The Diagnostic and Prognostic Values of MicroRNA-196a in Cancer

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Abstract

Background

miRNA-196a (miR-196a) was previously reported to be upregulated in cancers, and has the diagnostic and prognostic values in cancers. Whereas, the conclusion was still unclear according to the published data. To assess such a role of miR-196a in cancers, this study was conducted based on published data.

Methods

To identify the relevant published data, we searched articles in databases and then the relevant data was extracted to assess the correlation between miR-196a expression and diagnosis and survival for cancer patients, all data was analysis with the statistical software STATA.

Results

The pooled results showed that miR-196a was a valuable diagnostic biomarker in cancer (AUC=0.87, 95%CI: 0.84-0.90; sensitivity=0.73, 95%CI:0.64-0.81; specificity=0.90, 95%CI:0.81-0.95), which was consistent with the data from databases (breast cancer: miR-196a-3p: AUC=0.77, 95%CI: 0.74-0.79; miR-196a-5p: AUC=0.71, 95%CI: 0.66-0.75; pancreatic cancer: miR-196a-3p: AUC=0.80, 95%CI: 0.73-0.87; miR-196a-5p: AUC=0.61, 95%CI:0.51-0.71). In addition, miR-196a in tissues was an unfavorable survival prognosis biomarker (HR=2.54, 95%CI: 1.79-3.61, P Heterogeneity =0.000, I²=75.8%) and serum or plasma (HR=4.06, 95%CI: 2.67-6.18, P Heterogeneity =0.668, I²=0%), which was consistent with the data from databases (adrenocortical carcinoma: HR=5.70; esophageal carcinoma: HR=1.93; brain lower grade glioma: HR=2.91; GSE40267: HR=2.47, 95%CI: 1.2-5.07; TCGA: HR=1.82, 95%CI: 1.21-2.74; GSE19783: HR=4.24, 95%CI:1.18-18.06).

Conclusion

Our results demonstrated that miR-196a acts as a tumor suppressor with prognostic and diagnostic values for cancer.

Background

MicroRNAs(miRNAs), a kind of non-coding RNAs with 21-25 nucleotides, inhibit gene expression by targeting the 3′-untranslated region (3′-UTR) of target messenger RNA (mRNA) (1). In the past few decades, aberrant expression of miRNAs has been shown to play an important role in a variety of cancers (2). Meanwhile, studies of these molecules have led to the observation of clinically useful genetic biomarkers and novel therapeutic agents.

miR-196a, a member of the miR-196 family that has two members (miR-196a and miR-196b), comes from the transcription of two genomic loci, HOXC gene MIR196A2 and HOXB gene MIR196A1 (3). miR-196a-5p and miR-196a-3p are two molecules produced by pre-MIR196A2. Moreover,pre-MIR196A1 also can encode miR-196a-5p.

Previous studies have shown that miR-196a exert multiple functions in carcinogenesis and cancer progression. For example, miR-196a inhibited proliferation and invasion of hepatocellular carcinoma cells by targetingFOXO1(4). Meanwhile, miR-196a-2 polymorphism was associated with hepatocellular carcinoma recurrence after liver transplantation (5),and we also previously reported it was associated with occurrence of cancers (6).In addition, overexpression of miR-196a was associated with aggressive pathological features and shorter survival of patients with gliomas (7).

miR-196a was focused on cancers by numerous studies for its role in carcinogenesis and potential role in cancer diagnosis or survival prediction. Overexpression of miR-196a was reported in types of cancers, such as liver cancer (4), breast cancer (8), esophageal squamous cell carcinoma (9) and corrected with progression of hepatocellular carcinoma (10), thyroid carcinoma (11), esophageal carcinoma (12), etc. Moreover, the level of miR-196a-5p in serum was suggested to be served as a diagnostic biomarker for cancers, including non-small cell lung cancer (13), prostate cancer (14) and biomarker for prediction of cancers progression (15). Whereas, the conclusions of role of miR-196a in clinical application were not always consistent. Therefore, we conducted this meta-analysis according to published data and try to determine whether miR-196a is valuable biomarker for cancer diagnosis and prognosis.

Materials And Methods

Search strategy

In order to obtain all relevant articles, we used the keywords ("microRNA-196a" OR "miR-196a" OR "microRNA-196a") and ("carcinoma" OR "cancer" OR "tumor") to search in PubMed, Web of science, CNKI and other similar databases. In addition, we manually searched for related references in some additional papers and reviews.

