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Diagnostic and Prognostic Potential of Circulating miR-1301-3p, miR-106a-5p, miR-129-5p, miR-3613-3p, and miR-647 microRNAs in Gastric Cancer

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Abstract—Gastric cancer (GC) is one of the most common malignant tumors worldwide and ranks fifth in the structure of cancer mortality. MicroRNAs are involved in the pathogenesis and progression of GC as epigenetic factors, and are considered as potential noninvasive markers. We selected microRNAs involved in the regulation of epigenetic mechanisms in GC (miR-1301-3p, miR-106a-5p, miR-129-5p, miR-3613-3p, miR-647) and analyzed their expression in plasma of GC patients. To assess their diagnostic and prognostic potential, we estimated correlations of differential expression with clinical and pathological characteristics of GC tumors. The study included 65 plasma samples from the GC patients and 48 plasma samples obtained from the individuals without tumor lesions, which were used as a control group. The expression was analyzed by using real-time polymerase chain reaction (RT-PCR) method. When comparing the expression levels of selected microRNAs in the plasma of GC patients and the control group, significant differences were found for miR-1301-3p (p = 0.040), miR-106a-5p (p = 0.029), miR-129-5p (p < 0.0001), miR-647 (p < 0.0001). MiR-129-5p expression was significantly associated with the prevalence of a primary tumor (p = 0.002), with the development of metastases to regional lymph nodes (p = 0.003), and distant metastases (p = 0.003), as well as with the late clinical stage (p = 0.003). There was a significant correlation between the miR-3613-3p expression and the clinical stage of GC (p = 0.049). ROC analysis revealed that combining miR-106a-5p, miR-129-5p, miR-1301-3p, and miR-647 improves diagnostic and prognostic properties of the potential panel of markers.

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Keywords: miR-1301-3p, miR-106a-5p, miR-129-5p, miR-647, miR-3613-3p, microRNAs, gastric cancer, epigenetics, biomarker

INTRODUCTION

Gastric cancer (GC) is one of the most common types of malignant tumors and is characterized by high aggressiveness and mortality. In most patients, GC is diagnosed at the late stages, when size of the primary tumor is increased and development of the distant metastases occurs. Therefore, search for novel diagnostic and prognostic markers remains important for early diagnostics and effective treatment of GC. Markers that can be detected in noninvasively obtained biological material are of particular interest. Differential expression of circulating microRNAs (miRNAs) in the plasma of patients is considered as a potential marker for various diseases, including cancer [1-6].

MiRNAs are short (approximately 22 nucleotides) single-stranded non-coding sequences that control

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various physiological processes and play an important role in epigenetic regulation by suppressing translation of messenger RNA (mRNA). Differential expression of some miRNAs is associated with clinical characteristics of various types of tumors and their treatment, which makes them interesting for further study as prognostic biomarkers [7]. However, miRNAs have some limitations as markers, since their expression may vary depending on the tumor, type of biomaterial, as well as its storage conditions, which complicates the analysis.

Mutations in the known driver genes that are typical for many tumors are underrepresented in GC. Investigation of somatic mutations in the genes of epigenetic regulation in GC confirms their important role in the development and pathogenesis of GC [8]. Using the computer algorithm mirDIP [9] and analyzing previously published data, we selected miRNAs that target genes involved in epigenetic processes and demonstrate differential expression in various types of tumors. These include miR-1301-3p, miR-106a-5p, miR-129-5p, miR-647, miR-3613-3p.

Some of the selected miRNAs were described as participating in the development and progression of many types of tumors. In particular, miR-1301 suppresses migration and invasion of tumor cells in hepatocellular carcinoma [10], colorectal cancer [11], and GC [12, 13]. MiR-106a demonstrates an oncogenic effect and promotes proliferation and metastasis in GC [14, 15], prostate cancer [16], cervical cancer [17], and osteosarcoma [18]. MiR-129-5p, conversely, was described as a tumor suppressor in breast cancer [19], colon cancer [20], and GC [21]. Differential expression of miR-647 has been found in hepatocellular carcinoma [22], colorectal cancer [23], and GC [24]. Differential expression of miR-3613-3p was observed in the plasma of patients with retinoblastoma [25], in the plasma and tissues of patients with colorectal cancer [26] and GC [27].

The aim of this work was to study expression of the selected miRNAs in the plasma of GC patients with various clinical and pathological characteristics of tumor growth to evaluate their potential as biomarkers for GC.

