


Prognostic role of microRNA polymorphisms in advanced gastric cancer: a translational study of the Arbeitsgemeinschaft Internistische Onkologie (AIO)

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Background: To determine the prognostic role of selected microRNA (miRNA) polymorphisms in advanced gastric cancer (AGC).

Patients and methods: Six hundred and seventy-four AGC patients received 5-fluorouracil (F), leucovorin (L), oxaliplatin (O) or FL + cisplatin (P) or additional docetaxel (T) to FLO (FLOT) within four clinical trials. Polymorphisms of mir-26a1 (rs7372209), mir-27a (rs895819), mir-100 (rs1834306), mir-146a (rs2910164), mir-196-a2 (rs11614913), mir-219-1 (rs107822) and mir-423 (rs6505162) were genotyped. Variable selection for the final multivariate model (n = 487) was based on univariate and multivariate Cox-regression analyses with a cut-off P-value of ≤20%.

Results: Genetic factors significantly associated with overall survival (OS) were rs7372209 (mir-26a1) variant genotypes (hazard ratio, HR 1.307 [95% confidence interval (CI) 1.031–1.656], P = 0.0272), rs895819 (mir-27a) variant genotypes (HR 1.304 [95% CI 1.031–1.650], P = 0.0270) and rs11614913 (mir-196a2) variant genotypes (HR 0.791 [95% CI 0.625–1.000], P = 0.0497). Clinical factors with significant impact on OS were Eastern Cooperative Oncology Group (ECOG) 2 performance status (HR 1.880 [95% CI 1.254–2.820], P = 0.0023), curative surgery of advanced disease (HR 0.235 [95% CI 0.123–0.449], P < 0.0001) and addition of docetaxel in locally AGC patients (HR 0.348 [95% CI 0.145–0.838], P = 0.0301). Combined analyses revealed an improved OS in patients without any unfavourable genotype of 18 months compared with 14, 12 and 10 months in patients with 1, 2 and 3 unfavourable genotypes, respectively (P = 0.0257).

Conclusions: These data suggest a significant impact of selected miRNA polymorphisms on prognosis in AGC.

Key words: advanced gastric cancer, chemotherapy, microRNA, polymorphisms, prognostic factors,
Introduction

Gastric cancer (GC) is one of the most common causes of cancer-related death worldwide and the sixth most frequently diagnosed cancer in Europe [1, 2]. Gastric carcinogenesis is a multistep process, leading to genetic and epigenetic changes in oncogenes and tumour-suppressor genes in the gastric epithelium [3]. However, many steps in the molecular pathology remain unknown and need to be further characterized [4, 5]. The majority of GCs is diagnosed in an advanced stage and is thereby unresectable at the time of diagnosis. This fact contributes to the poor 5-year survival of ~10%–20% in Europe and the USA [6, 7]. The fundament of unresectable GC treatment is systemic chemotherapy. As to date, there is no globally established standard of care and different regimens are used in the Western countries and Japan [8]. Patients are treated either with a triplet consisting of 5-fluorouracil (5-FU), a platinum-compound and docetaxel or epirubicin, or with a doublet of 5-FU and a platinum-compound, respectively [9]. Improvement in the long-term outcome of treatment requires further studies with the focus of optimizing existing reference regimens including the identification of useful predictive and prognostic biomarkers as well as examining novel targeted agents [10]. The most recent and so far the only successful approach for a targeted therapy is the introduction of the monoclonal antibody trastuzumab in the treatment of advanced gastric cancer (AGC) [11].

During the last decade, growing interest has been shown in microRNAs (miRNAs), and it is now well documented that altered miRNA expression plays a role in the pathogenesis of many human malignancies, including GC [12–14]. miRNAs are transcribed and processed to primary miRNA (pri-miRNA) in the nucleus. The pri-miRNA is then converted to pre-miRNA and transported into the cytoplasm. Mature miRNA is produced by the enzyme Dicer from pre-miRNA and is incorporated in the RNA-induced silencing complex, which is produced by the enzyme Dicer from pre-miRNA and is incorporated in the RNA-induced silencing complex, which is supposed to regulate up to almost one-third of all known human transcripts [17]. miRNAs function as post-transcriptional negative regulators of gene expression and effectuate decreased target mRNA levels by either inducing mRNA cleavage or translational repression. miRNAs have been reported to affect numerous central cellular pathways, such as cell proliferation and apoptosis [18].