Inclusion and exclusion criteria

In order to indentify articles were suitable for this study, all enrolled articles should meet the including criteria: 1) patients reported in the article were all diagnosed with gold standard; 2) the detection of miR-196a was performed in serum, plasma, tissues or other human body fluids; 3) reported sufficient value related to the expression of miR-196a and prognostic value for overall survival (OS), progression-free survival (PFS), recurrence free survival (RFS), or disease-free survival (DFS); 4) provided sufficient data to calculate or extract the true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN).
Additionally, articles that meet one of the following terms will be removed: 1) non-English and non-Chinese publications; 2) insufficient diagnostic and prognostic data available for meta-analysis; 3) containing duplicate data.

Data extraction and checking

Two authors (M.X and B.P) independently completed database search, article quality evaluation, data extraction, and uncertain articles were evaluated by the third author (B.H). The extracted data includes author name, publication date, country and region of case, miRNA type, sample type, cancer type, sample size, sensitivity and specificity, cut-off value, HR and 95% CI, and follow-up time.

Statistical analysis

To evaluate the diagnostic value of miR-196a for cancer, the sensitivity and specificity of all included articles and the corresponding sample content were extracted, and then SROC curve were drawn based on original data of enrolled studies, and the area under curve (AUC) was used to evaluate the diagnostic value. Chi-square test and P test were applied to assess the heterogeneity across the studies.

In the prognostic meta-analysis, the pooled HR with 95% CI was calculated to evaluate the relationship between the level of miR-196a and the prognosis of cancer patients. Whereas, there were two studies that did not directly present available data (16, 17), herein, we obtained the value using the Kaplan-Meier survival curves according to the method reported by Tierney (18). Similarly, Chi-square test and P test were applied to evaluate the heterogeneity. If \( P<0.05 \) and \( I^2 \geq 50\% \), we use a fixed effects model, otherwise the random effects model was applied (19). To describe the publication bias, funnel plots, Begg’s and Egger’s tests was applied.

All data was carried out with the statistical software STATA (version 13.1) and \( P<0.05 \) is statistically significant.

Database analysis

To explore the dysregulated miR-196a in cancer patients, data of the serum samples were obtained from Gene Expression Omnibus (GEO) database. We accomplished a comprehensive analysis of miR-196a expression profiles in GSE113486 and GSE106817. Besides, we also explored the role of miR-196a in cancer prognosis prediction in the ENCORI (http://starbase.sysu.edu.cn/panCancer.php) and Kaplan-Meier Plotter databases (http://kmplot.com/analysis/index.php?p=service).

Results

Eligible studies

A total of 425 articles were searched from the three databases (PubMed, Web of science and CNKI) by using the keywords and 98 duplicated articles were removed by screening the title, abstract and author and then we excluded articles, including reviews, letters or not related to the topic according to the established criteria. Finally, after reading full-text of 44 articles meeting the including criteria, 21 of them with insufficient data and unrelated to the diagnosis and prognosis were removed. Finally, a total of 23 articles were enrolled in meta-analysis (Supplemental Figure 1), of which, seven studies were related to diagnosis (13, 20-25) (Table 1), and 17 studies were related to prognosis (7, 10-12, 16, 17, 23, 26-35) (Table 2), respectively.

To assess the quality of non-randomized researches, the Newcastle Ottawa Scale (NOS) was applied (36). We scored each article strictly according to the scoring standard, and those with a score greater than 6 were considered high-quality articles (Table 3).

Diagnostic meta-analysis

Study characteristics

A total of seven articles reported the role of miR-196a as a biomarker in cancer diagnosis (Table 1), and all of the samples of these studies were collected as serum and plasma. For ethnicity, there are three and four studies based on Europe population and Asia population, respectively. The quantitative real-time polymerase chain reaction (qRT-PCR) was used by all studies to detect miRNA expression.

Expression of miR-196a and diagnosis

In order to assess the diagnostic value of miR-196a for cancer, the pooled sensitivity (SEN) and specificity (SPE) were calculated, and forest plots were also drawn (Figure 1). The pooled AUC of seven studies involving serum or plasma was indicated miR-196a is a valuable diagnostic biomarker for cancers (AUC=0.87, 95% CI: 0.84-0.90; SEN=0.73, 95% CI: 0.64-0.81, \( P_{\text{Heterogeneity}}<0.001, I^2=73.94\% \); SPE=0.90, 95% CI: 0.81-0.95, \( P_{\text{Heterogeneity}}<0.001, I^2=79.17\% \)) (Figure 1, Table 4).

