA-AAGAC--ACGACGAu	|| || ||: | |||||||	aaaAAGAUCUUUAGAUUGCUGCUa
LINC02035	cgguuauaaagacACGACGAu	|||||||	gcuuuacuuaaaaUGCUGCUu
AC107068.1	cgguUAUAAAGAC---ACGACGAu	|| | ||| |||||||	uguuAUCAUCCUGGGAUGCUGCUu
LINC02434	cgguuauaaagacACGACGAu	|||||||	ggagggaccagauUGCUGCUu
AC106799.3	cgGUU-AUAAAGACACGACGAu	||| | || || ||||||	aaCAAUUCUUCCUUUGCUGCUu
AC093297.2	cgGUUAUAAAGAC---ACGACGAu	|| |:|| |||||||	ugCAACCGCUUUGGAUUGCUGCUu
AC010245.2	cgguuaUAAAGACACGACGAu	||| | ||||||	cuuuccAUUGAUCUGCUGCUg
EPB41L4A- AS1	cgguuauaaagacACGACGAu	|||||||	cugaaguguagacUGCUGCUa
LINC01184	cgGUUAUA--AAGAC-ACGACGAu	:|:|:| || || |||||||	uuUAGUGUCAUUAUGUUGCUGCUa
HCG17	cgguuauaaagacACGACGAu	|||||||	ugcuuugcccgccUGCUGCUg
LINC00473	cgguuaUAAA-GAC--ACGACGAu	|||| ||| |||||||	agggccAUUUCCUGUUUGCUGCUu
AC004852.2	cgGUUAUAA-AGACACGACGAu	|| ||| | ||||||	uuCAAAAUUGCCACUGCUGCUg
AC005537.1	cgGUUAUAAAGACACGACGAu	||||| : ||||||	cuCAAUAAAAU--UGCUGCUa
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	AC003092.1	cgGUUA-UA-AAGACACGACGAu	|| | || | || |||||||	cuCACUCAUCUGCUAUGCUGCUg
	CASC9	cgguuauaaagACACGACGAu	| ||||||	agauggacacaUUUGCUGCUu
	RNF139-AS1	cgguuAUAAAGACACGACGAu	||||| |||||||	uugguUAUUU-AAUGCUGCUu
	PVT1	cgguuauaAAGAC-ACGACGAu	| ||| |||||||	uuugcuucUCCUGUUGCUGCUa
	C9orf163	cgguuauaaaGACACGACGAu	|| |||||||	ccugguggggCUUUGCUGCUu
	LINC00707	cgguUAUAAAGAC---ACGACGAu	|| |||| |||||||	cugcAGACAUCUGGACUGCUGCUu
	LINC00843	cgguuauAAAGACACGACGAu	| | |||||||||	uccccguUCUGUGUGCUGCUg
	AC022400.5	cgguuauAAAGACACGACGAu	| | |||||||||	uccccguUCUGUGUGCUGCUg
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	AL160290.2	cggUUAUAAAGACACGACGAu	|: | || |||||||	cagAGGAGCUCCGUGCUGCUa
	AL158835.1	cgguUAUAAAGACACGACGAu	||||| | ||||||	auucAUAUUCAUUGCUGCUg
	NEAT1	cgguuauaaagacACGACGAu	|||||||	cucgccuucacgcUGCUGCUg
	AC008124.1	cggUUA--UAAAGACACGACGAu	||| | || ||||||||	caaAAUCCAAUCCU-UGCUGCUg
	AC024884.2	cgguuauaAAGACACGACGAu	| || |||||||	cagcuuugUACUAUGCUGCUc
	HELLPAR	cgguuauaaAGACACGACGAu	|| ||||||	ugcuucaaaUCAUUGCUGCUu
	AC055713.1	cgguuAUAA-AGACACGACGAu	|||| |: |||||||	uuggcUAUUAUUAAUGCUGCUa
	LINC00943	cgguuauAAAGACACGACGAu	|| || |||||||	uagucacUUCCUCUGCUGCUg
	AL139327.2	cgguuAUAAAGAC-ACGACGAu	| || | | ||||||	uuuuuUUUUCCAGAUGCUGCUg
	AL138820.1	cgguuauAAAGACACGACGAu	|| || |||||||	ucuugccUUACU-UGCUGCUu
	LINC00639	cgGUUAUA-AAGACACGACGAu	:|| || | |||||||	ugUAAGAUGUGAUUUGCUGCUc
	AL355916.1	cgguuauaaAGACACGACGAu	|| |||||||||	ucucugaccUCAGUGCUGCUg
	AL049775.1	cgguuauaaagacACGACGAu	|||||||	gaggcuguggggaaUGCUGCUu
	MEG3	cgGU-UAUAAAGACACGACGAu	|| ||:| | |||||||	caCACAUGUGGCCUUGCUGCUg
	MEG8	cgguuauaaaGACACGACGAu	|| |||||||	auuuaaccacCU-UGCUGCUg
