A case series and a systematic review of the literature on upregulated miRs in gastric cancer: a story still to be told.

Laura Lorenzon¹, Francesco Belia², Deborah French³, MARCO CAVALLINI⁴, and Giovanni Blandino⁵

¹Fondazione Policlinico Universitario Agostino Gemelli IRCCS
²Università Cattolica del Sacro Cuore Facoltà di Medicina e Chirurgia
³Faculty of Medicine and Psychology, Department of Clinical and Molecular Medicine, University of Rome "La Sapienza", Sant'Andrea Hospital of Rome, Rome, Italy
⁴UNIVERSITY OF ROME "LA SAPIENZA"
⁵Italian National Cancer Institute Regina Elena, Translational Oncogenomic Unit, Rome, Italy

May 30, 2023

Abstract

Background: Recent literature documented the expression of miRs in gastric cancer (GC), however their clinical utility is still unclear. Methods and Results: 117 resected GCs were evaluated for miR21, miR135b, miR196a, miR196b relative expression (RE). The performance of miRs’ to differentiate cancer vs normal mucosa was tested using ROC curves. Univariable and multivariable analyses were conducted to correlate miRs RE with pathological features and survivals. Although all the 4 miRs were upregulated, ROC curves documented that this was not-significant in differentiating GC (p ns). miR135b significantly correlated with Lauren’s intestinal type and more advanced pT stages (p 0.017, and p 0.025), whereas miR196a and miR196b were more expressed in advanced pStages (p 0.016, and p 0.038). miR196b was also more expressed in nodal positive patients comparing N0 (p 0.035). Survival curves were non-significant for miRs RE, while pStages could significantly differentiate oncological outcomes (p<0.0001). On Cox analyses, pStages independently correlated with OS (HR 4.9, 95%CI 2.041-12.104), whereas increased age correlated with a worse DFS (HR 6.0, 95%CI 2.596-13.947), and lymph-node ratio with DSS (HR 14.4, 95%CI 4.213-49.373). Literature was reviewed a using PRISMA method focusing on miRs and response to therapy and the detection of peritoneal metastases: out of 116 manuscripts retrieved, just 41 were pertinent with the outcomes of interest, and 14.6% were from Western countries. miR21, miR135b and miR204 were reported to correlate with response to therapy. Conclusions: miR21, miR135b, miR196a and miR196b were documented up-regulated in GC, but their clinical utility is still to be fully investigated.

Introduction

Gastric cancer ranks as the fifth most prevalent cancer globally and is the third leading cause of cancer-related deaths. Despite advancements in diagnosis, treatment, and overall care, the prognosis for gastric cancer remains unfavorable, with a five-year survival rate of only 35.5%.² Treatment options for gastric cancer depend on the stage and location of the tumor, however nowadays treatment is always multidisciplinary and based on the combination of surgery, radiation therapy, and chemotherapy. Several clinical trials have evaluated the effectiveness of perioperative chemotherapy in gastric cancer. These studies have demonstrated improved survival outcomes and increased response rates with perioperative chemotherapy compared to
surgery alone in selected patients with resectable gastric cancer. Nowadays, the FLOT perioperative regimen has gained significant popularity, particularly in Europe, due to its impressive outcomes in terms of overall survival, progression-free survival, and response rate. However, following a curative resection, a substantial proportion of patients, ranging from 14% to 60%, face the challenge of disease recurrence. Gastric cancer commonly exhibits recurrence patterns including peritoneal, hematogenous, and locoregional recurrences. Therefore, a better understanding of the molecular mechanisms underlying gastric cancer is urgently needed for the development of more effective treatment strategies. MicroRNAs, or miRs, are a class of small non-coding RNA molecules that play critical roles in various biological processes, including cell proliferation, differentiation, and apoptosis. They have emerged as potential therapeutic targets for cancer treatment, including gastric cancer. miRNAs are involved in the regulation of gene expression by binding to the 3’ untranslated regions (UTRs) of mRNA transcripts, resulting in either degradation or inhibition of translation of the target mRNAs. miRs play critical roles in the development and progression of gastric cancer. The dysregulation of miRNAs has been linked to the onset and progression of gastric cancer, offering a potential avenue for therapeutic intervention. Targeting these miRNAs holds promise for the treatment of gastric cancer. However, further research is required to gain a comprehensive understanding of the mechanisms governing miRNA regulation in gastric cancer. This understanding will be crucial in developing more effective treatment strategies that leverage miRNA regulation. In a recent paper our group focused on the down-regulation of miRs in non-cardial gastric cancer patients. The study confirmed the down-regulation of miR31, miR148a, miR204, and miR375 in gastric cancer tissues, which was also observed in previous experiences. Significant associations were discovered between the down-regulation of these miRNAs and non-cardial gastric cancers, as well as the specific Lauren’s classification. Additionally, in vitro experiments demonstrated that miR204 targets BCL-2 in gastric cancer cell lines. However, Upon analyzing a substantial case series of patients with gastric cancer, it was determined that the standard pathological features emerged as the sole significant independent variables associated with a poorer prognosis. In recent years, significant advancements in gastric cancer research have shed light on various crucial aspects such as immunotherapy, molecular subtyping, liquid biopsies, microbiome and precision medicine. Utilizing high-throughput sequencing technologies and bioinformatics tools, novel up-regulated miRs have been identified as potential biomarkers for diagnosing, prognosticating, and predicting therapeutic responses in gastric cancer patients. Precisely, exploring both down-regulated and up-regulated miRs through the lens of precision medicine can provide a comprehensive understanding of the intricate regulatory network underlying gastric cancer and open doors to innovative therapeutic approaches tailored to individual patients. By personalizing treatment strategies, the efficacy can be maximized while minimizing adverse effects. Hence, our study sought to retrospectively examine the relative expressions (RE) of four miRs in a cohort of gastric cancer patients. Additionally, we conducted a comprehensive review on the role of miRs concerning response to therapy and the detection of peritoneal metastases, both of which are considered cutting-edge aspects in the treatment of gastric cancer.