MATERIALS AND METHODS

Patients and biomaterial. The study involved 65 GC patients, including 36 men and 29 women, with average age of 64 years (range of years 40-83). All patients were diagnosed and undergone surgical treatment at the N. N. Burdenko Elective Surgery Clinic of the I. M. Sechenov First Moscow State Medical University. The material was annotated with indication of its localization, clinical stage, Loren classification and TNM classification, presence or absence of signet ring cells, as well as age, gender, and overall survival. Plasma samples from 48 healthy donors with no history of cancer were included in the investigation as a control group. All subjects gave their voluntary informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki and the protocol No. 04-19 approved by the Ethics Committee of Sechenov First Moscow State Medical University (Sechenov University) on March 6, 2019. Clinical and pathological characteristics of the patients and healthy donors are presented in Table 1.

RNA extraction and real-time polymerase chain reaction (RT-PCR). Total RNA was extracted from the samples by using Trizol (Life Technologies, USA) and a miRNeasy Mini Kit (Qiagen, Germany) according to the protocol suggested by manufacturers with small modifications. A NanoDrop 2000 micro-volume spectrophotometer (Thermo Fisher Scientific, USA) was used to estimate concentration and purity of the obtained RNA.

For each sample, cDNA was synthesized from 300 ng of total RNA using a MiScript II RT Kit (Qiagen) according to the recommended protocol. Real-time PCR was performed with a CFX96 Real-Time PCR Detection System (Bio-Rad, USA). Estimation of expression level was performed in three repetitions for each transcript and exogenous control cel-miR-39-3p, using a MiScript SYBR Green PCR Kit (Qiagen) according to the manufacturer's protocol. Primer sequences are listed in Table 2. Presynthesized miScript Primer Assay (Qiagen) primer was used for cel-miR-39-3p. The obtained Ct values were normalized and analyzed using the $2^{-\Delta Ct}$ method and were presented as relative expression units (REU) [28].

Statistical analysis. Statistical analysis of the results was performed by using Statistica 13.1 program

Table 1. Clinical and pathological characteristics of the participants

Variables	Patients (n = 65)	Healthy controls (n = 48)
Gender		
Female	29 (45%)	28 (58%)
Male	36 (55%)	20 (42%)
Age (years)		
<49	9 (14%)	16 (33%)
>=50	56 (86%)	32 (67%)

Table 1 (*cont.*)

Variables	Patients (n = 65)	Healthy controls (n = 48)
T		
T1	3 (5%)	
T2	24 (37%)	
T3	29 (44%)	
T4	9 (14%)	
N		
N0	32 (49%)	
N1	27 (42%)	
N2	4 (6%)	
N3	2 (3%)	
M		
M0	52 (80%)	
M1	13 (20%)	
Stage		
I	3 (5%)	
II	28 (43%)	
III	21 (32%)	
IV	13 (20%)	
Survival status (n = 63)		
Alive	61 (97%)	
Dead	2 (3%)	
Lauren classification (n = 58)		
Diffuse	51 (88%)	
Intestinal	7 (12%)	
Signet ring cells		
No	43 (66%)	
Yes	22 (34%)	
Tumor localization		
Antral region	6 (10%)	
Cardia	19 (29%)	
Body	40 (61%)	

Table 2. Primer sequences used to estimate microRNA expression

Primer	Sequence
miR-1301-3p	5′-AGCTGCCTGGGAGTGACTTC-3′
miR-106a-5p	5′-AAAAGTGCTTACAGTGCAGGTAGA-3′
miR-129-5p	5′-TTTTGCGGTCTGGGCTTGC-3′
miR-647	5′-TGGCTGCACTCACTTCCTTC-3′
miR-3613-3p	5′-ACAAAAAAAAAAGCCCAACCCTTC-3′

(StatSoft, USA). Normality of sample distribution was evaluated using the Shapiro–Wilk test (for sample sizes < 50 samples) or the Kolmogorov–Smirnov test (for sample sizes > 50 samples). In the case of normal distribution, the results are presented as a mean and standard deviation. When distribution was not normal, quantitative characteristics were described by using median (Me) and lower and upper quartiles (Q1-Q3). Comparison of the two groups by a quantitative indicator with normal distribution was performed using the Student's t-test; comparison of the two groups' variables with distribution differed from normal was performed by the Mann–Whitney test. The Kruskal–Wallis test was used when comparing three or more groups of variables, distribution of which differed from the normal.

A predictive model characterizing dependence of a variable on factors was developed using linear regression. The Kaplan–Meier method was used to analyze overall survival. To explore potential of microRNAs as a predictive biomarker, receiver operating characteristic curves (ROC-curves) were constructed, and area under the curve (AUC) was examined by calculating sensitivity and specificity at various threshold levels. Potentially useful predictors have been included in one-dimensional and multidimensional logistic regression analysis. $p < 0.05$ values were considered statistically significant.