	AL117190.1	cgguuauaaaGACACGACGAu	|| |||||||	auuuaaccacCU-UGCUGCUg
	LINC00638	cgGUUAUAAAGACACGACGAu	| | || | |||||||	gcCCAGCUGUCCAUGCUGCUg
	FAM30A	cgguuauaaagacACGACGAu	|||||||	agagggaaagggaUGCUGCUg
	AC012236.1	cgguuauaaagacACGACGAu	|||||||	agagaggggcaccUGCUGCUa
	AC020661.1	cggUUAUAAAGACACGACGAu	|| | | ||||||||	ugaAAGACAU-UGUGCUGCUg

	IQCH-AS1	cgGUUAUAAAGA------CACGACGAu	||||: || | |||||||||	ucCAAUGAUUGUGGUGACGUGCUGCUa
	AC068338.2	cggUUAUAAAGACACGACGAu	|| || | |||||||	acaAAGAUAAC-CUGCUGCUg
	LINC02128	cgguuauaaaGAC--ACGACGAu	||| |||||||||	uccgucagacCUGCCUGCUGCUu
	LINC00922	cgguuauaaagacACGACGAu	|||||||||	agcccugugagguUGCUGCUg
	AC040162.3	cgGUUAUAAAGACACGACGAu	| ||| | ||||||	ugCUAUA-AACACUGCUGCUu
	LINC01572	cgguUAUAAAGAC-ACGACGAu	|||| || ||||||	uaucAUAUACCCGCUGCUGCUa
	AC133540.1	cgguuauaAAGAC--ACGACGAu	| ||| |||||||	ccuuggccUGCUGGCUGCUGCUc
	FENDRR	cggUUAUAAAGAC-----ACGACGAu	|||| | ||| |||||||||	aggAAUA-UCCUGCAACCUGCUGCUc
	AC126696.3	cgguuauaaAGAC-ACGACGAu	|| | |||||||	aaagcuccaUCGGCUGCUGCUg
	LINC00324	cgguuauaAAGACACGACGAu	|| | |||||||	uccgcccgUUAU-UGCUGCUc
	RP1-178F10.3	cgguuauaaagacACGACGAu	|||||||||	uucuguuggcgccUGCUGCUg
	AC129926.1	cgGUU-AUAAAGAC-ACGACGAu	||: |: ||| ||||||	ucCAGCUGCCUCUGCUGCUGCUg
	SNHG25	cgguUAUAAAGACACGACGAu	|| | || ||||||	ugucAUCGUCCU-UGCUGCUu
	LINC00511	cggUUAUAAA-GACACGACGAu	|:|:||| || ||||||	gaaAGUGUUUCCUCUGCUGCUa
	AC090772.3	cggUUAUAAAGAC--ACGACGAu	| | || || ||||||	gugACUUUUGGUGGAUGCUGCUg
	AC068473.5	cgguuauAAAGACACGACGAu	| ||| |||||||	uuuugacUAUCUCUGCUGCUu
	LINC00662	cggUUAUAAAGAC-ACGACGAu	|||: | ||| ||||||	cugAAUGGUACUGCUGCUGCUg
	SCGB1B2P	cgguuauaaagacACGACGAu	|||||||||	cuagcucccgcccUGCUGCUg
	LINC00665	cgGUUAUAAAG-ACACGACGAu	:| |: | | | ||||||	gaUACUGCUCCUUUUGCUGCUu
	AC010624.2	cgguuauaaaGAC-ACGACGAu	||| |||||||	gguccccccaCUGCUGCUGCUa
	AC005261.1	cgguuauaaagacACGACGAu	|||||||||	cacugauuaaaacUGCUGCUu
	AL035252.3	cgguuauaaAGACACGACGAu	|| |||||||	cucuaggccUCACUGCUGCUu
	SMIM25	cgguuauaaagacACGACGAu	|||||||||	ccacugccaccacUGCUGCUc
	AP001432.1	cgGUUAUAAAGACACGACGAu	||:| || ||||||	cuCAGU-CAUCACUGCUGCUu
	LINC01547	cgguuauaAAGACACGACGAu	| || |||||||	cggccccgUCCUCUGCUGCUg
	AP001476.1	cgguuauaaagacACGACGAu	|||||||||	cgggcgagcccgaUGCUGCUc
	AP000547.3	cgGUUAUAAAGAC-ACGACGAu	|::|| ||| ||||||	ccCGGUA--CCUGCUGCUGCUc
	BX890604.1	cgguuauaaagacACGACGAu	|||||||||	cgaggccggcggcUGCUGCUa

	FAM239A	cgguuauaaagacACGACGAu	|||||||	cgaggccggcggcUGCUGCUa
	FAM239B	cgguuauaaagacACGACGAu	|||||||	uaccuggggccguUGCUGCUu
	XIST	cgGUUAUAAAGACACGACGAu	||||| | | | | |||||||	gcCAAUUUGUGUUUGCUGCUc
	MIR503HG	cggUUAUAAAGACACGACGAu	|||: | | | |||||||	uugAAUGACUGUAUGCUGCUu
miR-1249-3p	AC239800.3	acuucuucccccCUUCCCGCa	|||||||	gacucugaaaccaGAAGGGCGg
