RESEARCH ARTICLE

Prognostic significance of microRNA-101 in solid tumor: A meta-analysis

Xianxiong Ma*, Jie Bai*, Gengchen Xie, Yulin Liu, Xiaoming Shuai‡*, Kaixiong Tao‡*

Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, People’s Republic of China

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* kaixiongtao@hust.edu.cn (KT); xmshuai@hust.edu.cn (XS)

Abstract

MicroRNA-101 has been reported as an important factor in carcinogenesis of several malignant tumors. However, its actual role in prognosis among solid malignancies remains unclear. Accordingly, we performed this meta-analysis aiming to identify prognostic significance of miR-101 in solid tumor. Pooled hazard ratios (HRs) with 95% confidence intervals (CIs) for overall survival (OS) or disease-free survival (DFS)/metastasis-free survival (MFS)/progression-free survival (PFS)/relapse-free survival (RFS)/time-to progression (TTP) were estimated with random effects or fixed effects models on the basis of heterogeneity. Subgroup analysis, sensitive analysis and meta-regression analysis were also conducted to clarify the possible confounding factors and investigate the source of heterogeneity. Publication bias was evaluated by using Begg’s and Egger’s tests. A total of 21 studies containing 3753 cases were selected into our quantitative analysis via electronic database search. A lower expression of miR-101 was significantly associated with worse OS (HR = 0.66, 95%CI [0.52–0.85], P = 0.001) and PFS (HR = 0.70, 95%CI [0.51–0.95], P = 0.023) in patients with solid tumor. The under-expression of miRNA-101 is a credible indicator of poorer prognosis in several of solid malignancies.

Introduction

MicroRNAs (miRNA, miRs) are a subset of small non-coding RNA molecules that are approximately 18–22 nucleotides in length. MiRNAs play crucial regulatory roles in gene expression at the post-transcriptional level [1, 2]. The major mechanism of miRNA action is the interaction with the 3'-UTR of the targeted gene mRNA, followed by degradation of the mRNA or inhibition of mRNA protein translation. In human cancers, numerous studies have shown that the expression of miRNAs is deregulated and these miRNAs act as regulatory molecules in many biological processes, including differentiation, proliferation, and apoptosis of tumor cells [3–5]. Several miRNAs are downregulated in many tumors and appear to function as tumor suppressor genes [6]. Among these downregulated miRNAs, miR-101 is one of the most downregulated miRNAs in human cancers, multiple research studies have been exploring the prognostic
function of miR-101 in cancer patients in order to find a reliable biomarker to guide for cancer treatment[7, 8]. MiRNA-101, located on chromosome 1[65058434–65058508], is widely known as a tumor suppressive miRNA that is strongly downregulated in several cancers including neuroblastoma, gastric cancer, prostate cancer, nasopharyngeal carcinoma and hepatocellular carcinoma [9–11]. The aberrant expression of miR-101 not only has diagnostic implications but also can predict cancer patient survival [12]. Although an overwhelming majority of evidence has explored a negative prognostic value of miR-101 under-expression across miscellaneous neoplasms, the prognostic impact of miR-101 in malignancies remains controversial. Failed to draw a similar conclusion, Slattery et al[13] and Lv et al [14] presented a worse survival status of patients under stronger miR-101 expression, suggesting an astonishing positive prognostic significance in circumstance of miR-101 under-expression. Therefore, in the present study through gathering available evidence, we carried out an integrated meta-analysis as well as subgroups analysis to identify the relationship between miR-101 expression level and survival of cancer patients by pooling the hazard ratio (HR) from studies addressing the correlation between miR-101 and OS/PFS of patients with malignancies, aiming to provide more theoretical supports for targeted treatment.

Materials and methods

Search strategy

We performed a thorough search for available literatures in electronic databases of Pubmed, Embase and Web of science until April 2017, using the following words "(microRNA-101 OR miR-101 OR miR101 OR miRNA-101) AND (tumor OR neoplasm OR cancer OR carcinoma OR malignancy)". In order to avoid missing the potentially related articles, reference lists were also screened. Two authors independently carried out this procedure and any discrepancy was resolved by mutual discussion.

Selection criteria

Inclusion criteria were as follows: 1) studies exploring any of the solid tumor; 2) studies dealing with miR-101 expression and OS/DFS/PFS/RFS/MFS/TTP; 3) studies that categorized patients into low- and high-expression groups based on the miR-101 expression; 4) studies providing HR directly or key information to calculate HR indirectly, such as Kaplan-Meier curves and original survival data; 5) studies assessing miR-101 expression in tissue or blood.