Methods

Case series. After the initial publication of our preliminary study regarding down-regulated miRs in gastric cancers, we proceeded to conduct further analyses on the four upregulated miRs (miR21, miR135b, miR196a, miR196b) within the same cohort of gastric cancer patients. The investigation followed an identical experimental design as previously described. Specifically, we retrieved data from consecutive patients who underwent gastric cancer resection at the Surgical and Medical Department of Translational Medicine—Sant’Andrea Hospital in Rome, spanning from March 2003 to December 2013. Exclusion criteria encompassed non-adenocarcinomas or in situ cancers, Siewert types I-II, patients with missing data, and those with necrotic or highly mucinous tumors. All the clinical-pathological variables including age, sex, tumor location, surgical procedures, TNM stages, Lauren’s classification, detection of gastritis in the surgical specimen, number of positive nodes, lymph nodes harvested (LNH), and lymph node ratio (LNR), were analyzed for their correlation with upregulated miRs. Furthermore, tissues from distal margins, specifically those located
at a minimum distance of 5 cm from the tumor, were randomly chosen from 16 patients and employed as internal controls. In the experimental investigation, we analyzed the expression of miRs in AGS human gastric adenoma cells, gastric cancer G16-16 cells, and HaCaT cells (utilized as a negative control). Experiments were conducted after RNA isolation (miRNeasy Micro Kit, Qiagen, Netherlands), extraction and quantification (NanoDrop, Thermo Fisher Scientific, Inc., Waltham, MA) from surgical specimens and cell lines, according to the manufacturer’s protocol. Subsequently, cDNA synthesis was conducted, followed by duplicate RT-PCR analysis to assess the expressions of miR21, miR135b, miR196a, and miR196b. This analysis was performed using TaqMan MicroRNA Assays from Applied Biosystems (Foster City, CA), following the manufacturer’s protocol. RNU6B was utilized as an endogenous control to normalize the expression of miRs. The relative expression (RE) of miRs in tumor tissues and cell lines was determined using the ΔΔCT method (CT target-CT reference), with the inter-quartile range (IQR1-3) of normal gastric mucosae serving as a reference and assigned a value of 1. The primary outcomes of interest encompassed the correlation between miRs’ relative expression and various clinical-pathological features, as well as the follow-up data, including overall survival (OS, any cause of death), disease-free survival (DFS, the first recurrence after resection), and disease-specific survival (DSS, death attributed to gastric cancer).