Single-nucleotide polymorphisms (SNPs) are common DNA sequence variations, and there is recent evidence that SNPs in miRNAs may contribute to cancer susceptibility and progression [16]. In miRNA genes and genomic regions involved in miRNA processing, SNPs have the potential of interfering with normal miRNA gene regulation, leading to aberrant mRNA levels. Thus, such nucleotide substitutions can give rise to both loss-of-function and gain-of-function mutations in the miRNA, e.g. by changing the miRNA 5′/3′-UTR binding site [19].

Aim of this study

We have carried out an exploratory investigation on the effect of SNPs in miRNA genes in a large sample set consisting of patients with AGC enrolled in clinical phase II and III trials of the German ‘Arbeitsgemeinschaft Internistische Onkologie’ (AIO), with the future goal of improving individualized treatment and prognosis of this disease by identification of molecular markers with impact on therapy outcome.

Materials and methods

Study subjects

Six hundred and seventy-four patients with histologically confirmed GC were treated within four clinical trials. Of importance, only patients with at least one application of chemotherapy were included in this analysis. Patients who did not receive any chemotherapy due to withdrawal of informed consent or death prior treatment start were excluded from analysis as well as those with neoadjuvant treatment of localized disease (Figure 1).

The clinical trials investigated the efficacy and safety of either a doublet of platinum/5-FU (FLO or FLFP) or a triplet of oxaliplatin, 5-FU and docetaxel (FLOT) [20, 21]. In detail, FLP included biweekly cisplatin 50 mg/m² and weekly leucovorin 200 mg/m² and 5-FU 2000 mg/m², FLO biweekly oxaliplatin 85 mg/m², leucovorin 200 mg/m² and 5-FU 2600 mg/m² and FLOT biweekly oxaliplatin 85 mg/m², leucovorin 200 mg/m², 5-FU 2600 mg/m² and docetaxel 50 mg/m². All participants in the translational part gave written informed consent for genetic analyses, and the translational study was approved by the local ethics committees.

Selections of polymorphisms and genotyping

We extensively searched the literature and the dbSNP database and selected seven SNPs in functional regions of miRNA genes. The selection was based on the following criteria: a functional impact of the miRNA SNP has been studied in vitro or by association analyses in cancer patients; the miRNA has a hypothesized role in the pathogenesis of GC; genomic alterations or expression of the miRNA has a hypothesized association with susceptibility and/or prognosis in GC; the reported minor allele frequency was ≥0.10 (http://www.ncbi.nlm.nih.gov/projects/SNP). The selected miRNA genes with their suggested role cancer cells and the potential functional relevance of the analysed SNPs are listed in Table 1. Selected polymorphisms were rs7372209 (mir26a1), rs895819 (mir-27a), rs1834306 (mir-100), rs2910164 (mir-146a), rs11614913 (mir-196a2), rs107822 (mir-219-1) and rs6505162 (mir-423). DNA isolation was carried out as previously described using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) from whole blood samples [25]. Genotyping was carried out using custom-designed genotyping assays (Applied Biosystems, Darmstadt, Germany). Assay IDs are given in supplementary Table S1, available at Annals of Oncology online. Allelic discrimination was conducted with an Applied Biosystems 7500 Fast Real-Time PCR unit. For each run two non-template and three positive controls were added. Ten percentage of the samples were randomly chosen and genotyped in duplicate, with a concordance of 100%.