RESULTS

Abundance of miR-1301-3p, miR-106a-5p, miR-129-5p, and miR-647 is significantly different in the plasma of GC patients and healthy donors. To estimate diagnostic potential of the investigated miRNAs, the levels of expression were quantified in the plasma samples of GC patients and plasma samples of the individuals without tumor lesions at the time of the study. It was shown that abundance of miR-1301-3p in the plasma samples of GC patients was significantly reduced compared to the control group

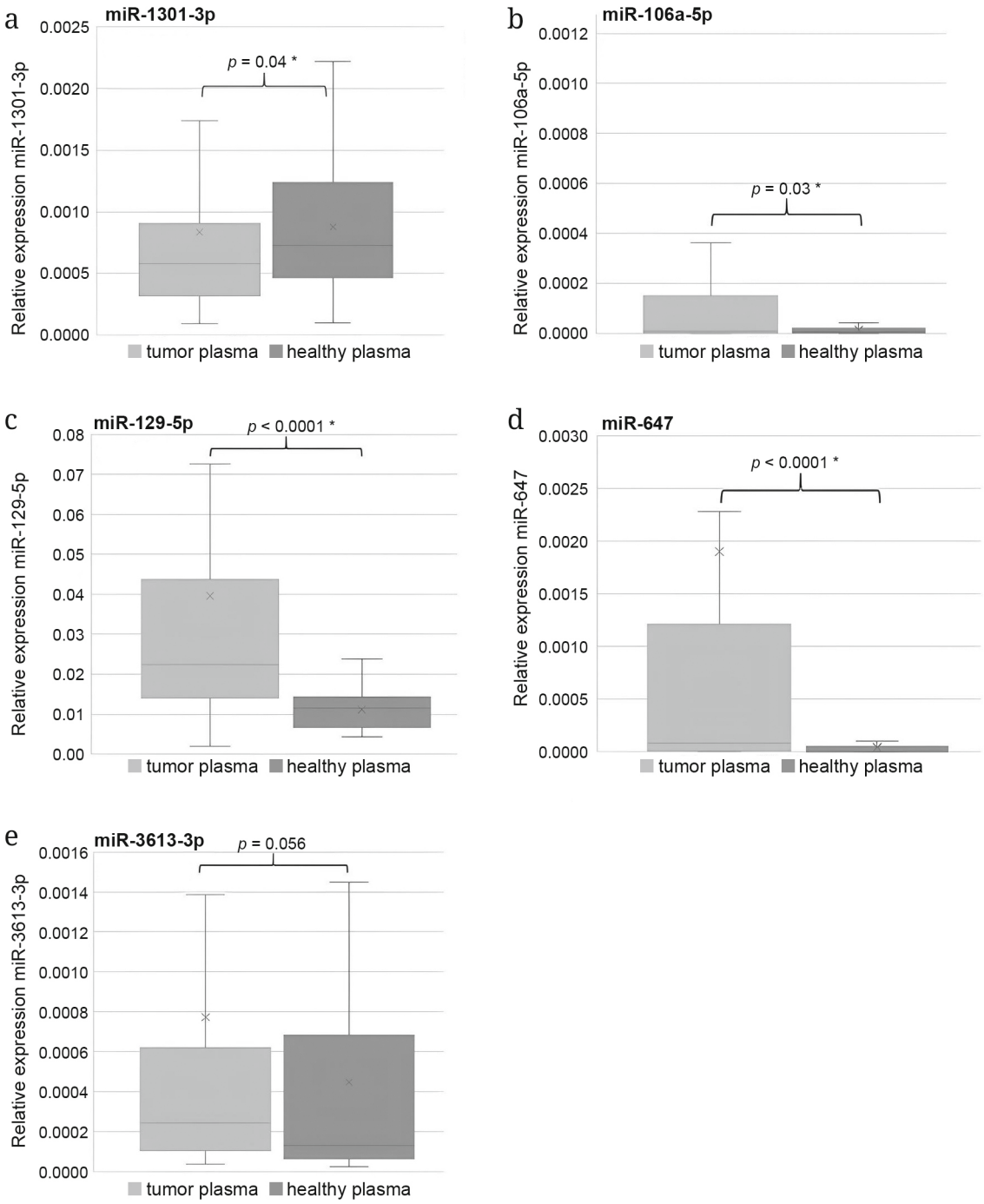

Fig. 1. Expression level of miR-1301-3p (a), miR-106a-5p (b), miR-129-5p (c), miR-647 (d), and miR-3613-3p (e) in the plasma of GC patients and healthy donors. * Statistically significant *p*-values.