In order to assess the diagnostic value of miR-196a for cancer of subgroups, we separated the studies according to sample size (more than 100 or not) and ethnicities (Asian or Caucasian), and subgroup analysis revealed that the results of subgroup stratified by sample size or ethnicity all have no differences (Asian: \( AUC=0.86, 95\% \text{ CI}: 0.83-0.89 \); \( SEN=0.73, 95\% \text{ CI}: 0.66-0.79, P_{\text{Heterogeneity}}=0.01, I^2=70.87\%, \text{SPE}=0.92, 95\% \text{ CI}: 0.81-0.97, P_{\text{Heterogeneity}}<0.001, I^2=86.60\% \); Caucasian: \( AUC=0.90, 95\% \text{ CI}: 0.87-0.92 \); \( SEN=0.85, 95\% \text{ CI}: 0.44-0.98, P_{\text{Heterogeneity}}<0.001, I^2=83.22\%, \text{SPE}=0.84, 95\% \text{ CI}: 0.64-0.94, P_{\text{Heterogeneity}}<0.03, I^2=67.12\% \); Sample size<100: \( AUC=0.91, 95\% \text{ CI}: 0.88-0.93 \); \( SEN=0.80, 95\% \text{ CI}: 0.50-0.94, P_{\text{Heterogeneity}}<0.001, I^2=75.86\%, \text{SPE}=0.87, 95\% \text{ CI}: 0.74-0.94, P_{\text{Heterogeneity}}<0.03, I^2=63.93\% \); Sample size>100: \( AUC=0.84, 95\% \text{ CI}: 0.80-0.87 \); \( SEN=0.73, 95\% \text{ CI}: 0.64-0.80, P_{\text{Heterogeneity}}<0.001, I^2=78.13\%, \text{SPE}=0.91, 95\% \text{ CI}: 0.77-0.97, P_{\text{Heterogeneity}}<0.001, I^2=89.45\% \)) which were consistent to overall results (Figure 2, Table 4).
Prognostic meta-analysis

Study characteristics and quality assessment

In order to explore the relationship between miR-196a expression and survival of cancer patients, a total of 17 articles were enrolled in this meta-analysis. Of enrolled studies, totally 12 studies were conducted with tumor tissue, only four articles involving in serum or plasma, and one article based on bone marrow samples (35). In addition, all of the 17 articles were based on Asians except for one based on Caucasian (31). All detection methods of included studies were based on qRT-PCR, shown in Table 2.

Expression of miR-196a and prognosis

The pooled results of all 12 studies conducted with tumor tissue showed that the increased expression of miR-196a was an unfavorable survival prognosis biomarker (high expression vs. low expression: HR=2.54, 95%CI: 1.79-3.61, \( P_{\text{Heterogeneity}}<0.001, \hat{I}^2=75.8\% \)). The similar result was also observed in the studies conducted with serum or plasma (high expression vs. low expression: HR=4.06, 95%CI: 2.67-6.18, \( P_{\text{Heterogeneity}}=0.668, \hat{I}^2=0\% \)) (Figure 3, Table 5).

To assess the pooled result further, we performed subgroup analyses according to survival data (OS or RFS) and the data resources (Published data or TCGA data), and the result showed that the pooled results of all subgroups (OS: HR=2.57, 95%CI: 1.60-4.12, \( P_{\text{Heterogeneity}}<0.001, \hat{I}^2=81.2\% \); RFS: HR=2.94, 95%CI: 1.77-4.87, \( P_{\text{Heterogeneity}}=0.497, \hat{I}^2=0\% \); Published data: HR=2.67, 95%CI: 2.02-3.53, \( P_{\text{Heterogeneity}}=0.071, \hat{I}^2=40.5\% \); Data from TCGA: HR=5.03, 95%CI: 3.51-7.20, \( P_{\text{Heterogeneity}}=0.604, \hat{I}^2=0\% \)) were similar to the overall pooled result (Figure 3, Table 5).