The following were the exclusion criteria: 1) studies on myelomas, lymphomas, or leukemia; 2) duplicated or overlapped studies; non-original articles, such as reviews, articles or letters; 3) laboratory studies on cell lines or animals level; 4) studies on a set of microRNAs but not miR-101 alone; 5) studies with a sample-size less than 20 participants.

Qualitative assessment

Newcastle-Ottawa Scale(NOS)[15] was adopted to evaluate the quality of each eligible article. The scale was revised with certain adaptive modifications to match the practical needs of the pooled analysis. There are three aspects contained in the scale: selection, comparability, and outcome. Stars awarded for each quality item serve as a quick visual assessment. Stars are awarded such that the highest quality studies are awarded up to nine stars. Studies with more than 6 stars were considered as of high quality. Otherwise, studies were excluded from the final meta-analysis.
Data extraction

All eligible publications were reviewed by Shuai and Bai. The following details of each article were recorded: first author’s name, publication year, cancer type, treatment, sample size, stage of disease, miR-101 test method, the cutoff value to discriminate high or low expression of miR-101, sample sources, follow-up time, extracting method of HR, outcome, NOS and et al. The HR value was extracted directly if it was calculated by a multivariate analysis. Otherwise, the results from univariate analysis were also allowed in the meta-analysis. If both multivariate analysis and univariate analysis were not available, Kaplan-Meier curves were used to extract HR value by using the described method[16].

Statistical analysis

The heterogeneity of the studies included in this meta-analysis was assessed by the Q statistic test and the I² statistic test, where I² more than 50% indicated evidence of heterogeneity[17]. The random-effects model was selected when I² was significant (>50%); otherwise, the fixed-effects model was selected. Publication bias was examined using Begg’s funnel plot and Egger’s linear regression test. P<0.05 was considered significant[18]. All analyses were performed with STATA version 12.0 software (Stata Corporation, College Station, TX).

Results

Study selection

In total, 291, 331, 683 records were identified from Pubmed, Web of Science and Embase. According to the selection criteria, most of the preliminarily included entries were eliminated on account of duplicated data, inappropriate article type or inadequate original information. Finally, a total of 21[13, 14, 19–37] observational studies consisting of 3753 cases were retained for subsequent pooling calculation. None of the eligible entries scored less than six by NOS. Fig 1 displayed the selection workflow of all eligible studies in our meta-analysis.

Characteristics of included studies

The majority of included studies were carried out in China (n = 15), the other six studies were conducted in Netherlands (n = 2), USA, Japan, India, and Norway. None of the eligible entries scored less than six by NOS, indicating a high methodological quality across all studies. The cancer types included HCC, BTCC, NSCLC, GBC, CRC, LSCC, GBM, ESAC, PDAC, astrocytoma and glioma. Four study clearly stated the research-related treatment, as shown in Table 1, three studies did not clarify whether patient received adjuvant therapy after surgery, most of studies (n = 14) did not receive any adjuvant treatment after surgery. Study sample sizes ranged from 21 to 1134, qRT-PCR (n = 19) and microarray (n = 2) were used to assess miR-101 expression, and cutoff value varied among studies with median expression of miR-101 the most widely used. 15 studies enrolled patients with stages I-IV and five studies explored with stages I-III (n = 4) or stages III-IV (n = 1), only one study did not specify the stage of disease in the study population. The source of miR-101 came from tissue (n = 20) and blood (n = 1). 13 HRs were reported in the present analysis. The other 8 HRs were estimated by analyzing K-M curves. About a half of HRs (n = 11) were calculated by using a multivariate analysis and the remaining 10 records either did not clarify the calculating methods or were computed by using univariate analysis. 19 studies provided data on OS and 9 studies provided on DFS/PFS/RFS/MFS/TTP with respect to outcome.
Correlation of miR-101 expression with OS and subgroup analysis

Highly significant heterogeneity ($I^2 = 68.6\%$, $p < 0.001$) was detected when 19 studies were pooled. To make a conservative estimate, a random-effect model rather than a fixed-effect model was used to account for the highly significant inter-study heterogeneity. The pooled HR ($HR = 0.66$, 95%CI [0.52–0.85], $P = 0.001$) suggested that lower expression level of miR-101 significantly predicted poorer OS in patients with solid tumor (Fig 2).