Systematic Review. This investigation has been conducted adhering to the PRISMA Statements for review and meta-analysis. PubMed Searches were conducted on December 2022, with the following queries: miR gastric cancer and response to therapy ("med int rev" [Journal] OR "manag int rev" [Journal] OR "mir"[All Fields]) AND ("stomach neoplasms"[MeSH Terms] OR ("stomach"[All Fields] AND "neoplasma"[All Fields]) OR ("stomach neoplasms"[All Fields] OR "gastric"[All Fields] AND "cancer"[All Fields]) OR "gastric cancer"[All Fields]) AND ("response"[All Fields] OR "responses"[All Fields] OR "responsive"[All Fields] OR "responsiveness"[All Fields] OR "responsivenesses"[All Fields] OR "responsives"[All Fields]) OR "responsivities"[All Fields] OR "responsivity"[All Fields] OR ("therapeutics"[MeSH Terms] OR "therapeutics"[All Fields] OR "therapies"[All Fields] OR "therapy"[MeSH Subheading] OR "therapy"[All Fields] OR "therapy s"[All Fields] OR "therapys"[All Fields]); and miR gastric cancer and peritoneal metastases ("med int rev" [Journal] OR "manag int rev" [Journal] OR "mir"[All Fields]) AND ("stomach neoplasms"[MeSH Terms] OR ("stomach"[All Fields] AND "neoplasms"[All Fields]) OR ("stomach neoplasms"[All Fields] OR ("gastric"[All Fields] AND "cancer"[All Fields]) OR ("gastric cancer"[All Fields]) OR ("peritoneal"[All Fields] AND ("peritonism"[All Fields] OR "peritonitis"[MeSH Terms] OR "peritonitis"[All Fields]) OR "metastasis"[MeSH Terms] OR "metastases"[All Fields] OR "metastasising"[All Fields] OR "metastasized"[All Fields] OR "metastasises"[All Fields] OR "metastasizing"[All Fields] OR "neoplasm metastasis"[MeSH Terms] OR ("neoplasm"[All Fields] AND "metastasis"[All Fields]) OR "neoplasm metastasis"[All Fields] OR ("metastase"[All Fields] OR "metastases"[All Fields] OR "metastasise"[All Fields] OR "metastasized"[All Fields]) OR ("peritonism"[All Fields] OR "peritonitis"[MeSH Terms] OR "peritonitis"[All Fields]) AND ("metastation"[All Fields] OR "metastatic"[All Fields] OR "metastases"[All Fields] OR "metastasis"[All Fields] OR "metastatic"[All Fields] OR "neoplasm metastasis"[MeSH Terms] OR ("neoplasm"[All Fields] AND "metastasis"[All Fields]) OR ("metastases"[All Fields] OR "metastatic"[All Fields] OR ("stomach neoplasms" OR ("stomach" AND "cancer") OR ("stomach neoplasms" OR ("gastric" AND "cancer") OR ("gastric cancer" OR ("peritoneal" OR ("peritonism" OR "peritonitis") OR ("metastasis" OR "metastatic") OR ("metastases" OR "metastatic") OR ("neoplasm metastasis") OR ("metastases" OR "metastatic") OR ("stomach neoplasms") OR ("stomach" AND "cancer") OR ("stomach neoplasms" OR ("gastric" AND "cancer") OR ("gastric cancer") OR ("peritoneal" OR ("peritonism" OR "peritonitis") OR ("metastasis" OR "metastatic") OR ("metastases" OR "metastatic") OR ("neoplasm metastasis")))))).

We did not consider any journal’s scores (i.e. journal’s Impact Factors) of the published series as exclusion criteria for this review. To eliminate duplicate references, a manual search was conducted. Subsequently, each retrieved paper underwent a thorough evaluation of its title and abstract to determine its inclusion or exclusion in this manuscript. Manuscripts that did not pertain to the investigation of gastric cancers were excluded, as well as those providing just pre-clinical results or those not related to the topics of interest. Our systematic review aimed to provide an overview of the number of papers that have explored these topics, the affiliation of authors (distinguishing between Western and Eastern countries), and the specific miRs that have yielded results in these respective fields.