Heterogeneity and sensitivity analyses

For the meta-analysis of diagnosis, among the studies conducted with serum or plasma, there was a significant heterogeneity across the enrolled studies (\( P_{\text{Heterogeneity}}=0.00, \hat{I}^2=73.9\% \)) and subgroup of sample size (n<100: \( P_{\text{Heterogeneity}}=0.00, \hat{I}^2=75.86\% \); n>100: \( P_{\text{Heterogeneity}}=0.00, \hat{I}^2=78.13\% \)) and ethnicity (Asian: \( P_{\text{Heterogeneity}}=0.01, \hat{I}^2=70.87\% \); Caucasian: \( P_{\text{Heterogeneity}}=0.00, \hat{I}^2=83.22\% \)). Therefore, a meta-regression was conducted based on sample size, ethnicity, year of publication. The results suggested that heterogeneity was mainly derived from sample type (\( P<0.001 \)) (Figure 4).

For prognosis analysis, no significant heterogeneity among the studies involving serum or plasma. Whereas, there was a significant heterogeneity across the studies with tumor tissue (\( P_{\text{Heterogeneity}}=0.00, \hat{I}^2=75.80\% \)) and subgroup of studies with OS (\( P_{\text{Heterogeneity}}=0.00, \hat{I}^2=81.20\% \)), which may be due to the difference of data resources in that the heterogeneity was decreased (HR=2.67, 95%CI: 2.02-3.53, \( P_{\text{Heterogeneity}}=0.071, \hat{I}^2=40.5\% \)) when three studies come from the database were removed (11, 31, 33). Additionally, to assess the stability of the pooled result, sensitivity-analysis was conducted by omitting each study and the result revealed that no single study deleting changed the significance of the pooled result (Figure 4).

Publication bias

To test the publication bias of the studies based on diagnosis, Deeks’ funnel plot asymmetry test was used. The funnel plots of the studies related diagnosis were symmetrical, indicating no publication bias of these studies was presented (\( t=0.24, P=0.816 \)). Additionally, the Egger’s and Begg’s tests were performed for the studies related to prognosis, the similar results was observed (\( t=1.16, P=0.260 \)), shown in Figure 5.

Diagnostic and prognostic analysis based on the database

In order to verify the diagnostic role of miR-196a in serum of patients with cancer, we searched two datasets in the GEO database (GSE113486 and GSE106817) containing expression of miR-196a in breast cancer, pancreatic cancer patients and corresponding normal controls, and results showed that the AUC of miR-196a-3p (AUC=0.77, 95%CI: 0.74-0.79) and -5p (AUC=0.71, 95%CI: 0.66-0.75) showed favorable diagnostic value for breast cancer. Additionally, the similar results were also observed in pancreatic cancer (miR-196a-3p: AUC=0.80, 95%CI: 0.73-0.87; miR-196a-5p: AUC=0.61, 95%CI: 0.51-0.71), which were consistent to the pooled results of this study.

In order to verify the prognostic role of miR-196a for cancer, we searched in ENCORI, which contains survival and differential expression analysis of miRNAs, lncRNAs, pseudogenes and mRNAs and in Kaplan-Meier Plotter database, which includes the effect of mRNA, miRNA, protein on survival in 21 cancer types. As shown in figure6, the prognostic HR values of miR-196a-5p in patients with adenocortical carcinoma, esophageal carcinoma, and brain lower grade glioma were 5.70 (\( P=6.9e-5 \)), 1.93 (\( P=0.012 \)) and 2.91 (\( P=4.5e-9 \)), respectively. In addition, the results of Kaplan-Meier Plotter database showed that high expression of miR-196a predicted unfavorable OS of breast cancer patients (GSE40267: HR=2.47, 95%CI: 1.2-5.07, \( P=0.011 \)), (TCGA: HR=1.82, 95%CI: 1.21-2.74, \( P=0.0034 \)) and (GSE19783: HR=4.24, 95%CI: 1.18-0.06, \( P=0.033 \)), respectively. Therefore, all the results from databases supported the pooled results based on published data.

Discussion

In this meta-analysis, a total of 23 articles were included to explore the role of miR-196a in cancer diagnosis and prognosis. The pooled results showed that the increase of miR-196a could be used as a diagnosis and prognosis biomarker for cancers.