Given that the substantial heterogeneity exhibited in the trials aggregated with respect to the OS, meta-regression and subgroups analyses were conducted to explore the heterogeneity of covariates including country, tumor type, test method, cutoff, extracting method, multivariate analysis (Table 2). Subgroups analysis by country explored that lower miR-101 expression status was identified as a worse prognostic marker in China group ($HR = 0.60$, 95%CI [0.51–0.70], $I^2 = 49.9\%$, $P = 0.014$), but not in non-China group. According to subgroups of different cancer types, the subgroups (HCC & CRC) that significant heterogeneity was found show no significant HR(HCC HR = 0.72, 95%CI[0.28–1.86], $I^2 = 87.1\%$, $P < 0.001$; CRC HR = 1.22, 95%CI[0.40–3.67], $I^2 = 89.3\%$, $P < 0.001$, fixed-effects model), which was completely opposite to BC & NSCLC & other group(BC HR = 0.76, CI[0.65–0.89], $I^2 = 0$, $P = 0.486$; NSCLC...
Table 1. Characteristics of the included articles.

| Study       | Country | Cancer type | Treatment | NO. of patients | Stage | Test method | Cutoff | Sample source | Follow-up (months) | Extracting method | Multiple analysis | Male/ female | Mean age | Outcome | NOS |
|-------------|---------|-------------|-----------|-----------------|-------|-------------|--------|---------------|-------------------|-------------------|------------------|--------------|----------|---------|--------|-----|
| Zheng 201517 | China   | HCC         | Surgery   | 163             | I-IV  | qRT-PCR     | ROCB   | Blood         | 70                | Report            | Yes              | 136/27       | NA       | OS      | 8      |
| Zhang 201218 | China   | HCC         | Surgery   | 130             | I-IV  | qRT-PCR     | Median | Tissue        | 8.6yE            | Report            | Yes              | 96/34        | NA       | OS/DFS  | 9      |
| Zhang 201419 | China   | BTCC        | Surgery   | 72              | I-IV  | qRT-PCR     | 1.45NF | Tissue        | every 3m          | Report            | Yes              | 42/30        | 57       | OS      | 8      |
| Ye 201620    | China   | NSCLC       | NA        | 105             | I-IV  | qRT-PCR     | Median | Tissue        | NA               | Report            | Yes              | 67/38        | NA       | OS      | 8      |
| Lv 201621    | China   | HCC         | Surgery   | 78              | I-III | qRT-PCR     | Average | Tissue      | 60                | Report            | Yes              | 63/15        | 53       | OS/RFS  | 9      |
| Chandra 201722 | India  | BC          | NA        | 37              | I-IV  | qRT-PCR     | Median | Tissue        | NA               | Report            | No               | NA          | NA       | OS/MFS  | 8      |
| Bao 201623    | China   | GBC         | Surgery   | 53              | I-IV  | qRT-PCR     | NA     | Tissue        | 40                | Report            | Yes              | 18/35        | NA       | OS      | 8      |
| Shen 201324   | China   | HCC         | NA        | 154             | NA    | Microarray  | NA     | NA           | K-M              | No                | NA               | NA          | NA       | OS      | 7      |
| Stalley 201625 | USA    | CRC         | Surgery   | 1134            | I-IV  | Microarray  | NA     | Tissue        | 60.4              | Report            | No               | 597/537     | 65.4     | OS      | 8      |
| Li J 201526   | China   | BC          | Surgery   | 111             | I-III | qRT-PCR     | NA     | Tissue        | NA               | K-M              | No               | NA          | NA       | OS/DFS  | 7      |
| Li M 201526   | China   | LSCC        | Surgery   | 80              | I-IV  | qRT-PCR     | Median | Tissue        | 60                | K-M              | No               | 56/24        | NA       | OS      | 7      |
| Li X 201326   | China   | Gliomas     | Surgery   | 50              | I-IV  | qRT-PCR     | 8E     | Tissue        | 30                | K-M              | No               | 34/16        | 41       | OS      | 7      |
| Tian 201627   | China   | GBM         | S+R+C     | 70              | I-IV  | qRT-PCR     | Average | Tissue      | NA               | K-M              | No               | 33/37        | NA       | OS/PFS  | 7      |
| Gao 201528    | China   | CRC         | Surgery   | 735             | I-IV  | qRT-PCR     | NA     | Tissue        | 56(m)F            | Report            | Yes              | NA          | NA       | OS/DFS  | 8      |
| Hiroki 200929 | Japan   | ESAC        | Surgery   | 21              | I-IV  | qRT-PCR     | NA     | Tissue        | 23(m)            | Report            | Yes              | NA          | 64.9(m) | OS/DFS  | 8      |
| Schee 201230  | Norway  | CRC         | Surgery   | 193             | I-III | qRT-PCR     | Median | Tissue        | NA               | K-M              | No               | 112/81       | NA       | MFS     | 7      |
| Maffouh M 201431 | Norway | CRC         | Surgery   | 25              | III-IV| qRT-PCR     | Median | Tissue        | NA               | K-M              | No               | NA          | NA       | OS      | 6      |
| Liu C 201732   | China   | Astrocytoma | S+R+C     | 80              | I-IV  | qRT-PCR     | NA     | Tissue        | NA               | Report            | Yes              | 36/54        | NA       | OS      | 7      |
| Luo L 201133   | China   | NSCLC       | Surgery   | 45              | I-III | qRT-PCR     | 0.54   | Tissue        | NA               | K-M              | No               | 29/16        | NA       | OS      | 7      |
| Zhang S 201534 | China   | CRC         | Surgery   | 172             | I-IV  | qRT-PCR     | Median | Tissue        | NA               | Report            | Yes              | 99/73        | NA       | OS      | 8      |
| Jansen 201235  | Netherlands | BC       | S+C       | 245             | I-IV  | qRT-PCR     | Median | Tissue        | 89(10–165)       | Report            | Yes              | 0/235       | NA       | TTP     | 8      |