(p = 0.040) (Fig. 1a). A statistically significant increase in the miR-106a-5p expression was found in the plasma samples of GC patients compared to the control (p = 0.029) (Fig. 1b). Abundance of the miR-129-5p was significantly higher in the plasma of GC patients compared to the control (p < 0.0001) (Fig. 1c), as well as abundance of the miR-647 (p < 0.0001) (Fig. 1d). No significant difference was found for the miR-3613-3p (p = 0.056) (Fig. 1e).

Expression levels of miR-129-5p and miR-3613-3p in the GC plasma samples are associated with clinical and pathological characteristics of GC patients. To determine significant factors related to the clinical course of GC, we analyzed potential associations between the expression of circulating miRNAs and clinical and pathological characteristics of the GC patients.

It was found that the increased expression of miR-129-5p was associated with the larger tumor size

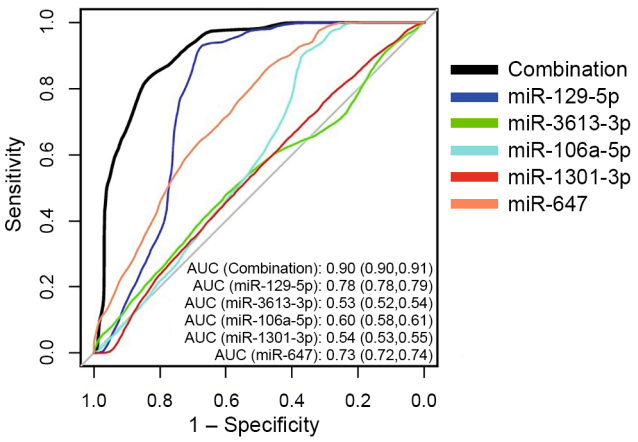

Fig. 2. Associations of abundance of miR-106a-5p, miR-129-5p, miR-3613-3p, miR-1301-3p, miR-647 in the plasma of GC patients and healthy donors, as well as combinations of miRNAs that showed the best results (miR-129-5p, miR-1301-3p, miR-647).

(T3-T4 group) according to the TNM classification (p = 0.002), metastasis to regional lymph nodes (N1-3) (p = 0.003), distant M1 metastases (p = 0.003), as well as later GC stages (stage III-IV) (p = 0.003). MiR-3613-3p was also associated with the advanced stages of GC (stage III-IV) (p = 0.049). When comparing with other characteristics (gender, age, overall survival, Loren classification, the presence of the signet ring cells), no significant correlations were found. Presence of the signet ring cells is a characteristic of the gastric signet ring cell carcinoma with low differentiation of tumor cells. It is an unfavorable histological subtype of GC that progresses rapidly, metastasizes early, and has worse prognosis compared to the other subtypes.

There were also no significant associations when comparing expression of the miR-106a-5p, miR-647, and miR-1301-3p in the plasma of GC patients with different clinical and pathological characteristics. The results of all estimated correlations are presented in Table S1 in the Online Resource 1.

Diagnostic value of miRNAs was confirmed by ROC-curves and analysis of the AUC. To estimate potential diagnostic and prognostic value of the miRNAs in GC, ROC-curves were used. When analyzing differentiation between the groups of GC patients and the healthy donors, the best result was shown for combination of miR-129-5p, miR-1301-3p, and miR-647, for which AUC was 0.90 (confidence interval [95% CI]: 0.90-0.91), sensitivity 83%, and specificity 83%, accuracy 83% (Fig. 2).

Expression of miR-1301-3p, miR-106a-5p, miR-129-5p, miR-647, and miR-3613-3p in the plasma of GC patients was compared with their clinical and pathological characteristics. MiRNAs that showed significant differences were further analyzed by one-dimensional logistic regression analysis to calculate AUC with statistical significance level of more than 0.5.

The ROC-curves and AUC values obtained for all investigated miRNAs and clinical and pathological characteristics of GC patients are presented in Fig. 3. For each of the characteristics, combination of only those miRNAs that showed the best combined result is shown. In particular, the AUC for miR-106a-5p, miR-129-5p, miR-647 and the primary tumor size T was 0.72 (confidence interval [95% CI]: 0.71-0.74), sensitivity 70%, specificity 62% and accuracy 65% (Fig. 3a).