For diagnosis meta-analysis, in this study, a total of seven diagnosis-related articles were included, the overall and subgroups pooled result showed that miR-196a could be used as a diagnostic marker for cancer. In fact, the oncogene role of miR-196a in cancer has been reported in previous studies, and it combined with other miRNAs can improve the efficiency of cancer diagnosis. For example, miR-196a and miR-148a could act as candidate biomarkers for early gastric cancer diagnosis (37). Similarly, miR-10a-5p and miR-196a-5p can serve as non-invasive biomarkers for non-small cell lung cancer (13). Additionally, miR-
miR-196a combined with miR-1202 could serve as biomarkers for evaluating the effectiveness of endometrial cancer treatment (38). In addition, results from databases were consistent to the pooled results, indicating miR-196a has promising clinical application in cancer diagnosis.

The mechanisms of overexpression of miR-196a in cancer have been illustrated by previous studies. In breast cancer, miR-196a could be transcriptly regulated by the binding of ERα to its promoter region and DNA methylation within the HOXC locus negatively related with the expression of miR-196a, which supporting miR-196a could be regulated in a repressive epigenetic modification (39). Moreover, a time delay was found in the precursor MIR196A2 gene into mature MIR196A processing, suggesting the overexpression of miR-196a was regulated post-transcriptionally (31).

In this meta-analysis, a significant heterogeneity among enrolled diagnosis related studies was presented in the overall and subgroup results, which was aroused from the sample type, suggesting that the level of miRNAs may be affected according the sample type. Actually, the difference of miRNAs level in serum and plasma has been reported previously, which may be attributed to the some detectable miRNAs were from platelets (40).

Regarding the role of miR-196a in the prognosis of cancer, the overall and subgroups pooled results showed that miR-196a could be used as a prognostic marker for cancer. Actually, miR-196, regarding as an oncogene, has been investigated with several biological function related tumor progression. High expression of miR-196a was associated with shorter OS of gastric cancer patients, which may be attributed to the downregulating of its targeted gene p27kip1 (16). Moreover, miR-196a was associated with tumor progression by down-regulation of SPRR2C, S100A9, and KRT5 (41). Additionally, in colorectal cancer, miR-196 could promote metastasis and prognosis in human by inhibiting HoxB8, and it can also decrease the sensitivity of cancer cells to chemotherapy with FOLFOX4 (42).

For the meta-analysis of prognosis, the pooled results of this article indicated that high expression of miR-196a predicted the poor prognosis of cancer. Whereas, a significant heterogeneity was presented among studies, which could be eliminated by removing three studies coming from the database that two were thyroid cancer data from TCGA database and one breast cancer data from GEO database. Specifically, in the breast cancer study, the HR of miR-196 to survival of patients was opposite for the ER+ pre-menopausal (HR = 0.342, 95%CI: 0.1534-0.7623) and ER+ post-menopausal (HR = 1.599, 95%CI: 1.0806-2.3652), which may be a source of heterogeneity. More important, the original data of these three studies was based on high throughput platform, which was different with other studies based on qRT-PCR, may contribute to the heterogeneity. In short, the pooled results of published data or results of databases all supported that high expression of miR-196a predicted the poor prognosis of cancer.

Admittedly, there have been previous meta-analysis articles regarding the role of miR-196a in cancer diagnosis and prognosis. For example, the prognostic value of miR-196a was assess in Asian cancer patients (43). Compared with this article, the novelty of our research was that we included more recent studies, regarding European population and Asian population, indicated the conclusion of this study was robust, that we also retrieve the data of related databases to confirm our pooled results, which was consistent each other, indicating our result was based on a larger size of sample, and that we further proved the feasibility of miR-196a as a cancer diagnostic biomarker in serum or plasma based on published data and data of databases, indicating our study was relative more comprehensive. In addition, compared with the study regarding the polymorphism locates at the coding region of miR-196a, our study discussed the expression of miR-196a (44), and our previous study has discussed association between the miR-196a polymorphism and cancer risk (46).

Although, the result of meta-analysis was objective and strong, several limitations of this article should be addressed. Firstly, the HR and corresponding 95% CIs of two articles were extracted from survival curves, which may be inaccurate and have an impact on the final results. Secondly, all the studies published in English or Chinese were included, which may lead to the language bias. Thirdly, the results of this meta-analysis lack experiments to confirm, which should be validated by future study.

Conclusion

In short, our study confirm that miR-196a can be used as a diagnostic and prognostic marker for cancers.