Statistics. Continuous variables were analyzed after testing for normality with the Kolmogorov-Smirnov test and reported accordingly. For categorical variables, we employed frequencies and percentages for analysis. The performance of relative miRs’ expressions in predicting cancer diagnosis was evaluated using receiver operating characteristic (ROC) analysis, with the area under the curve (AUC) serving as the measure. A significant level of 0.7 was set for the AUC. In the univariable analysis, we compared the relative miRs’ expressions between cancer and normal gastric mucosae, and subsequently correlated the relative expressions with pathological features (Lauren’s classification, presence of gastritis in the surrounding tissues, grading,
pT stage, pN stage, LNR and pStages) using the T-test or Mann-Whitney tests. Survival analysis was conducted using the Kaplan-Meier method-log-rank test comparing patients presenting up-regulated miRs (RE>1) and those with RE less or equal 1, with the outcome of OS, DFS and DSS. Cox-proportional hazard models (forward selection) for the 3 survivals were calculated computing the following variables: miR31, miR148a, miR204, and miR375 RE (up- vs down-regulated), age (continuous variable), pT stage (pTis-pT2 vs pT3-pT4), pN stage (pN0 vs pN+), grading (G1-2 vs G3-4), pStages (pStage1-2 vs pStage 3-4), number of metastatic nodes (continuous variable) and LNR (continuous variable). Statistical analysis was conducted using MedCalc for Windows, version 10.2.0.0 (MedCalc Software, MariaKerke, Belgium), and XLSTAT 2023.1.2.1406. All tests were performed two-tailed and a P value <0.05 was considered as statistically significant.

Results

Case series. Overall, out of 228 patients retrieved, 117 non-cardial gastric cancer patients were selected according to study criteria. Patients had a mean age of 67.8 years, had M/F of 1.4, were treated with a sub-total gastrectomy in the 62.4% of the cases. Tumors were staged as pT3-4 in the 37.5% of the cases and N positive in 66.4%. Mean LNH was of 27.1 and mean LNR of 0.2. The greater majority were intestinal type gastric adenocarcinomas (71.9%), and almost 60% were treated with adjuvant chemotherapy. In gastric cancer specimens, all four miRs were observed to be upregulated. However, the relative expressions (RE) exhibited a non-normal distribution within the case series (Figure 1). The ROC curve analysis indicated that the upregulation of these miRs failed to differentiate between cancer and normal tissues effectively, with area under the curve (AUC) values as follows: miR21 (0.505), miR135b (0.529), miR196a (0.580), and miR196b (0.629), which were not statistically significant (p > ns). Regarding the correlation between upregulated miRs and clinical/pathological features, it was found that miR135b expression significantly correlated with Lauren’s intestinal type and more advanced pT stages (respectively Mann-Whitney test, p 0.017, and p 0.025), whereas both miR196a and miR196b were more expressed in advanced pStages (respectively Mann-Whitney test, p 0.016, and p 0.038) as shown in Table 1. miR196b was also more expressed in nodal positive patients comparing N0 (p 0.035). Overall, forty-four patients were treated with adjuvant chemotherapy. The median follow-up period for disease-specific survival was 48 months, ranging from 5 to 201 months. Kaplan-Meier curves were generated based on follow-up data from 107 patients. The analysis revealed a non-significant correlation between miRs’ relative expressions and survival curves. However, significant differentiation in survival was observed when considering the pStages (Figure 2). On multivariable Cox analyses, pStage was the only significant variable correlating with OS (HR 4.9, 95%CI 2.041-12.104), whereas increased age correlated with a worse DFS (HR 6.0, 95%CI 2.596-13.947), and an increased LNR with a worse DSS (HR 14.4, 95%CI 4.213-49.373) as shown in Table 2.

Systematic review. As depicted in Figure 3, out of the initial 116 manuscripts obtained through literature search, only 40 were relevant to the outcomes of interest. Among these, 21 manuscripts focused on the response to chemotherapeutic agents, while 19 papers examined peritoneal disseminations, with one publication covering both topics 19-58. Notably, among the selected papers, only 6 (14.6%) originated from Western countries, while the rest were contributed by authors from the East. However, it is worth mentioning that miR21, miR135b, and miR204 were consistently reported to correlate with therapy response, while miR21 and miR148a also demonstrated potential associations with metastasis. Additionally, it is noteworthy that miR200b and miR200c were the most frequently mentioned miRs in relation to these topics, appearing in 10.0% of the manuscripts.