Analysis of the ROC-curve of miR-106a-5p, miR-129-5p, miR-647 and the development of metastases to regional lymph nodes showed an AUC of 0.76 ([95% CI]: 0.75-0.77), sensitivity of 88% and specificity of 56%, accuracy of 71% (Fig. 3b). The AUC for miR-106a-5p, miR-129-5p and the development of distant metastases was 0.76 ([95% CI]: 0.75-0.8), sensitivity 89% and specificity 60%, accuracy 84% (Fig. 3c). The AUC for miR-106a-5p, miR-647 and signet ring cells was 0.64 ([95% CI]: 0.63-0.66), sensitivity 96% and specificity 30%, accuracy 74% (Fig. 3d).

Combination of miR-106a-5p, miR-129-5p, and miR-647 and clinical stage of the GC patients gave the AUC of 0.72 ([95% CI] 0.71-0.73), sensitivity 76%, specificity 53%, accuracy 64% (Fig. 3e).

DISCUSSION

In recent years, more and more studies confirm important role of ncRNAs in the GC pathogenesis. Aberrant expression of some of them could be considered as one of the key events in carcinogenesis, which makes them promising biomarkers.

To analyze the miRNA-target gene pairs, we used the computer algorithm mirDIP (http://ophid.utoronto.ca/mirDIP/), which is able to integrate data on almost 152 million target pairs collected from 30 different resources, therefore providing high degree of reliability [9]. As a result, 407 miRNAs were predicted, and each of them can regulate several genes of epigenetic regulation simultaneously. Based on the obtained data, miR-106a-5p, miR-129-5p, miR-3613-3p, miR-647, and miR-1301-3p were selected for further investigation as potential regulators of some genes of epigenetic regulation. Among them are genes involved in DNA methylation/demethylation, histone modifications, and chromatin remodeling (Fig. 4). In the previous work, we have demonstrated importance of somatic mutations in these genes for the GC development and progression [8], whereas this study was devoted to the role of epigenetic factors as biomarkers for GC.

A significant advantage of miRNAs is their ability to circulate in body fluids. Serum or plasma samples