Abbreviations

miRNAs: MicroRNAs; NSCLC: non-small cell lung cancer; ORC: oral cancer; IPMNs: Intraductal Papillary Mucinous Neoplasm of the Pancreas; GC: gastric cancer; PanNET: Pancreatic Neuroendocrine Tumor; PanIN2/3: pancreatic intrapithelial neoplasia grade2-3; SpFPC: sporadic pancreatic ductal adenocarcinoma; FPC: familial pancreatic cancer; ESCC: Esophageal Squamous Cell Carcinoma; AUC: the area under curve; SEN: sensitivity; SPE: specificity; TP: true positives; FP: false positives; FN: false negatives; TN: true negatives; OS: overall survival; DFS: disease free survival; RFS: relapse-free survival; OSCC: Oral squamous cell carcinoma; HCC: Hepatocellular Carcinoma; GIST: Gastrointestinal Stromal Tumors; AML: acute myeloid leukaemia; EOC: epithelial ovarian cancer; PanNET: Pancreatic Neuroendocrine Tumor; CRC: colorectal cancer; GC: gastric cancer; PDAC: Pancreatic Ductal Adenocarcinoma.

Declarations

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None.

Authors' contributions

None.

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**Availability of data and materials**

They could be achieved upon reasonable request to the authors.

**Conflicts of interest**

The authors declare that there are no conflict of interests.

**Consent for publication**

All author approved the publication of this paper.

**Declarations**

Ethics approval and consent to participate.

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Tables

Table 1 Characteristics and methodology assessment of 7 studies included in the diagnosis meta-analysis
| First author | Year | City | Ethnicity | Sample type | Cancer type | Case/Control | AUC | SEN (%) | SPE (%) | TP | FP | FN | TN | Cut-off value |
|--------------|------|------|-----------|-------------|-------------|--------------|-----|---------|---------|----|----|----|----|----------------|
| Min (13)     | 2018 | China| Asian     | Serum       | NSCLC       | 80/75        | 0.785| 67.86%  | 77.57%  | 54 | 17 | 26 | 58 | Median  |
| Lu (21)      | 2015 | China| Asian     | Plasma      | ORC         | 90/53        | 0.864| 66.70%  | 96.20%  | 60 | 2  | 30 | 51 | 29.9   |
| Wang (24)    | 2009 | America| Caucasian | Plasma | Pancreatic Cancer | 28/19 | 0.69 | 43%    | 84%    | 12 | 3  | 16 | 16 | NM    |
| Slater1 (22) | 2014 | Germany| Caucasian | Serum | PanIN2/3 | 5/10 | 0.64 | 100%  | 60%    | 5  | 4  | 0  | 6  | 7.51  |
| Slater2 (22) | 2014 | Germany| Caucasian | Serum | Sp-FPC | 9/10 | 0.97 | 90%    | 89%    | 8  | 1  | 1  | 9  | 7.96  |
| Slater3 (22) | 2014 | Germany| Caucasian | Serum | FPC | 10/10 | 0.99 | 90%    | 100%   | 9  | 0  | 1  | 10 | 7.96  |
| Tsai (23)    | 2016 | China| Asian     | Plasma     | GC          | 98/126       | 0.864| 69.50%  | 97.60%  | 68 | 3  | 30 | 123| 1.153 |
| Pan (25)     | 2020 | China| Asian     | Serum       | Cervical cancer | 158/60 | 0.835| 84.2%  | 80.3%  | 133| 12 | 25 | 48 | 3.84  |
| Liu (20)     | 2020 | China| Asian     | Plasma       | Pancreatic Cancer | 40/40 | 0.865| 72.5%  | 92.5%  | 29 | 3  | 11 | 37 | 1.56  |

NSCLC, non-small cell lung cancer; ORC, oral cancer; IPMNs: Intraductal Papillary Mucinous Neoplasm of the Pancreas; GC, gastric cancer; PanNET, Pancreatic Neuroendocrine Tumor; PanIN2/3, pancreatic intrapithelial neoplasia grade2-3; Sp-FPC sporadic pancreatic ductal adenocarcinoma; FPC, familial pancreatic cancer; ESCC, Esophageal Squamous Cell Carcinoma; AUC, the area under curve; SEN, sensitivity; SPE, specificity; TP, true positives; FP, false positives; FN, false negatives; TN, true negatives.