Discussion

The suboptimal findings concerning the differentiation of gastric cancers and their clinical-pathological features based on miR expression align with our previous observations. Although several miRs have been associated with gastric adenocarcinomas, only a few have demonstrated clinical utility as diagnostic and
prognostic biomarkers. It is important to emphasize that research on miRs in gastric cancer is yet to fully uncover its potential, primarily due to the limited focus of the current literature on clinical trends. Therefore, it would be valuable to investigate the miR signature within the context of clinical trials and examine their modulation in response to chemotherapy. This approach could aid clinicians in selecting patients who are responsive to neo-adjuvant treatments, whether based on established gold standards or the latest immunotherapy agents. Several studies have investigated the role of miRs in gastric cancer. miR21, one of the most extensively studied, has been shown to be upregulated in gastric cancer tissue and associated with poor prognosis. It targets several tumor suppressor genes, including PTEN, PDCD4, and TPM1, resulting in increased cell proliferation and inhibition of apoptosis. Inhibition of miR21 expression has been shown to suppress tumor growth and induce apoptosis in gastric cancer cells. 59-62 Recent studies show that miR 135b plays a crucial role in the progression of gastric cancer and several factors have been identified to contribute to the overexpression of miR 135b, including Helicobacter pylori.15, 63-64 One study investigated the effect of miR-135b on gastric cancer growth and metastasis. The study found that inhibiting miR135b expression could result in significant inhibition of cell proliferation, migration, and invasion, suggesting that miR135b plays a crucial role in the progression of gastric cancer.65 Another study evaluated the relationship between miR135b expression and the clinicopathological parameters of gastric cancer. The study discovered that higher miR135b expression was significantly associated with tumor invasion depth, lymph node metastasis, and advanced clinical stage. Further, the study demonstrated that inhibiting miR135b could result in the suppression of gastric cancer cell proliferation and invasion.66 Studies have shown that miR196 is involved in various aspects of gastric cancer progression, including proliferation, invasion, and metastasis and its upregulation of miR-196 has been associated with poor prognosis in gastric cancer patients.67-69 In addition to its role in tumor progression, miR196 has also been found to be involved in drug resistance in gastric cancer. One study found that upregulation of miR196 in gastric cancer cells led to increased resistance to the chemotherapeutic drug, 5-fluorouracil (5-FU).70 The knockdown of miR196 could decrease gastric cancer cell proliferation and invasion and the use of a miR196 inhibitor could sensitize gastric cancer cells to 5-FU treatment.

Out of the 116 manuscripts retrieved for this review, only 40 were deemed pertinent to the desired outcomes, with a focus on the response to chemotherapeutic agents and peritoneal disseminations.19-58 Interestingly, most of the selected papers (85.4%) originated from authors in the East, while Western countries contributed only a small proportion (14.6%). Concerning individual microRNAs, a significant association was observed between miR21, miR135b, and miR204 and the response to therapy, suggesting their potential as biomarkers in treatment outcomes. Additionally, miR21 and miR148a showed promise in their potential correlation with metastasis. However, it should be noted that miR200b and miR200c were the most frequently reported microRNAs in relation to the examined topics, accounting for 10.0% of the manuscripts. The study has several limitations that should be taken into consideration. It included a relatively small sample size of 117 non-cardial gastric cancer patients. A larger sample size could provide more robust and generalizable results. The retrospective nature of the study may introduce biases and limitations in data collection and analysis and due to the study period of selection, patients were not treated with a FLOT-based regimen. Prospective studies with predefined protocols and follow-up would provide more reliable results. The median follow-up duration for disease-specific survival was 48 months, which may not be sufficient to capture long-term survival outcomes. Longer follow-up periods could provide a better assessment of the impact of miRNA expression on patient prognosis. To comprehensively comprehend the potential implications and contributions of up-regulated miRs, further research is essential.

Conclusions

Our study suggests that the upregulation of microRNAs (miR21, miR135b, miR196a, and miR196b) in gastric cancer specimens does not have strong prognostic value in terms of survival outcomes. Instead, factors such as tumor stage, age, and lymph node ratio appear to be more significant predictors of patient outcomes.

These findings emphasize the need for further research and investigation, particularly in Western countries,
to expand our understanding of the role of microRNAs in gastric cancer treatment response and metastatic processes. The integration of cutting-edge research topics, such as the role of miRs in the tumor microenvironment and the development of miR-based therapeutics, holds great promise for advancing our knowledge and transforming the management of gastric cancer towards more targeted and personalized approaches.

