Prognosis Value of Low microRNA-34a Expression in Human Gastrointestinal Cancer: A Meta-Analysis

Yan-Ling Chen  
First Affiliated Hospital of Soochow University  
https://orcid.org/0000-0002-3196-1053

Xiao-Lin Liu (✉ lx55@foxmail.com)  
The First Affiliated Hospital of Soochow University

Ling Li  
First Affiliated Hospital of Soochow University

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Abstract

Background: Mounting evidence shows that microRNA-34a (miR-34a) is involved in cancer prognosis. Therefore, we summarize the predictive role of miR-34a for survival in patients with gastrointestinal cancers (GICs).

Methods: All the eligible studies were searched by PubMed, Web of Science and EMBASE and survival results were extracted. Then, the hazard ratio (HR) with corresponding 95% confidence intervals (CIs) was calculated to evaluate the prognostic role of miR-34a in GICs. The association between miR-34a expression and clinicopathological characteristics was estimated by odds ratio (OR) and 95%CIs.

Results: A total of 20 studies were included in this meta-analysis. For overall survival (OS), the lower miR-34a expression significantly predicted poorer outcome in GICs, with the pooled HR of 1.86 (95% CI: 1.52-2.28, P<0.01). For disease-free survival (DFS), progressive-free survival (PFS), and recurrence-free survival (RFS), the lower miR-34a expression revealed worse DFS/PFS/RFS with the pooled HR of 1.86 (95% CI: 1.31–2.63, P < 0.01). Significant relation of differentiation/TMN stage/lymphatic metastasis and the expression level of miR-34a was identified.

Conclusion: This meta-analysis reveals that lower miR-34a expression is significantly connected with worse OS and DFS/PFS/RFS of GICs patients. In addition, miR-34a expression level is relatively lower in patients with lymph node metastasis than patients without, and decreased miR-34a expression level is linked to poor tumor differentiation and late TMN stage. MiR-34a may become a new factor for prognosis prediction and progression of GICs.

1. Background

Gastrointestinal cancers (GICs) account for the major cancer-related deaths around the world, especially in developing countries[1]. Previous studies have shown that stomach, esophageal, liver, and colorectal cancers were commonly identified as the leading causes of cancer deaths[2]. Nowadays, common treatments for GICs contain surgeons, neoadjuvant chemoradiotherapy, and adjuvant chemoradiotherapy and immunotherapy, however, the therapeutic effects are limited in patients with advanced stages. Therefore, there is an urgent need for early detection of GICs and recognition of high-risk patients with poor prognosis.

MicroRNAs (miRNAs) are small-molecule RNAs with a length of 19 to 25 nucleotides that regulate post-transcriptional silence of target genes by combining with the 3'-untranslated region (3'-UTR) of target messenger RNA[3]. MiRNAs participate in various biological processes including cell multiplication, differentiation, apoptosis and cell cycle regulation[4]. Studies have reported that miRNAs are abnormally expressed in tumors and have strong diagnostic and prognostic values[5].

MicroRNA-34a (miR-34a), a member of miR-34 family, has been verified abnormally expressed in various tumors, including esophageal cancer (EC) [6], gastric cancer (GC)[7], colorectal cancer (CRC)[8], hepatocellular carcinoma (HCC)[9], pancreatic cancer (PC)[10], gallbladder cancer (GBC)[11], and other cancers[12]. Based on recent studies, miR-34a has been considered closely related to gastrointestinal cancer multiplication[13], invasion[14] and metastasis[15], which beared on the animate biological roles of miR-34a in cellular signal pathways, such as MAPK/Ras pathway[16], Wnt/β-Catenin pathway[17], PI3K/Akt pathway[18], SIRT1/p53 pathway[19], FoxM1/c-Myc pathway[20] and etc. However, the prognostic accuracy of miR-34a in GICs was inconsistent among these studies. Yuxin Hu et al[21], Hui WT et al[22], and Yang B et al[23] reported that the low expression level of miR-34a predicted a worse survival rate in GICs patients. On the contrary, Osawa S et al[24], Zhang X et al[25] and Mojin Wang et al[26] found that GICs patients were benefited from down-regulated miR-34a. Aimed to systematically assess the prognostic value of miR-34a in GICs and discuss the association between miR-34a expression and clinicopathological characteristics, we performed a meta-analysis on the basis of all published relevant studies.