Table 2 The main features of 17 included studies in prognostic meta-analysis
| First author | Year | Country | Ethnicity | Sample type | Cancer type | Case | Outcome | HR     | (95%CIs)   | P value | Cut-off value | Micro type |
|--------------|------|---------|-----------|-------------|-------------|------|---------|--------|------------|---------|--------------|------------|
| Tsai (23)    | 2016 | China   | Asian     | Plasma      | GC          | 98   | OS      | 3.057(M) | 1.100-8.495 | 0.032   | Median       | miR-1      |
| Lee(28)      | 2015 | Korea   | Asian     | Tissues     | PanNET      | 37   | OS      | 16.267(M) | 1.732-153.789 | 0.015   | 1.279        | miR-1      |
| Kong(17)     | 2011 | China   | Asian     | Serum       | PDAC        | 33   | OS      | 2.67(U)  | 0.6-11.86   | 0.007   | -5.22        | miR-1      |
| Fu(11)       | 2018 | China   | Asian     | Tissues     | Thyroid cancer | 530* | OS      | 5.111(M) | 3.724-7.706 | 0.008   | Median       | miR-16a-156 |
| Liu(29)      | 2013 | China   | Asian     | Tissues     | OSCC        | 95   | OS      | 2.57(M)  | 1.20-5.48   | 0.02    | Median       | miR-1      |
| Wang(10)     | 2019 | China   | Asian     | Tissues     | HCC         | 83   | RFS     | 2.395(M) | 1.207-4.752 | 0.0125  | Median       | miR-1      |
| Niinuma(32)  | 2012 | Japan   | Asian     | Tissues     | GIST        | 132  | OS      | 9.1(M)   | 3.5-23.7    | <0.001  | 1.4          | miR-1      |
| Guan(7)      | 2015 | China   | Asian     | Tissues     | Glioma      | 63   | OS      | 1.8(M)   | 1.2-2.8     | 0.005   | Median       | miR-1      |
| Zhang(35)    | 2018 | China   | Asian     | Bone marrow | AML        | 124  | OS      | 1.845(M) | 0.996-3.417 | 0.052   | Median       | miR-1      |
| Fan1(26)     | 2015 | China   | Asian     | Tissues     | EOC         | 146  | OS      | 2.731(M) | 0.804-9.637 | 0.025   | NM           | miR-1      |
| Fan2(26)     | 2015 | China   | Asian     | Tissues     | EOC         | 146  | RFS     | 2.432(M) | 0.638-8.537 | 0.076   | NM           | miR-1      |
| Tang(33)     | 2018 | China   | Asian     | Tissues     | Thyroid Cancer | 514* | OS      | 2.864(M) | 0.065-4.881 | 0.147   | NM           | miR-16a-156 |
| Milevskiy1(31)| 2019 | Australia| Caucasian | Tissues     | ER+ breast cancer | -* | OS      | 0.342(M) | 0.1534-0.7623 | 0.0091  | NM           | miR-1      |
| Milevskiy2(31)| 2019 | Australia| Caucasian | Tissues     | ER+ breast cancer | -* | OS      | 1.599(M) | 1.0806-2.3652 | 0.0195  | NM           | miR-1      |
| Liu(30)      | 2015 | China   | Asian     | Serum       | Cervical Cancer | 105 | OS      | 3.510(M) | 1.961-6.874 | 0.025   | NM           | miR-1      |
| Ge1(27)      | 2014 | China   | Asian     | Tissues     | CRC         | 126  | OS      | 4.691(M) | 1.688-10.318 | 0.001   | NM           | miR-1      |
| Ge2(27)      | 2014 | China   | Asian     | Tissues     | CRC         | 126  | RFS     | 4.668(M) | 1.632-10.261 | 0.001   | NM           | miR-1      |
| Zhang1(34)   | 2014 | China   | Asian     | Serum       | Osteosarcoma | 105 | OS      | 6.28(M)  | 1.62-13.39  | 0.01    | 4.86         | miR-1      |
| Zhang2(34)   | 2014 | China   | Asian     | Serum       | Osteosarcoma | 105 | RFS     | 6.95(M)  | 1.63-14.82  | 0.01    | 4.86         | miR-1      |
| Sun(16)      | 2012 | China   | Asian     | Tissues     | GC          | 31   | OS      | 2.90(U)  | 0.47-17.90  | <0.001  | Median       | miR-1      |
| Wu1(12)      | 2017 | China   | Asian     | Tissues     | Esophageal carcinoma | 120 | OS      | 1.985(M) | 1.256-2.961 | 0.019   | Median       | miR-1      |
| Wu2(12)      | 2017 | China   | Asian     | Tissues     | Esophageal carcinoma | 120 | DFS    | 1.927(M) | 1.343-2.671 | 0.016   | Median       | miR-1      |

*data from TCGA. OS: overall survival; DFS: disease free survival; RFS: relapse-free survival; OSCC: Oral squamous cell carcinoma; HCC: Hepatocellular Carcinoma; GIST: Gastrointestinal Stromal Tumors; AML, Acute Myeloid Leukaemia; EOC, epithelial ovarian cancer; PanNET, Pancreatic Neuroendocrine Tumor; CRC, colorectal cancer; GC,gastric cancer;PDAC, Pancreatic Ductal Adenocarcinoma.