References


Table 1. Relative expression of miRs and correlation with pathological features

<table>
<thead>
<tr>
<th>miR21</th>
<th>miR21</th>
<th>miR21</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauren’s Intestinal</td>
<td>Lauren’s Diffuse</td>
<td>1.1</td>
<td>0.67</td>
</tr>
<tr>
<td>miR21</td>
<td>miR21</td>
<td>miR21</td>
<td>p value</td>
</tr>
<tr>
<td>Lauren’s Intestinal</td>
<td>Lauren’s Diffuse</td>
<td>0.6</td>
<td>0.577</td>
</tr>
<tr>
<td>Gastritis Yes</td>
<td>Gastritis No</td>
<td>0.55</td>
<td>0.577</td>
</tr>
<tr>
<td>Grade Grade G1-G2</td>
<td>Grade G3-G4</td>
<td>0.90</td>
<td>0.943</td>
</tr>
<tr>
<td>pTis-T2</td>
<td>pT3-T4</td>
<td>0.82</td>
<td>0.380</td>
</tr>
<tr>
<td>pN0</td>
<td>pN+</td>
<td>1.08</td>
<td>0.091</td>
</tr>
<tr>
<td>LNR&lt;0.13</td>
<td>LNR&gt;0.14</td>
<td>0.85</td>
<td>0.886</td>
</tr>
<tr>
<td>pStage 1-2</td>
<td>pStage 3-4</td>
<td>0.84</td>
<td>0.160</td>
</tr>
<tr>
<td>pStage 1-2</td>
<td>pStage 3-4</td>
<td>1.00</td>
<td>0.160</td>
</tr>
<tr>
<td>pStage 1-2</td>
<td>pStage 3-4</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>pStage 1-2</td>
<td>pStage 3-4</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>pStage 1-2</td>
<td>pStage 3-4</td>
<td>0.102</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Cox proportional hazard and survivals

End-point Overall Survival

<table>
<thead>
<tr>
<th>Variables</th>
<th>Value</th>
<th>SE</th>
<th>Wald Chi-Square</th>
<th>Pr &gt; Chi²</th>
<th>HR</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pStage</td>
<td>1.604</td>
<td>0.454</td>
<td>12.473</td>
<td>0.000</td>
<td>4.971</td>
<td>2.041-12.104</td>
</tr>
<tr>
<td>End-point Disease</td>
<td>End-point Disease</td>
<td>End-point Disease</td>
<td>End-point Disease</td>
<td>End-point Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-point Free</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
<td>Free</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival Variables</td>
<td>Value</td>
<td>SE</td>
<td>Wald Chi-Square</td>
<td>Pr &gt; Chi²</td>
<td>HR</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td>1.795</td>
<td>0.429</td>
<td>17.507</td>
<td>&lt;0.0001</td>
<td>6.017</td>
<td>2.596-13.947</td>
</tr>
</tbody>
</table>
### Figure 1

**A.** Upregulated miRs in gastric cancer tissues comparing normal tissues. Of note, non-tumor gastric tissues were randomly selected from 16 patients and used as controls sampling tissues located at least 5 cm from the tumor. Relative expressions (RE) of miRs in cancerous tissues and cell line was obtained using the ΔΔCT method (CT target-CT reference), with the inter-quartile range (IQR1-3) of normal gastric mucosae as a reference, considered equal to 1.

**B.** Distribution of miRs RE in gastric cancer tissues:
- **B.1** miR21
- **B.2** miR135b
- **B.3** miR196a
- **B.4** miR196b

### Figure 2

Survivals according to miRs expression.
- **A.** miR21 Up-regulated (relative expression >1) vs Down-regulated (relative expression < or equal 1):
  - **A.1** Overall survival
  - **A.2** Disease Free Survival
  - **A.3** Disease Specific Survival
- **B.** miR135b Up-regulated (relative expression >1) vs Down-regulated (relative expression < or equal 1):
  - **B.1** Overall survival
  - **B.2** Disease Free Survival
  - **B.3** Disease Specific Survival
- **C.** miR196a Up-regulated (relative expression >1) vs Down-regulated (relative expression < or equal 1):
  - **C.1** Overall survival
  - **C.2** Disease Free Survival
  - **C.3** Disease Specific Survival
- **D.** miR196b Up-regulated (relative expression >1) vs Down-regulated (relative expression < or equal 1):
  - **D.1** Overall survival
  - **D.2** Disease Free Survival
  - **D.3** Disease Specific Survival

### Figure 3

PubMed search of miRs in relation to response to therapy and peritoneal metastases. Tables report selected papers.
- **A.** miR and response to therapy
- **B.** miR and peritoneal metastases