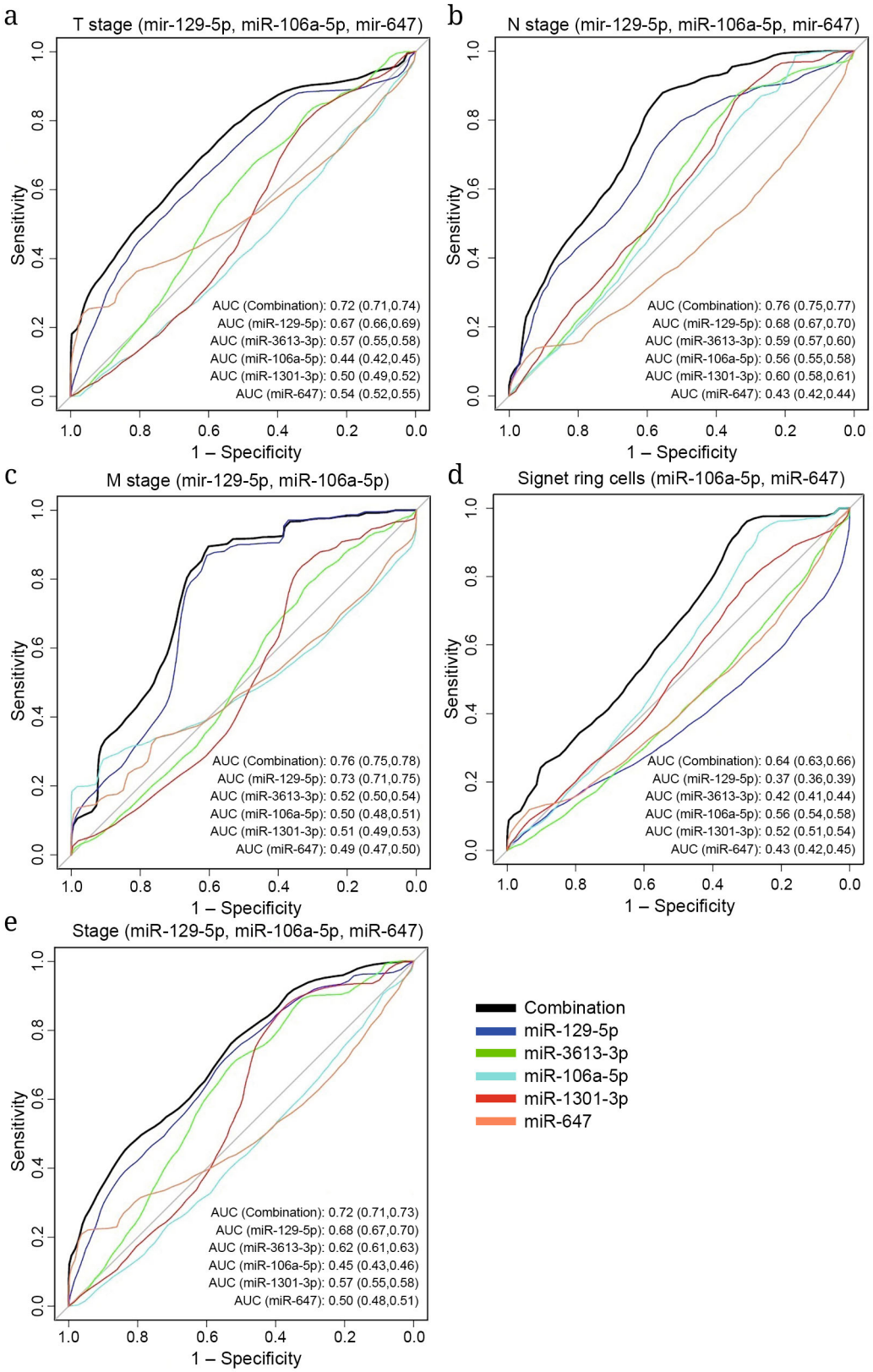

Fig. 3. Associations of miR-106a-5p, miR-129-5p, miR-3613-3p, miR-1301-3p, miR-647 with clinical and pathological characteristics of the GC patients, as well as combinations of miRNAs that showed the best results: miR-106a-5p, miR-129-5p, miR-647 with the primary tumor size (a), miR-106a-5p, miR-129-5p, miR-647 with the development of metastases to regional lymph nodes (b), miR-106a-5p, miR-129-5p with the development of distant metastases (c), miR-106a-5p, miR-647 with signet ring cells (d); miR-106a-5p, miR-129-5p, miR-647 with the clinical stage (e). The receiver operating characteristic (ROC-curves).

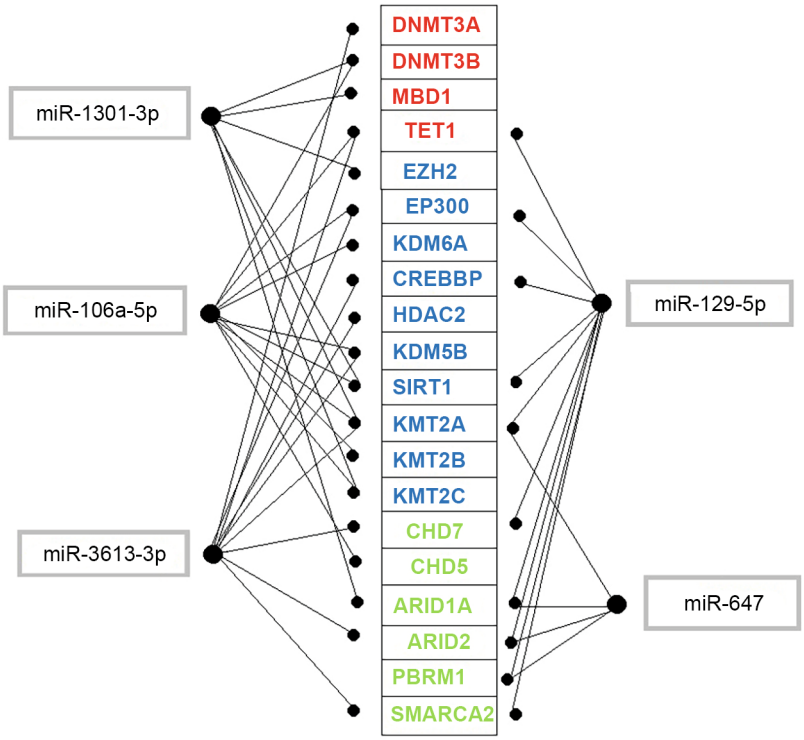

Fig. 4. MiRNAs and their target genes involved in epigenetic regulation (genes associated with DNA methylation are highlighted in red, genes regulating histone modifications are highlighted in blue, genes regulating chromatin remodeling are highlighted in green).

are relatively easily available, and miRNAs are able to withstand adverse physiological conditions, including pH changes, high temperature, and freeze/thaw cycles. Therefore, circulating miRNAs in plasma are considered as promising biomarkers for non-invasive diagnosis and prognosis. Expression patterns of miRNA in plasma allow identification of various types of tumors, including GC. To assess diagnostic and prognostic potential of the selected miRNAs (miR-1301-3p, miR-106a-5p, miR-129-5p, miR-647, and miR-3613-3p), we investigated their expression in the plasma of GC patients compared to the healthy donors, as well as their potential associations with clinical and pathological characteristics.