2. Methods

2.1. Literature Search
We searched PubMed, Web of Science and Embase database to identify relevant studies before January 1, 2020. The following keywords were used: 'microRNA-34a', 'miR-34a', 'cancer', 'neoplasm', 'oesophageal', 'stomach', 'colorectal', 'colon', 'pancreatic', 'hepatocellular', 'liver', 'gallbladder', 'prognosis', 'survival', 'hazard ratio', 'gastrointestinal', which were combined with 'AND' or 'OR'. The results were limited to papers published in English.

2.2. Selection Criteria

Studies were included in based on the following conditions: (1) the diagnoses of GICs were confirmed by histopathology; (2) measured expression of miR-34a in tissue or blood, and divided into high and low level; (3) reported the survival outcome directly or provided survival data from Kaplan-Meier survival curves. Exclusion criteria are the following: (1) reviews, laboratory studies or letters; (2) lacked key information about survival outcomes or unable to calculate, such as HR or 95% CI.

2.3. Data Extraction and Quality Assessment

Two investigators (Yan-Ling Chen and Xiao-Lin Liu) independently extracted the data from all eligible references, including first author, published time, country, tumor type, sample type, test method, TNM stage, follow-up time and cut-off value, HRs of miR-34a for OS and/or DFS, PFS, RFS, and 95% CIs. In addition, the data of clinical characteristics were collected from studies reported that. All eligible studies were retrospective. The Newcastle-Ottawa Scale (NOS) was used to assess the quality. The range of scores is 0 to 9, and score more than 6 was considered as high quality[27]. Any disagreement achieved consensus finally by discussion.

2.4. Statistical Analysis

We used RevMan 5.3 (Cochrane Collaboration, Oxford, UK) and STATA 12.0 (StataCorp LP, College Station, TX, USA) to conduct the statistical analysis. The pooled HR and corresponding 95% CI were used to evaluate the prognostic value of low miR-34a expression in GICs. The heterogeneity among studies was calculated using Cochran's Q test and Higgins's I^2 statistic. If P > 0.05 or I^2 ≤ 50%, we considered no significant heterogeneity existed, the fixed-effect model was used; if P ≤ 0.05 or I^2 > 50%, the random-effect model was used. Some studies didn't provide the HR and 95% CI directly, we obtained the key points and the relevant data from Kaplan-Meier survival curves by utilizing Engauge Digitizer 4.1 software, then calculated HR and corresponding 95% CI following Tierney's method [28]. Publication bias was assessed by funnel plots and Egger's tests. Besides, we performed a sensitivity analysis by removing studies one by one to assess the influence of single study. The association between miR-34a expression and clinicopathological characteristics was evaluated by the pooled OR and 95% CI.

3. Results

3.1. Literature search

A total of 1196 records were obtained in the beginning. 825 studies were excluded because of duplication. 282 records were excluded after screening the titles and abstracts. According to the selection criteria, 19 studies were identified as eligible finally, including 2 EC, 5 GC, 4 HCC, 4 PC, 3 CRC, 1 GBC. As one of the studies contains two different groups, 20 independent experiments were included to quantitatively analyze. The flow diagram of the study selection is shown in Fig. 1.