Table 3 Newcastle–Ottawa quality assessments scale
First author | Year | Quality indicators from Newcastle–Ottawa Scale Scores
--- | --- | ---
Tsai (23) | 2016 | 1 2 3 4 5 6 7 8
Lee(28) | 2015 | 1 2 3 4 5 6 7
Kong (17) | 2011 | 1 2 3 4 5 6 7
FU(11) | 2018 | 1 2 3 4 5 6 7
Liu(29) | 2013 | 1 2 3 4 5 6 7
Wang(10) | 2019 | 1 2 3 4 5 6 7
Ninnuma(32) | 2012 | 1 2 3 4 5 6 7
Guan(7) | 2015 | 1 2 3 4 5 6 7
Zhang(35) | 2018 | 1 2 3 4 5 6 7
Fan(26) | 2015 | 1 2 3 4 5 6 7
Tang(33) | 2018 | 1 2 3 4 5 6 7
Milevskiy(31) | 2019 | 1 2 3 4 5 6 7
Liu(30) | 2015 | 1 2 3 4 5 6 7
Ge(27) | 2014 | 1 2 3 4 5 6 7
Zhang(34) | 2014 | 1 2 3 4 5 6 7
Sun(1616) | 2012 | 1 2 3 4 5 6 7
Wu(12) | 2017 | 1 2 3 4 5 6 7

1. Representativeness of the exposed cohort; 2. Selection of the non-exposed cohort; 3. Ascertainment of exposure; 4. Outcome of interest not present at start of study; 5. Control for important factor or additional factor; 6. Assessment of outcome; 7. Follow-up long enough for outcomes to occur; 8. Adequacy of follow up of cohorts

Table 4 Results of diagnostic meta-analysis

| Variables | Subgroup | Case/Control | Pooled results |
| --- | --- | --- | --- |
|  |  |  | AUC(95%CI) | Sensitivity(95%CI) | I²(%) | P | Specificity(95%CI) | P |
| Serum and Plasma | - | 518/403 | 0.87(0.84-0.90) | 0.73(0.64-0.81) | 73.94 | 0.00 | 0.90(0.81-0.95) | 79.17 | 0.00 |
| Serum and Plasma | Asian | 466/354 | 0.86(0.83-0.89) | 0.73(0.66-0.79) | 70.87 | 0.01 | 0.92(0.81-0.97) | 86.60 | 0.00 |
| Serum and Plasma | Caucasian | 52/49 | 0.90(0.87-0.92) | 0.85(0.44-0.98) | 83.22 | 0.00 | 0.84(0.64-0.94) | 67.12 | 0.03 |
| Serum and Plasma | Sample size<100 | 92/89 | 0.91(0.88-0.93) | 0.80(0.50-0.94) | 75.86 | 0.00 | 0.87(0.74-0.94) | 63.93 | 0.03 |
| Serum and Plasma | Sample size>100 | 426/314 | 0.84(0.80-0.87) | 0.73(0.64-0.80) | 78.13 | 0.00 | 0.91(0.77-0.97) | 89.45 | 0.00 |

Table 5 Results of prognostic meta-analysis

| Variables | Subgroup | Pooled HR(95%CI) | I²(%) | P |
| --- | --- | --- | --- | --- |
| Serum and Plasma | - | 4.06(2.67-6.18) | 0 | 0.668 |
| Tissues | - | 2.54(1.79-3.61) | 75.8 | 0.000 |
| Tissues | OS | 2.57(1.60-4.12) | 81.2 | 0.000 |
| Tissues | RFS | 2.94(1.77-4.87) | 0 | 0.497 |
| Tissues | Published data | 2.67(2.02-3.53) | 40.5 | 0.071 |
| Tissues | Data from TCGA | 5.03(3.51-7.20) | 0 | 0.604 |