In our study, the expression level of miR-1301-3p in the plasma samples of GC patients was significantly lower compared to the control group (p = 0.040). It has been shown previously that overexpression of miR-1301-3p suppresses migration, invasion, and proliferation of GC cells [12, 13]. In addition, miR-1301 expression was associated with survival of the patients with hepatocellular carcinoma [29], however, in our study, no significant associations with the clinical and pathological characteristics of the GC patients were found.

We found a significant increase of the miR-106a-5p expression level in the plasma of GC patients compared to the control (p = 0.029), which is in concordance with the previous studies. Wang et al. showed that overexpressed miR-106a could play an oncogenic role in the GC carcinogenesis [14, 30]. In another study, the miR-106a expression level was significantly higher in the plasma samples of GC patients compared with the plasma from healthy donors [31], which is in concordance with our results. Some authors confirmed that the increased miR-106a expression is associated with metastasis and epithelial-mesenchymal transition [32], as well as with tumor differentiation, lymph node metastasis, and tumor size [31]. In our study, no correlations of the miR-106a-5p expression with clinical and pathological characteristics of the GC patients were found.

The miR-129-5p expression was significantly different in the plasma samples of GC patients compared with the control group (p < 0.0001). Both increased and decreased expression was observed in different samples, but the average value was higher in the GC patients than in the control group. This result differs from the values obtained by other researchers, as miR-129-5p expression has been reported to be decreased in the tumor tissues and blood of the GC patients compared with the corresponding non-tumor adjacent tissues and healthy donors [33]. The miR-129-5p expression level was significantly lower in the GC tissues than in the adjacent non-tumor tissues, and also lower in the GC cell lines compared

with the normal epithelial cells of gastric mucosa [34]. MiR-129-5p suppresses invasion and proliferation of the GC cells [35]. Yu et al. found that the increased level of miR-129-5p in the GC cells delays the cell cycle at the G0/G1 phase, showing suppressive activity against the tumor [36]. Some studies also mentioned both increase and decrease of the miR-129-5p expression [37], as it was in our investigation, but it was mainly related to the tumor tissues of hepatocellular carcinoma.

As a result of our statistical analysis, it was shown that the miR-129-5p expression was significantly associated with the primary tumor size, development of metastases to regional lymph nodes and distant metastases, as well as with the clinical stage of GC. The miR-129-5p expression has previously been associated with the tumor size and invasion of lymph nodes, and poor prognosis in the GC patients [38].

The level of miR-647 expression was significantly different in the plasma samples of GC patients compared with the control group ($p < 0.0001$). MiR-647 is known to act as a tumor suppressor in GC, suppressing invasion and metastasis [39]. We did not find a significant statistical association of the miR-647 expression level in plasma of the GC patients with clinical and pathological characteristics, although in some studies the miR-647 expression was significantly changed in the patients with GC metastases in the lymph nodes [40]. Previously, we reported association of the miR-647 expression with the primary tumor size of GC patients, but we did not find significant differences between the expression levels in the tumor and adjacent non-tumor tissues of GC patients and the sectional samples of gastric tissue (control) [41].

In our study, no significant difference in the miR-3613-3p expression was found between the plasma samples of GC patients and the control group ($p = 0.056$), although it was previously demonstrated that the miR-3613-3p level was significantly lower in the tumor tissues and serum of the patients with breast cancer. Functional studies have shown that miR-3613-3p could act as a tumor suppressor and restrain its progression by regulating the cell cycle [42]. In addition, it was found that miR-3613-3p overexpression causes significant suppression of several genes with tumor suppression potential (encoding apoptotic protease activating factor 1 (APAF1), Dicer, DNA fragmentation factor β subunit, von Hippel–Lindau protein, and neurofibromin 1) in the human neuroblastoma cells [43]. Analysis of correlations of the miR-3613-3p levels in plasma with the clinical and pathological characteristics of the GC patients demonstrated a tendency to be increased at the late stages of GC (stage III+IV) with low statistical significance ($p = 0.049$). Small amount of the available data and their ambiguity suggests an unclear role of miR-3613-3p in GC [27].

At present, profiles of miRNA expression are being actively studied in different types of tumor to form a system that will allow determining presence of a tumor, its stage and presence of metastases, as well as assessing sensitivity to the chosen therapy. There are numerous papers presenting miRNAs as potential markers that could be used for diagnosis and prognosis of tumors progression with high sensitivity and specificity [44-46]. Many studies have shown that using combination of miRNAs as a marker generally increases its sensitivity and specificity, and also allows combining diagnostic and prognostic properties of the individual miRNAs providing a more accurate assessment of the disease in each specific case.