3.2. Characteristics of the eligible studies

The main features of eligible studies are summarized in Table 1, and the summary of HRs and their 95% CIs are shown in Table 2. The eligible articles were published between 2011 to 2019, including 1691 participants with OS data and 676 participants with DFS/PFS/RFS data from China, America, Japan, Scotland, Slovakia. The types of GICs included EC, GC, CRC, HCC, PC, and GBC. Quantitative real-time PCR (qRT-PCR) was extensively used in whole studies to assess the expression of miR-34a. Tumor tissues were the most commonly used sample, except for Long L-M's study [37] in which plasma samples were used. Among included studies, 8 studies have reported HR and the corresponding 95% CI directly, and the HR and 95% CI of remaining 12 studies were calculated by Kaplan-Meier survival curves.
| Study                  | Year | Country    | Tumor type | Design | Sample | Num. | Stage | Cut-off | Follow-up time | Test method | Outcome  |
|------------------------|------|------------|------------|--------|--------|------|-------|---------|----------------|-------------|----------|
| Yuxin Hu et al [21]    | 2011 | America    | EC         | R      | Tissue | 99   | I-IV  | Median  | > 250          | qRT-PCR     | OS/DFS   |
| Lin X et al [29]       | 2015 | China      | EC         | R      | Tissue | 111  | I-IV  | Median  | NR             | qRT-PCR     | OS       |
| Osawa S et al [24]     | 2011 | Japan      | GC         | R      | Tissue | 37   | II-III| 70%     | 60             | qRT-PCR     | OS       |
| Hui WT et al [22]      | 2015 | China      | GC         | R      | Tissue | 76   | I-III | Mean    | > 60           | qRT-PCR     | OS       |
| Wei B et al [30]       | 2015 | China      | GC         | R      | Tissue | 157  | I-IV  | NR      | > 100          | qRT-PCR     | OS       |
| Zhang H et al [31]     | 2015 | China      | GC         | R      | Tissue | 137  | I-IV  | 2.44    | 68             | qRT-PCR     | OS       |
| Yang B et al [23]      | 2015 | China      | GC         | R      | Tissue | 50   | I-IV  | Median  | 60             | qRT-PCR     | OS       |
| Li XY et al [32]       | 2015 | China      | HCC        | R      | Tissue | 114  | I-IV  | ROC     | 90             | qRT-PCR     | OS/PFS   |
| Yang F et al [33]      | 2013 | China      | HCC        | R      | Tissue | 30   | NR    | Mean    | 60             | qRT-PCR     | OS       |
| Cui X et al [34]       | 2015 | China      | HCC        | R      | Tissue | 120  | NR    | Median  | 60             | qRT-PCR     | OS/RFS   |
| Xu X et al [20]        | 2015 | China      | HCC        | R      | Tissue | 75   | I-IV  | Median  | 60             | qRT-PCR     | OS/RFS   |
| Ohuchida K et al [35]  | 2011 | Japan      | PC         | R      | Tissue | 90   | NR    | NR      | <100           | qRT-PCR     | OS       |
| Jamieson N.B et al [36]| 2012 | Scotland   | PC         | R      | Tissue | 72   | NR    | Median  | 48             | qRT-PCR     | OS       |
| Long L.-M et al [37]   | 2016 | China      | PC         | R      | plasma | 159  | I-IV  | Mean    | 24             | qRT-PCR     | OS       |
| Zhixia Sun et al [38]  | 2018 | China      | PC         | R      | Tissue | 139  | I-IV  | Mean    | 60             | qRT-PCR     | OS       |
| Zhang X et al [25]     | 2017 | China      | CRC        | R      | Tissue | 84   | I-IV  | 2       | 36             | qRT-PCR     | OS       |
| Kristina Hasakova et al [39] | 2019 | Slovakia   | CRC        | R      | Tissue | 64   | I-IV  | Median  | 100            | qRT-PCR     | OS       |
| Gao J et al [40]       | 2014 | China      | CRC        | R      | Tissue | 205  | II-III| 0.307   | <80            | qRT-PCR     | DFS      |
| Gao J et al [40]       | 2014 | China      | CRC        | R      | Tissue | 63   | II-III| 0.307   | <80            | qRT-PCR     | DFS      |