Statistical analysis

Deviation from Hardy–Weinberg Equilibrium (HWE) and pair-wise linkage disequilibrium (LD) were analysed using R (Version 2.3.1, http://www.r-project.org/). All SNPs except of rs6505162 (mir-423) showed no deviation from the HWE. There was no LD between the analysed polymorphisms. Exploratory association analysis between the independent variables and the dependent variable overall survival (OS) was based on Cox-regression.
models. Independent variables were Eastern Cooperative Oncology Group (ECOG) performance status, age, sex, metastases, disease sites, addition of docetaxel (FLOT versus FLO or FLP), surgery following chemotherapy and genotype information of the analysed miRNA polymorphisms. Heterozygous and homozygous variant genotypes were grouped and compared with homozygous wild types. All factors were studied univariately and in a corresponding multivariate Cox-regression model. Univariate analyses only included patients with complete datasets for the selected clinical and genetic parameters to ensure 100% concordance with individuals of the multivariate analyses \((n = 487)\). Risk factors were assessed as relevant to be mutually included in the final model if the \(P\)-value was <20%. Relevant effects in either analysis were studied further for treatment (docetaxel) interaction. Finally, we defined a multivariate Cox-regression model that consists of the former relevant exploratory factors and relevant interactions to treatment. We assessed any effect in the multivariate Cox-regression model as statistically significant if the corresponding \(P\)-value was <5%. HRs with their corresponding 95% CIs of the final multivariate model are shown in Figure 2. There was no significant association between the other analysed polymorphisms and OS. Combined analyses of rs7372209 (mir-26-a1), rs895819 (mir-27-a) and rs11614913 (mir-196-a2) that could be identified as independent prognostic factors revealed an improved median OS in patients without any unfavourable genotype \((n = 72)\) of 18 (95% CI 14–21) months, compared with 14 (95% CI 12–18), 12 (95% CI 10–15) and 10 (95% CI 8–15) months in patients with 1 \((n = 192)\), 2 \((n = 169)\) or 3 \((n = 54)\) unfavourable genotypes, respectively. The log-rank test indicated a statistically significant difference between the survival rates over time \((P = 0.0257, \text{Figure 3})\).

**results**

Four hundred and eighty-seven patients were assessable for exploratory association analyses. Genotype frequencies are given in supplementary Table S1, available at *Annals of Oncology* online. The median age of the population was 67 years. Twenty-eight percent of patients received FLO, 14% FLP and 58% FLOT, with an overall median OS of 14 months (95% CI 12–15). Further patient’s characteristics are summarized in Table 2.

Four hundred and eighty-seven patients were assessable for OS. Multivariate Cox-regression analysis revealed that three of the analysed mir polymorphisms were statistically significant associated with OS. These SNPs were rs7372209 located in the 5’_untranslated region_ of mir-26a \((P = 0.0272)\), rs895819 in the pre-miRNA of mir-27-a1 \((P = 0.0270)\) and rs11614913 in the pre-miRNA of mir-196-a2 \((P = 0.0497)\). Additionally, surgical resection following chemotherapy \((P < 0.0001)\) and ECOG performance status of 2, compared with 0 at study entry, were significantly \((P = 0.0023)\) associated with improved OS. A significant association between the addition of docetaxel to platinum/5-FU and improved OS was observed only in locally advanced AGC patients \((P = 0.0301)\). HRs with their corresponding 95% CIs of the final multivariate model are shown in Figure 2. There was no significant association between the other analysed polymorphisms and OS. Combined analyses of rs7372209 (mir-26-a1), rs895819 (mir-27-a) and rs11614913 (mir-196-a2) that could be identified as independent prognostic factors revealed an improved median OS in patients without any unfavourable genotype \((n = 72)\) of 18 (95% CI 14–21) months, compared with 14 (95% CI 12–18), 12 (95% CI 10–15) and 10 (95% CI 8–15) months in patients with 1 \((n = 192)\), 2 \((n = 169)\) or 3 \((n = 54)\) unfavourable genotypes, respectively. The log-rank test indicated a statistically significant difference between the survival rates over time \((P = 0.0257, \text{Figure 3})\).

**discussion**

With this exploratory analysis, we were able to identify three miRNA polymorphisms (rs7372209 in the pri-miRNA of mir-26a1, rs895819 in the pre-miRNA of mir-27-a1 and rs11614913 in the pre-miRNA of mir-196-a2) as independent prognostic factors in AGC patients.