Note
A: surgery + radiotherapy + chemotherapy
B: Categorized based on receiver operating curve
C: based on 1.45 fold of normal expression
D: did not state the definition of cutoff
E: 8.6Years
F: median follow-up.

Abbreviations: HCC hepatocellular carcinoma; BTCC bladder transitional cell carcinoma; NSCLC non-small-cell lung cancer; BC breast cancer; CRC colorectal cancer; GBC gallbladder carcinoma; LSCC laryngeal squamous cell carcinoma; GBM glioblastoma multiforme; ESAC endometrial serous adenocarcinoma; PDAC pancreatic ductal adenocarcinoma.

https://doi.org/10.1371/journal.pone.0180173.t001
HR = 0.56, 95%CI[0.34–0.93], I² = 0, P = 0.976; Other HR = 0.54, 95%CI[0.43–0.67], I² = 0, P = 0.692); With respect to subgroups by different test methods, both significant heterogeneity and HR were found in qRT-PCR group (HR = 0.61, 95%CI[0.49–0.77], I² = 56%, P = 0.003), higher heterogeneity and no significant HR was reported in Microarray group (HR = 1.35, 95%CI[0.21–8.62], I² = 93.6%, P < 0.001); Subgroup analysis by Cutoff indicating that both Median (HR = 0.73, 95%CI[0.63–0.85], I² = 15.9%, P = 0.312) and Other (HR = 0.61, 95%CI [0.43–0.86], I² = 66.7%, P = 0.001) groups predict poor prognosis with under-expression of miR-101, in contrast, the Average (HR = 1.33, 95%CI[0.23–7.83], I² = 93.5%, P < 0.001) group with significant heterogeneity shows no significant HR. In the subgroup analysis based on extracting methods, The Report group (HR = 0.73, 95%CI [0.52–1.02], I² = 79.4%, P < 0.001) with significant heterogeneity found no significant HR, on the contrary, the K-M group
(HR = 0.54, 95%CI [0.40–0.72], I² = 0, P = 1) with significant HR found no significant heterogeneity. Similar to test method subgroups analysis, both groups show relative high heterogeneity in the subgroup analysis of multivariate analysis, significant HR was only found in Yes group (HR = 0.62, 95%CI [0.43–0.90], I² = 70.7%, P < 0.001).

**Correlation of miR-101 expression with DFS/PFS/RFS/MFS/TTP**

Nine eligible studies were adopted to pool HRs for DFS/PFS/RFS/MFS/TTP. With obvious statistical heterogeneity (I² = 74.7%, P < 0.001) (Fig 3), a random effect model was used to pool HRs. The result showed that low miR-101 expression was associated with negative outcome in patients with solid tumor (HR = 0.70, 95%CI [0.51–0.95], P = 0.023). Additionally, data were analyzed based on DFS, MFS, and Other. Patients with low miR-101 expression had a significantly shorter DFS (HR = 0.47, 95%CI [0.35–0.62], I² = 51.1%, P = 0.072, fixed-effect model) and MFS (HR = 0.76, 95%CI [0.60–0.97], I² = 53.1%, P = 0.144, fixed-effect model). Despite the lack of significant difference, a similar trend was observed for Other group (HR = 0.96, 95% [0.58–1.61], I² = 76.2%, p = 0.015, random-effect model).