Abbreviations: CRC, colorectal cancer; DFS, disease-free survival; EC, esophageal cancer; GBC, gallbladder cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; NR, no report; OS, overall survival; PC, pancreatic cancer; PFS, progressive-free survival; qRT-PCR, quantitative real-time PCR; R, retrospective; RFS, recurrence-free survival.
3.3. Overall survival is associated with miR-34a expression
We analyzed the association between low expression of miR-34a and OS at first, and remarkable heterogeneity between studies was found ($I^2 = 58.7\%$, $P = 0.001$, Table 3). Therefore, a random effects model was used to compute the pooled HR and corresponding 95% CI. The result showed that lower expression level of miR-34a significantly predicted worse OS, with the pooled HR of 1.86 (95% CI: 1.52–2.28; Fig. 2A).
|                        | No. of studies | No. of patients | Pooled HR (95% CI) | Meta regression p-value | Heterogeneity |
|------------------------|----------------|----------------|--------------------|-------------------------|---------------|
|                        |                |                | Fixed             | Random                  |               |
| Overall                | 18             | 1691           | 1.600 (1.44–1.77)  | 1.86 (1.52–2.28)        | 58.7% 0.001   |
| Ethnicity              |                |                |                   |                         | 0.806         |
| Asian                  | 15             | 1456           | 1.58 (1.42–1.76)  | 1.82 (1.48–2.24)        | 55.2% 0.005   |
| Caucasian              | 3              | 235            | 1.86 (1.25–2.76)  | 2.20 (0.90–5.37)        | 78.6% 0.009   |
| Sample Size            |                |                |                   |                         | 0.979         |
| ≥ 100                  | 7              | 937            | 1.51 (1.34–1.69)  | 1.61 (1.35–1.92)        | 36.1% 0.153   |
| < 100                  | 11             | 754            | 1.98 (1.59–2.48)  | 2.00 (1.37–2.93)        | 63.2% 0.002   |
| NOS Scores             |                |                |                   |                         | 0.978         |
| ≥ 8                    | 11             | 1100           | 1.53 (1.36–1.71)  | 1.75 (1.42–2.16)        | 49.5% 0.031   |
| < 8                    | 7              | 591            | 2.00 (1.56–2.55)  | 1.87 (1.20–2.93)        | 65.8% 0.008   |
| Specimen               |                |                |                   |                         | 0.933         |
| tissue                 | 17             | 1532           | 1.57 (1.41–1.75)  | 1.87 (1.50–2.33)        | 60.2% 0.001   |
| plasma                 | 1              | 159            | 1.88 (1.34–2.63)  | 1.88 (1.34–2.63)        | - -           |
| Cancer Types           |                |                |                   |                         | 0.494         |
| EC                     | 2              | 210            | 1.69 (1.04–2.74)  | 1.87 (0.88–4.00)        | 45.6% 0.175   |
| GC                     | 5              | 457            | 1.33 (1.13–1.57)  | 1.25 (0.59–2.65)        | 68.3% 0.013   |
| HCC                    | 4              | 339            | 1.60 (1.33–1.92)  | 1.84 (1.30–2.59)        | 48.7% 0.119   |
| PC                     | 4              | 460            | 2.27 (1.77–2.89)  | 2.59 (1.69–3.97)        | 57.1% 0.072   |

Abbreviations: 95% CI, 95% confidence interval; CRC, colorectal cancer; EC, esophageal cancer; GBC, gallbladder cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; HR, hazard ratio; NOS, Newcastle-Ottawa Scale; PC, pancreatic cancer.
### Table 1: Summary statistics for the meta-analysis

| Study | No. of studies | No. of patients | Pooled HR (95% CI) | Meta regression p-value | Heterogeneity |
|-------|----------------|-----------------|--------------------|-------------------------|---------------|
| CRC   | 2              | 148             | 1.59 (1.03–2.47)   | 0.0%                    | 0.556         |
| GBC   | 1              | 77              | 2.37 (1.11–5.06)   | -                       | -             |

Abbreviations: 95%CI, 95% confidence interval; CRC, colorectal cancer; EC, esophageal cancer; GBC, gallbladder cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; HR, hazard ratio; NOS, Newcastle-Ottawa Scale; PC, pancreatic cancer.