	AL391069.4	acuucuucccccCUUCCCGCa	|||||||	cgcuggguuccuaGAAGGGCGc
	TERC	acuucuuccccCCCUUCCCGCa	| |||||||	uaacccuaacuGAGAAGGGCGu
	AC008392.1	acuucuuccccccUUCCCGCa	|||||||	ccucaguacuguaaAAGGGCGg
	LINC01547	acuucuuccccccUUCCCGCa	|||||||	ucggcuucagaauaAAGGGCGc
	XIST	acuucuuccccccUUCCCGCa	|||||||	ucugcaguuaagcuAAGGGCGu
miR-5195-3p	LINC01355	ucGGGGGAGUCU-C-UUGACCUa	| |::| | | | |||||||	caCACUUUAAAAUGUAACUGGAu
	AL365181.2	ucgggggagUCU-CUUGACCUa	:|| |||||||	agaacagcgGGAGGAACUGGAg
	AC097634.1	ucGGGGGAGUC--U-CUUGACCUa	|::: |||| | |||||||	uuCUUUAUCAGUAAUCAACUGGAa
	MUC20-OT1	ucggggGAGUCUCUUGACCUa	|| :| |||||||	caugggCUGGGCCAACUGGAa
	AC093297.2	ucgggggAGUCUC--UUGACCUa	| :|:| |||||||	uuuagaaUAGGGGCAAACUGGAa
	AL008729.2	ucGGGGGAGUCUC-UUGACCUa	| ||| : || |||||||	gaCACCCAGGAAGCAACUGGAa
	AC008080.1	ucgGGGGAGUCUCUUGACCUa	:||| || |||||||	cagCUCCU--GA-AACUGGAa
	AC016831.5	ucgggGGAGUCUCUUGACCUa	:|| | |||||||	cuuugCUUCCCAUAACUGGAa
	CASC9	ucgGGGGAGUCUCUUGACCUa	||::|| |||||||	gaaCCUUUC----AACUGGAu
	PVT1	ucggGGGAGUCUCUUGACCUa	|| || |:|||||||	guugCCAUCUGGGAACUGGAu
	C9orf170	ucgggggagUCUCUUGACCUa	||||||||||	auguggggAGAGAACUGGAg
	LINC00707	ucgggggaGUCUCUUGACCUa	|||| |||||||	ugcagugcCAGAAAACUGGAa
	LINC01468	ucGGGGGAGUCUCUUGACCUa	:|| |:: | |||||||	caCUCCAUUGUA-AACUGGAa
	MALAT1	ucgggggaGUCUCUUGACCUa	::|| |||||||	guuugaagUGGAAAACUGGAa
	AP003486.1	ucgggggagucuCUUGACCUa	|||||||	uacaaagaaguuGAACUGGAu
	AC008124.1	ucggggGAGUCUCUUGACCUa	| ||| ||||||	gaugugaUGAGA-AACUGGAg
	AC083805.1	ucGGGGGAGUCUCUUGACCUa	|| ||: |||||||	gcCCAACUUUCCUAACUGGAg
	ATP2B1-AS1	ucGGGGGAGUCUCUUGACCUa	:: |: |||||||	cuCUUAAUUCCAGAACUGGAa
	HELLPAR	ucgggggagucucUUGACCUa	|||||||	cauacguaucauuAACUGGAa

MAPKAPK5-AS1	ucGGGGGAGUCUCUUGACCUa	|:||:|| | |||||||	uaCUCCUUCUGCCAACUGGAa
LINC01089	ucgggggagucUCUUGACCUa	| |||||||	gaaacuagauuAAAACUGGAa
MEG3	ucGGGGGAGUCUCUUGACCUa	||: || || |||||||	caCCUGCU-AGCAAACUGGAg
IQCH-AS1	ucgggggagucucUUGACCUa	|||||||	uagaaaauacaccAACUGGAa
LINC00052	ucgggggagucucUUGACCUa	|||||||	aaaaccaaaugccAACUGGAg
AC092127.1	ucgGGGGAGUCU--C---UUGACCUa	||::|::|| | |||||||	agaCCUUUUGGAAGGCUUAACUGGAu
AC005920.3	ucgggggaGUCUCUUGACCUa	::| ||||||||	agagugaaUGGUGAACUGGAc
LINC00662	ucgggggagucUCUUGACCUa	| |||||||	auuuuauaauaAAAACUGGAa
AC012313.1	ucgggggagucucUUGACCUa	|||||||	caugaguucccccAACUGGAg
BX640515.1	ucgGGGGAGUCU---CUUGACCUa	|::|| ||: |||||||	uugCUUCUAAGGAUUAAACUGGAa
LINC01671	ucggGGGAGUCUCUUGACCUa	||| |||||||||||	gaagCCCAGAGAGAACUGGAc
BX890604.1	ucggggGAGUCU--CUUGACCUa	|| ||| ||||||||	gagaugCUGAGAAGGAACUGGAg
LINC01204	ucggGGGAGUCUCUUGACCUa	|| ||| |||||||	cugaCCAUCA-CUAACUGGAu
JPX	ucggggggagUC-UC-UUGACCUa	|| || |||||||	ucaaaggaaAGAAGCAACUGGAa