Construction of ROC-curves is widely used to assess diagnostic potential of biomarkers both individually and in panels. We performed a one-dimensional logistic regression analysis for miR-1301-3p, miR-106a-5p, miR-129-5p, miR-647, and miR-3613-3p in the plasma of GC patients and healthy donors, as well as in association with the clinical and pathological characteristics of the GC patients. As a result, the assumption that combination of miRNAs in panels increases their specificity and sensitivity was confirmed. In particular, the use of miR-129-5p, miR-1301-3p, and miR-647 combination in analysis of differentiation between the GC patients and healthy donors showed the AUC values of 0.9 and sensitivity and efficacy values above 0.8, which significantly exceeded the AUC values for each individual miRNA (0.78, 0.54, and 0.73, respectively). The combination of miR-106a-5p, miR-129-5p, and miR-647 increases sensitivity and specificity when estimating the primary tumor size, clinical stage of the disease, development of metastases to regional lymph nodes, and distant metastases, although the results of ROC analysis show average efficacy. In particular, we have revealed potential of the panel of miR-129-5p, miR-1301-3p, miR-647 as a diagnostic system, and the miR-106a-5p, miR-129-5p, miR-647 panel as a predictive model for several clinical characteristics of the patients with GC.

It is known that analysis of the ROC-curve for miR-106a has previously demonstrated high sensitivity and specificity in the diagnosis of GC [31, 47]. In the study of gastric juice samples from GC patients, the AUC for miR-129-1-3p and miR-129-2-3p was 0.639 and 0.651, respectively, while for their combination the AUC reached 0.656 [48]. High efficiency and sensitivity of the miR-647 level in the serum of GC patients as a diagnostic biomarker [49] and of the miR-1301-3p level in the papillary thyroid cancer [50] has been also demonstrated previously.

MiRNAs play a complex role in oncogenesis, therefore their mechanisms of action and functions still remain not fully understood. Besides, there are some limitations in using circulating miRNAs as diagnostic

and prognostic biomarkers. Among them are low concentrations of miRNAs in plasma and serum, insufficiently accurate methods for quantifying miRNAs, and lack of the standard methods for normalization. Overcoming of these limitations is associated with certain challenges. Our data suggest potential diagnostic and prognostic significance of miR-106a-5p, miR-129-5p, miR-1301-3p, and miR-647 in gastric carcinogenesis, however, this requires further investigation.

CONCLUSIONS

Like the other types of tumors, patients with GC have better prognosis when it is diagnosed at an early stage and a patient obtains an optimal treatment. Thus, application of effective diagnostic and prognostic biomarkers for early diagnosis could increase long-term survival of GC patients. A profile of differentially expressed miRNAs associated with clinical and pathological characteristics of GC patients in plasma or serum samples could be used for noninvasive diagnosis and postoperative monitoring. In our work, we revealed statistically significant differences between the expression levels of miR-1301-3p, miR-106a-5p, miR-129-5p, and miR-647 in the plasma of GC patients and in the control group. Moreover, associations with clinical and pathological characteristics of the GC patients were found for miR-129-5p and miR-3613-3p. Combining of miR-129-5p, miR-1301-3p, and miR-647 into a panel improved their diagnostic properties, and combination of miR-106a-5p, miR-129-5p, and miR-647 increased their sensitivity and specificity in predicting several clinical and pathological characteristics. Thus, our data suggest potential diagnostic and prognostic value of the miR-106a-5p, miR-129-5p, miR-1301-3p, and miR-647 panel in GC, which requires additional investigation.

Abbreviations. AUC, area under the curve; GC, gastric cancer; ROC-curves, receiver operating characteristic curves.

Supplementary information. The online version contains supplementary material available at https://doi.org/10.1134/S000629792460385X.

Contributions. M. V. Nemtsova and I. V. Bure – concept and curation of the work; E. A. Vetchinkina, E. B. Kuznetsova, E. A. Alekseeva, and N. S. Esetov – experiments; A. E. Kiseleva – obtaining plasma samples and processing tables with clinical and pathological characteristics of patients; A. I. Kalinkin – computational analysis of the results; E. A. Vetchinkina, M. V. Nemtsova, and I. V. Bure – writing text of the paper.

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Ethics approval and consent to participate. The study was conducted in accordance with the Declaration of Helsinki and the protocol No. 04-19 approved by the Ethics Committee of Sechenov First Moscow State Medical University (Sechenov University) on March 6, 2019. All subjects gave their voluntary informed consent for inclusion before they participated in the study.

Conflict of interest. The authors of this work declare that they have no conflicts of interest.

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