Interestingly, the addition of docetaxel to FLO only improved OS in patients with locally advanced disease in this reported patient cohort. There was no significant improvement of OS in patients with metastatic GC. However, our analyses
were not designed for verifying a beneficial effect of additional docetaxel to platinum/5-FU in AGC in the first-line setting, especially due to the cross-comparison of different clinical trials that were conducted between 2003 and 2009. Therefore, this finding remains on the hypothesis level and final conclusions cannot be drawn with respect to the usage of docetaxel in AGC in the first-line setting. Surgical resection in curative intention of advanced disease following chemotherapy turned out to have a strong impact on OS. A total of 39 (8%) patients of which 38 had metastatic disease underwent surgery in curative intention following chemotherapy, which was at the investigator’s choice and not foreseen within the conducted clinical trials. Currently, surgery of metastatic disease does not represent standard of care in the management of GC and is mainly applied to patients on an individualized manner, based on the patient’s clinical course and by the biological behaviour of the tumour disease. Indeed, few reports describe a beneficial effect of surgery in the metastatic setting [26, 27]. However, the role of surgery in the metastatic setting needs evaluation in prospective clinical trials and is, e.g., the objective of an ongoing prospective randomized trial comparing gastrectomy, metastasectomy plus systemic chemotherapy with systemic chemotherapy alone (The GYMSSA Trial, NCT00941655) [28].

Table 1. Overview of selected miRNA genes and analysed SNPs

<table>
<thead>
<tr>
<th>Gene</th>
<th>rs number</th>
<th>SNP location</th>
<th>microRNA function</th>
<th>Suggested effect of the SNP in gastric cancer pathogenesis</th>
<th>Suggested functional relevance of the SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>mir-26-a1</td>
<td>rs7372209</td>
<td>5’UTR</td>
<td>Inhibition of cell growth and cell cycle progression by</td>
<td>Oncogenic</td>
<td>Decreased activity of mir-26-a1 leading to decreased apoptosis and increased cancer cell proliferation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(i) downregulation of cyclin E1 and CDK6 leading to pRb dephosphorylation [37]</td>
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<tr>
<td></td>
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<td></td>
<td>(ii) downregulation of cyclin D2 and EZH2 [38]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(iii) upregulation of CDK inhibitor expression [38]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mir-27a</td>
<td>rs895819</td>
<td>Pre-miRNA</td>
<td>Promotion of cell growth and proliferation by</td>
<td>Oncogenic</td>
<td>Increased level of mir-27a leading to increased cancer cell proliferation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(i) downregulation of ZBTB10 leading to reduced Sp1 expression [40]</td>
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<tr>
<td></td>
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<td></td>
<td>(ii) upregulation of cyclin D1 and downregulation of p21 [42]</td>
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<td></td>
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<td></td>
<td>(iii) downregulation of anti-apoptotic genes like BCL-2 [44]</td>
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<td></td>
<td></td>
<td></td>
<td>(iv) upregulation of P-glycoproteins [42, 44]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mir-100</td>
<td>rs1834306</td>
<td>5’UTR</td>
<td>Inhibition of cell growth and proliferation by</td>
<td>Oncogenic</td>
<td>Decreased expression of mir-100 leading to increased cancer cell proliferation and drug resistance</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>(i) downregulation of plk-1 [36]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(ii) downregulation of IGFR1 [36]</td>
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<tr>
<td>mir-146a</td>
<td>rs2910164</td>
<td>Pre-miRNA</td>
<td>Inhibition of cell proliferation and migration by</td>
<td>Oncogenic</td>
<td>Decreased expression of mir-146a leading to cancer cell proliferation and invasiveness</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(i) downregulation of EGFR and IRAK1 [50]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mir-196-a2</td>
<td>rs11614913</td>
<td>Pre-miRNA</td>
<td>Inhibition of apoptosis and promotion of cell proliferation by</td>
<td>Tumor suppressing</td>
<td>Impaired binding of mature mir-196a2 to its target mRNA leading to pro-apoptotic effects</td>
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<tr>
<td></td>
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<td></td>
<td>(i) post-transcriptional negative regulation of annexin A1 expression [45]</td>
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<tr>
<td>mir-219-1</td>
<td>rs107822</td>
<td>5’UTR</td>
<td>Inhibition of cell proliferation by</td>
<td>Oncogenic</td>
<td>Decreased expression of mir-219-1 leading to enhanced cancer cell proliferation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(i) downregulation of GPC3 [51]</td>
<td></td>
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<tr>
<td>mir-423</td>
<td>rs6505162</td>
<td>Pre-miRNA</td>
<td>Promotion of cell proliferation by</td>
<td>Tumor suppressing</td>
<td>Decreased function of mir-423 leading to decreased cell proliferation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(i) downregulation of PABPC1 [52]</td>
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</tbody>
</table>