In order to explicate the heterogeneity in OS, subgroup analysis was conducted by ethnicity (Asian and Caucasian), sample capacity (≥ 100 and < 100), NOS scores (≥ 8 and < 8), specimen (plasma and tissue) and tumor types (EC, GC, CRC, HCC and PC). As a result, the homogeneity was achieved in the CRC group (I² = 0.00%, P = 0.556; Table 3) and the correlation was obvious (HR = 1.59, 95% CI: 1.03–2.47, Fig. 2C). What's more, there were significant correlation between the expression level of miR-34a and OS in Asians (HR = 1.82, 95% CI: 1.48–2.24, Fig. 2B), sample capacity greater than or equal to 100 (HR = 1.61, 95% CI: 1.35–1.92, Fig. 2D) or less than 100 (HR = 2.00, 95% CI: 1.37–2.93, Fig. 2D), NOS scores equal to or greater than 8 (HR = 1.75, 95% CI: 1.42–2.16, Fig. 2F) or less than 8 (HR = 1.87, 95% CI: 1.20–2.93, Fig. 2F), specimen removed the plasma (HR = 1.87, 95% CI: 1.50–2.33, Fig. 2E), HCC (HR = 1.84, 95% CI: 1.30–2.59, Fig. 2C), and PC (HR = 2.59, 95% CI: 1.69–3.97, Fig. 2C) by random effect model. As shown in Table 3, the significance might be vanished in Caucasian and EC groups when fixed effects model turned into random effect model. Moreover, the heterogeneities were still evident among subgroups, except for the CRC group. To analyse heterogeneity ulteriorly, meta regression was performed, but it made no sense to explain the variation of HRs (p = 0.806 for ethnicity, p = 0.979 for sample capacity, p = 0.978 for NOS scores, p = 0.933 for specimen, p = 0.494 for cancer types, Table 3). Moreover, the sensitivity analysis was performed to assess the contribution of each study and no study seemed to make a difference to the pooled results (Fig. 2G). In addition, publication bias was evaluated by funnel plots and Egger’s tests. As shown in Fig. 2H, the funnel plots showed no obvious asymmetry, and the Egger’s tests revealed no significant publication bias existed (P = 0.058).

### 3.4. Tumor progression is associated with miR-34a expression

To evaluate the association between miR-34a expression and DFS/PFS/RFS, 6 studies were included in this analysis, and the data revealed that low miR-34a expression predicted a worse outcome with a combined HR of 1.86 (95% CI: 1.31–2.63) via a random effect model (P = 0.001, I² = 76.6%; Fig. 3A). To explain the heterogeneity, we performed subgroup analysis by DFS, PFS and RFS, which showed the significant correlation with the expression of miR-34a (HR = 2.50, 95% CI: 1.27–4.92 for DFS; HR = 1.54, 95% CI: 1.26–1.90 for RFS; Fig. 3B). What’s more, the homogeneity was achieved in the RFS group. Then, the sensitivity analysis was performed by removing studies one by one to assess the influence of single study. As shown in Fig. 3C, the stability of the entire study was not influenced by individual study. Finally, funnel plots and Egger’s tests were implemented to evaluate the publication bias. The funnel plot was roughly symmetric (Fig. 3D) and the P values of Egger’s tests was 0.909. Therefore, no evidence for significant publication bias existed.

### 3.5. Correlation between miR-34a levels and clinicopathological features in GICs

For obtaining relevant statistics to evaluate the relation between miR-34a expression levels and different clinicopathological characteristics, seven studies containing 647 patients of GICs were screened out. As shown in Table 4, we observed significant association between expression level of miR-34a and lymphatic metastasis (OR = 3.231, 95% CI: 2.237–4.666; Fig. 4A), differentiation degree (OR = 2.228, 95% CI: 1.538–3.228; Fig. 4B) via fixed effects model, and TMN stage (OR = 2.896, 95% CI: 1.302–6.442; Fig. 4C) via a random effect model. There were no significant correlation identified between miR-34a levels and gender (OR = 0.776, 95% CI: 0.566–1.065) or tumor sizes (OR = 0.736, CI: 0.460–1.177). The heterogeneity was disappeared in the
group of gender ($I^2 = 0.00\%, P = 0.888$), lymphatic metastasis ($I^2 = 0.00\%, P = 0.754$) and medium in the group of tumor size ($I^2 = 20.5\%, P = 0.284$), differentiation degree ($I^2 = 35.7\%, P = 0.169$) but obvious in the TNM stage group ($I^2 = 74.4\%, P = 0.004$). Sensitivity analysis was applied to the clinic characteristic analysis including lymphatic metastasis (Fig. 4D), differentiation degree (Fig. 4E) and TNM stage (Fig. 4F), suggesting no study had significant impacts on the results.