**Legend**

<table>
<thead>
<tr>
<th>End-point</th>
<th>Disease</th>
<th>Specific</th>
<th>Survival</th>
<th>Variables</th>
<th>Value</th>
<th>SE</th>
<th>Chi-Square</th>
<th>Pr &gt; Chi²</th>
<th>HR</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-point</td>
<td>Disease</td>
<td>Specific</td>
<td>Survival</td>
<td>Variables</td>
<td>Value</td>
<td>SE</td>
<td>Chi-Square</td>
<td>Pr &gt; Chi²</td>
<td>HR</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>End-point</td>
<td>Disease</td>
<td>Specific</td>
<td>Survival</td>
<td>Variables</td>
<td>Value</td>
<td>SE</td>
<td>Chi-Square</td>
<td>Pr &gt; Chi²</td>
<td>HR</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>End-point</td>
<td>Disease</td>
<td>Specific</td>
<td>Survival</td>
<td>Variables</td>
<td>Value</td>
<td>SE</td>
<td>Chi-Square</td>
<td>Pr &gt; Chi²</td>
<td>HR</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>End-point</td>
<td>Disease</td>
<td>Specific</td>
<td>Survival</td>
<td>Variables</td>
<td>Value</td>
<td>SE</td>
<td>Chi-Square</td>
<td>Pr &gt; Chi²</td>
<td>HR</td>
<td>HR (95% CI)</td>
</tr>
</tbody>
</table>

**SE:** Standard Error

**Table:**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Value</th>
<th>SE</th>
<th>Chi-Square</th>
<th>Pr &gt; Chi²</th>
<th>HR</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRN</td>
<td>2.669</td>
<td>0.628</td>
<td>18.065</td>
<td>&lt;0.0001</td>
<td>14.422</td>
<td>4.213-49.373</td>
</tr>
</tbody>
</table>

*Figure 1.* Overexpressed miRs in gastric cancer.
Figure 2. Survival curves according to miRs relative expression in gastric cancers

A. miR121

B. miR195b

C. miR196a

D. miR196b

E. Stages
Figure 3. Published systematic review of risks and response to therapy and peritoneal metastases

A. Published search
- 68 articles concluded
- 37 were excluded
- 31 were relevant

B. Published search
- 62 articles concluded
- 28 were excluded
- 34 were relevant

<table>
<thead>
<tr>
<th>Article</th>
<th>Journal</th>
<th>Year</th>
<th>Study Design</th>
<th>Risk Factors</th>
<th>Response to Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>JAMA</td>
<td>2020</td>
<td>Retrospective</td>
<td>Age, Gender</td>
<td>Surgical intervention</td>
</tr>
<tr>
<td>2.</td>
<td>NEJM</td>
<td>2021</td>
<td>Prospective</td>
<td>Tumor stage, Histology</td>
<td>Chemotherapy, Radiation</td>
</tr>
<tr>
<td>3.</td>
<td>Cancer</td>
<td>2022</td>
<td>Case-control</td>
<td>Genetic factors, Environment</td>
<td>Immunotherapy, Targeted therapy</td>
</tr>
<tr>
<td>4.</td>
<td>JCO</td>
<td>2023</td>
<td>Randomized trial</td>
<td>Oncolytic viruses, Gene therapy</td>
<td>Palliative care, Supportive care</td>
</tr>
</tbody>
</table>

10 articles analyzed

C. Published search
- 64 articles concluded
- 32 were excluded
- 32 were relevant

<table>
<thead>
<tr>
<th>Article</th>
<th>Journal</th>
<th>Year</th>
<th>Study Design</th>
<th>Risk Factors</th>
<th>Response to Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>JAMA</td>
<td>2021</td>
<td>Retrospective</td>
<td>Age, Gender</td>
<td>Surgical intervention</td>
</tr>
<tr>
<td>2.</td>
<td>NEJM</td>
<td>2022</td>
<td>Prospective</td>
<td>Tumor stage, Histology</td>
<td>Chemotherapy, Radiation</td>
</tr>
<tr>
<td>3.</td>
<td>Cancer</td>
<td>2023</td>
<td>Case-control</td>
<td>Genetic factors, Environment</td>
<td>Immunotherapy, Targeted therapy</td>
</tr>
<tr>
<td>4.</td>
<td>JCO</td>
<td>2024</td>
<td>Randomized trial</td>
<td>Oncolytic viruses, Gene therapy</td>
<td>Palliative care, Supportive care</td>
</tr>
</tbody>
</table>

20 articles analyzed