CDK, cyclin-dependant kinase; EGFR, epidermal growth factor receptor; GPC3, glypican; IGFR, insulin-like growth factor receptor; IRAK, interleukin-1 receptor-associated kinase; PABPC1, polyadenylate-binding protein cytoplasmic; plk, Polo-like kinase; pRb, retinoblastoma protein; UTR, untranslated region; ZBTB, Zinc finger and BTB domain containing.
An additional important clinical parameter with significant impact on OS was the number of involved sites at study entry. Unfortunately, we only had exact information on this parameter in 357 (73%) cases. However, when including this parameter in our multivariate analyses, it turned out to have some impact on OS with a HR of 1.137 (95% CI 1.008–1.283, $P = 0.0373$) for each additional involved site. The reported significant impact on OS of surgical resection following chemotherapy, ECOG 2 and the miRNA polymorphisms such as rs7372209 (mir-26a1) and rs895819 (mir-27-a1) remained significant (data not shown). Only rs11614913 (196-a2) failed the significance level in this smaller patient cohort with a $P$-value of 0.0637 (HR 0.772, 95% CI 0.587–1.01). We decided to primarily analyse and report the results of the larger cohort, due to its higher power for the identification of potential predictive or prognostic variables.

To our knowledge, this is the first report on a comprehensive association analysis between miRNA polymorphisms and prognosis in AGC. miRNAs are supposed to play an important role in aetiology, progression and prognosis of cancer [29]. A differential gene expression of miRNAs has already been shown for various cancers and even a cancer classification by miRNA expression profiles seems to be possible [30, 31]. Additionally, miRNAs have been identified as oncogenes as well as tumour-suppressor genes [13, 32–34]. The discovery of polymorphisms in miRNA genes has opened a new field of cancer research with implications for epidemiology, carcinogenesis and pharmacogenomics [16, 19]. miRNA polymorphisms can occur in the $5'$-UTR of their target genes affecting binding of miRNA to their target sequence, and in the $3'$-UTR of the mRNA affecting its gene expression, in the pre- or pri-miRNA affecting its biogenesis or, relatively rare, in the mature miRNA consisting of the $5'$-seed and the $3'$-mismatch tolerant region affecting the miRNA level and function [19]. miRNA polymorphisms in GC have so far only been studied in susceptibility studies with very limited data on their functional aspects, and few are suspected to be associated with GC risk [14, 35].

We have identified three potentially prognostic factors out of a set of seven miRNA polymorphisms. The presence of the variant alleles of rs7372209 (mir-26a1) and rs895819 (mir-27-a1) were associated with a worse prognosis in our AGC cohort. In line with our data, the variant allele of rs7372209 (mir-26a1) has been shown to be associated with worse treatment outcome in a cohort of 61 patients with metastatic colon cancer. Here, the authors reported time-to-progression and overall response data, but not OS data [36]. The underlying molecular mechanisms of this congruent observation in metastatic colon cancer and AGC remain to be cleared. Moreover, the functional impact of rs7372209 on mir-26a1 expression and/or function is unknown. mir-26a1 has been shown to inhibit cancer cell proliferation [37–39]. The underlying mechanisms seem to be complex and include the downregulation of cyclins D2, D3, E1, of EZH2 and of cyclin-dependant kinases (CDK4 and CDK6) and the upregulation of CDK inhibitors [37, 38]. Chen et al. [39] reported the induction of cell cycle arrest in hepatic carcinoma cells by ectopic expression of mir-26a. The report by Zhu et al. underlines this finding by showing an increased expression of mir-26a in quiescent cells and a decrease during cell proliferation. Also demonstrated in this report is a direct suppression of cyclin E1 and CDK6, resulting in a reduced phosphorylation of retinoblastoma protein [37].

Lu et al. [38] described miR-26a as growth suppressive in nasopharyngeal carcinoma and postulated that its suppressive effects are mediated mainly by repressing EZH2 expression. We speculate that the variant T allele of rs7372209 leads to an impaired mir-26a1 expression or function.