### Table 4

**Overall analysis of miR-34a expression association with clinicopathologic characteristics**

| Clinicopathological characteristics | Num. of studies | Num. of patients | Pooled OR (95%CI) | Heterogeneity |
|-------------------------------------|-----------------|------------------|-------------------|---------------|
|                                     |                 |                  | Fixed Random      | $I^2$ p-value  |
| Gender (male vs. female)            | 7               | 647              | 0.776 (0.566–1.065) | 0.777 (0.565–1.067) | 0.0% 0.888 |
| Tumor Size ($\leq 5$ vs $>5$ cm)   | 3               | 326              | 0.736 (0.460–1.177) | 0.284 (0.433–1.288) | 20.5% 0.284 |
| Lymphatic Metastasis (YES vs. NO)   | 6               | 571              | 3.231 (2.237–4.666) | 3.200 (2.210–4.635) | 0.0% 0.754 |
| TNM stage (III + IV vs. I + II)    | 5               | 458              | 2.468 (1.698–3.588) | 2.896 (1.302–6.442) | 74.4% 0.004 |
| Differentiation (poor vs. others)   | 6               | 597              | 2.228 (1.538–3.228) | 2.373 (1.430–3.938) | 35.7% 0.169 |

Abbreviations: 95%CI, 95% confidence interval; Fixed, fixed effects model; OR, Odds ratio; Random, random pooling model.

### 4. Discussion

In the last few decades, miRNAs have attracted increasing interest among investigators as potential biomarkers for cancer diagnosis and prognosis. Many clinical trials have demonstrated that miRNAs play a pivotal role in tumor development via regulating the expression of target genes and tumor suppressors or directly wielding their functions as oncogenes or tumor suppressors[41] [42]. It has been reported that miR-34a influenced tumor biological activities by targeting several genes or signal pathways, such as CCND1 in EC[43], PDGFR in GC[18], HMGB1 in CRC[44], XIST in PC[45] and etc. Recently, a systemic review has summarized numerous studies in which reported miR-34a has diagnostic and prognostic value in GICs[46]. However, among these studies, two opposing views were presented on whether patients could benefit from the high expression of miR-34a. Hao Wu et al[7], Milad Asadi et al[47] and Yan Zhou et al[48] showed the down-regulation of miR-34a was linked to a poor prognosis in GICs patients, while Hiyoshi Y et al[8], and Mojin Wang[26] reported patients were benefited from down-regulated miR-34a. The prognostic value of miR-34a in GICs has been illustrated in lots of studies, but the particular prognosis role of miR-34a in GICs remains unclear. As far as we know, this is the most overall meta-analysis exploring the clinical value of miR-34a in patients with GICs.

This meta-analysis discussed 20 papers and contained 2367 patients in total. Among these studies, 18 studies including 1691 patients provided the relevant statistics of OS. By the random effect model, the results showed that the decreased miR-34a expression was association with poorer outcome of GICs patients. To explain the potential sources of heterogeneity, subgroup analyses were performed. As a result, the homogeneity was reached in the group of CRC, and the OS of CRC group was found to be greatly associated with the miR-34a expression levels. Though the expression level of miR-34a in CRC patients remains controversial, there are several potential mechanisms suggested how low expression of miR-34a could induce unfavorable
outcome of CRC. It has been reported that miR-34a served a key role in suppressing CRC metastasis by targeting and regulating Notch signaling[25]. Also, miR-34a might be an important tumor suppressor of CRC progression by targeting FMNL2 and E2F5[49]. Besides, miR-34a inhibits recurrence of CRC through inhibiting cell growth, migration and invasion, inducing cell apoptosis and cell cycle arrest in a p53-dependent manner[40].

As shown in Table 3, the associations between miR-34a expression levels and OS were also significant in other subgroups. In addition, there is a closer relationship between low miR-34a level and poor OS in patients with PC (HR = 2.59, 95% CI:1.69–3.97). Empirically, HR > 2 is considered as strongly predictive [50]. As for the possible mechanism, Long Li-Min et al reported that miR-34a significantly inhibited the tumor growth of PC tumors by suppressing Notch1, Notch2 and Notch4 expression [37]. Since the heterogeneities within the subgroups were still significant, meta regression was performed to illustrate the influence of different factors including ethnicity, sample capacity, specimen, NOS scores and tumor classification, but there was no factor significantly affect the variation of HR. The analysis of tumor progression and miR-34a expression revealed that low miR-34a expression predicted a worse outcome, especially in DFS (HR = 2.50, 95% CI: 1.27–4.92). According to our research, we could infer that the decreased expression level of miR-34a is closely related to worse prognosis in patients with GICs. But for the EC and GC, the results were still not stable and required more comprehensive studies to further research the miR-34a prognostic value in GICs.

To evaluate the association between miR-34a and the clinical characteristics, seven articles including 647 patients were enrolled. Significant relations were observed between miR-34a expression levels and differentiation/TMN stage/lymphatic metastasis by fixed or random effects model was identified. Applying sensitivity analyses, there was no study had significant impacts on the results. Based on the findings, it suggested that patients with decreased miR-34a expression are more likely to develop lymphatic metastasis, and decreased miR-34a expression level was linked to poor tumor differentiation and late TMN stage.

Though this meta-analysis revealed that miR-34a was a promising biomarker of GICs, several potential limitations of this study should be considered. Firstly, the number of studies included was limited, leading to the relative lack of studies in subgroup analyses, for example, there is only one article reported PFS. Secondly, patients were all Asian and Caucasian, lacking data from other regions, which might result in ethnic bias. Thirdly, the cut-off value among studies were different, we didn't have absolute criteria to assess whether the expression of miR-34a is low or not, impacting the statistical power of analysis. Finally, some HRs and 95% Cis were calculated according to the data extracted from survival curves, so it’s difficult to exclude the influence of confounding bias.

### 5. Conclusion

In conclusion, our study demonstrates that lower miR-34a expression is significantly associated with poorer OS and DFS/PFS/RFS and may be a novel prognostic biomarker in GICs. Moreover, miR-34a expression level is relatively lower in patients with lymph node metastasis, and decreased expression level of miR-34a is related to poor tumor differentiation and late TMN stage. Further multicenter prospective clinical studies are needed to validate the association between miR-34a and prognosis of GICs.

### Abbreviations

95%CI, 95% confidence interval; CRC, colorectal cancer; DFS, disease-free survival; EC, esophageal cancer; GBC, gallbladder cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; HR, hazard ratio; NOS, Newcastle-Ottawa Scale; NR, no report; OR, Odds ratio; OS, overall survival; PC, pancreatic cancer; PFS, progressive-free survival; qRT-PCR, quantitative real-time PCR; R, retrospective; RFS, recurrence-free survival.

### Declarations

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**


Availability of data and materials

The authors declare that all data used or analysed during the current study are available on reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

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Authors’ contributions

Study design: YC. Data collection: YC and XL. Data analysis: YC, XL and LL. Manuscript composition: YC. Manuscript revision: XL. Tables Drafting: YC and LL. Figures Drafting: YC and XL. All authors read and approved the final manuscript.

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Authors’ information

1Department of Gastroenterology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, China.

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Figures
Figure 1

Flow diagram of the study selection process.
Figure 2

The association between miR-34a expression levels and (A) overall survival; subgroup analyses of (B) ethnicity (Asian and Caucasian), (C) cancer types (EC, GC, HCC, PC, CRC), (D) sample size (≥100 and < 100), (E) specimen (plasma and tissues), (F) NOS scores (≥8 and < 8); (G) sensitivity analysis for HR of OS; (H) publication bias evaluation for OS.
Figure 3

The association between miR-34a expression levels and (A) DFS/PFS/RFS; (B) subgroup analyses of DFS/PFS/RFS; (C) sensitivity analysis for HR of DFS/PFS/RFS; (D) publication bias evaluation for DFS/PFS/RFS. Gao J*, study containing two different groups.
Figure 4

The association between miR-34a expression levels and (A) lymphatic metastasis, (B) tumor differentiation degree, (C) TNM stages; sensitivity analyses for ORs of clinicopathological characteristics, such as (D) lymphatic metastasis, (E) tumor differentiation degree, (F) TNM stages.

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