In contrast, the functional impact of the variant C allele in rs895819 (mir-27a) seems to be elucidated and is supposed to lead to a higher mir-27a amount in the cell [40]. Sun et al. investigated the role of rs895819 in GC and could demonstrate an increased risk for GC in individuals with the variant C allele. Moreover, they were able to show a significant association of the mir-27a variant genotypes (C/C) with lymph node metastasis and carried out further functional analyses showing that the variant genotype is responsible for elevated mir-27a levels and reduced mRNA of the Zinc finger and BTB domain containing...
Figure 3. Kaplan–Meier plot for overall survival (OS) according to the number of unfavourable genotypes. Five unfavourable genotypes in three genes were identified by the multivariate Cox-regression analysis (see Figure 2). In detail, these genotypes were the two mir-26a1 non-wild-type genotypes, the mir-196a2 wild-type and the two mir-27a non-wild-type genotypes. Median OS was 18 (95% CI 14–21), 14 (95% CI 12–18), 12 (95% CI 10–15) and 10 (95% 8–15) months in patients with 0, 1, 2 or 3 unfavourable genotypes, respectively.
10 (ZBTB10), which is known to promote the expression of the transcription factor SPI [40]. In addition, Katada et al. [41] have described a higher expression of mir-27a in undifferentiated GC tissues and found a significant association between higher mir-27a expression and lymph node metastasis. Two other studies investigating the role of mir-27a in GC have shown that a downregulation of mir-27a could inhibit the proliferation of cancer cells in vitro and in vivo, respectively [42, 43]. Supplementary data are provided by Zhang et al. showing a downregulation of BCL-2 and other anti-apoptotic genes as well as of P-glycoprotein by mir-27a, and by Zhao and Hu showing that a downregulation of mir-27a leads to decreased expression of P-glycoprotein resulting in an accumulation of cytostatics in the cancer cells [42, 44]. These findings together with our results implicate that mir-27-a1 has an important role in the molecular pathogenesis and progression of GC and deserves further evaluation of its role in GC prognosis.

miR-196a is known to promote cell proliferation and to suppress apoptosis by post-transcriptional negative regulation of annexin A1 expression [45]. Our analyses revealed that the variant T allele of rs11614913 (mir-196-a2) was associated with improved OS, but this polymorphisms turned out to have the weakest effect in our patient cohort and the statistical significance was borderline. Our results confirm previous findings that the variant allele is associated with a better prognosis in GC and, among others, also in breast and lung cancer [46–48]. Very interestingly, Hu et al. carried out comprehensive functional analyses of rs11614913 in non-small cell lung cancer (NSCLC). The authors reported a significantly decreased survival in carriers of the wild type C/C genotype. In an analysis of 23 human lung cancer tissue samples, this genotype was also associated with a statistically significant increase in mature mir-196a expression, but not with changes in levels of the precursor, suggesting enhanced processing of the pre-miRNA to its mature form. Moreover, they could demonstrate that the rs11614913 SNP can affect the binding of mature mir-196a2-3p to its target mRNA [48]. Taken together, rs11614913 seems to lead to an impaired mir-196a2 function, suggesting a tumour-suppressing role of this SNP in cancer cells. The authors conclude that rs11614913 may be a prognostic biomarker for NSCLC and, although there are no corresponding analyses in GC, we hypothesize that similar mechanisms might be present in GC and our results implicate that rs11614913 deserves further evaluation in AGC.

Taken together, each individual SNP of the identified three miRNA polymorphisms had a week prognostic value in our study. However, the combined analyses of these three miRNA polymorphisms revealed a tremendous difference of 8 months median survival between patients without any unfavourable genotype and those with unfavourable genotypes in all of the three identified polymorphisms. In conclusion, the identified miRNA polymorphisms, especially when applied in a combined fashion, may have the potential to become useful tools in the future and might be of help for a better guidance of treatment decisions in GC patients. Nevertheless, at the current state, we cannot draw final conclusions on how to use these data in clinical practice. To make this possible, prospective studies with an upfront biomarker-trial design are needed. According to the suggested revised determination of levels of evidence (LOE) for biomarker studies, our study can be assigned to the category B as proposed by Simon et al. [49] with the need of a validation in an independent dataset and corresponds to the LOE II.

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disclosure
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