### Table 2. Pooled HRs for OS according to subgroup analysis.

| Subgroup          | NO. of studies | Heterogeneity | P-value | HR(95%CI)        | Meta-regression |
|-------------------|----------------|---------------|---------|-----------------|----------------|
|                   |                |               |         |                 | adj R²          | P-value       |
| Country           |                |               |         |                 |                |              |
| China             | 15             | 49.9%         | <0.001  | 0.60(0.51–0.70) | 7.02%          | 0.260         |
| Non-China         | 4              | 68.6%         | <0.001  | 0.91(0.36–2.32) |                |              |
| Tumor type        |                |               |         |                 | -8.15%         | 0.614         |
| HCC               | 4              | 87.1%         | <0.001  | 0.72(0.28–1.86) |                |              |
| BC                | 2              | 0             | 0.486   | 0.76(0.65–0.89) |                |              |
| CRC               | 3              | 89.3%         | <0.001  | 1.22(0.40–3.67) |                |              |
| NSCLC             | 2              | 0             | 0.976   | 0.56(0.34–0.93) |                |              |
| Other             | 8              | 0             | 0.692   | <0.001          | 0.54(0.43–0.67) |              |
| Test method       |                |               |         |                 | 13.12%         | 0.123         |
| qRT-PCR           | 17             | 56.6%         | 0.003   | 0.61(0.49–0.77) |                |              |
| Microarray        | 2              | 93.6%         | <0.001  | 1.35(0.21–8.62) |                |              |
| Cutoff            |                |               |         |                 | -10.08%        | 0.849         |
| Median            | 6              | 15.9%         | 0.312   | 0.73(0.63–0.85) |                |              |
| Average           | 2              | 93.5%         | <0.001  | 1.33(0.23–7.83) |                |              |
| Other             | 11             | 66.7%         | 0.001   | 0.61(0.43–0.86) |                |              |
| Extracting method |                |               |         |                 | -0.79%         | 0.393         |
| Report            | 12             | 79.4%         | <0.001  | 0.73(0.52–1.02) |                |              |
| K-M               | 7              | 0             | 1       | 0.54(0.40–0.72) |                |              |
| Multivariate method|              |               |         |                 | -8.02%         | 0.637         |
| Yes               | 10             | 70.7%         | <0.001  | 0.62(0.43–0.90) |                |              |
| No                | 9              | 68.6%         | <0.001  | 0.72(0.50–1.05) |                |              |
| Overall           | 19             | 68.6%         | <0.001  | 0.66(0.52–0.85) |                |              |

Note

- a) random-effects model
- b) fixed-effects model.

https://doi.org/10.1371/journal.pone.0180173.t002
Sensitivity analysis

Sensitivity analysis was performed by sequentially eliminating individual studies, indicating that there was not a single study that significantly contributed to heterogeneity both for OS (Fig 4) and DFS/PFS/RFS/MFS/TTP (Fig 5). Furthermore, a meta-regression was also conducted to explore the potential factors that are responsible for heterogeneity in OS. The results showed that the above factors could partly explain the heterogeneity but did not reach statistical significance (Table 2).

Publication bias

The Begg’s funnel plots, Egger’s test and Begg’s test were used to detect publication bias in the meta-analysis. Although the funnel plot revealed relative big publication bias in OS, but P-value of Begg’s and Egger’s tests were 0.861 and 0.166, respectively, showing no evidence for significant publication bias (Fig 6). Similarly, P-value of Begg’s and Egger’s tests for DFS/PFS/RFS/MFS/TTP were 0.297 and 0.765, no significant publication bias was detected, either (Fig 7).
Numerous profiling studies have demonstrated that miRNA expression levels failed to agree in various types of cancers, miRNAs can be potential biomarkers for cancer prognosis. Increasing data favor the potential use of miR-101 as a cancer prognostic predictor. Recently, genome-wide miRNA expression profiling studies revealed that miR-101 is widely present in various tissues and organs, and its aberrant expression was reported in various cancers including HCC [14, 19, 20, 25], CRC [13, 30], breast cancer [23, 26], NSCLC [22], gliomas [28], and et al. It is indubitable that miR-101 is an important cancer-related miRNA. More and more evidence has demonstrated that miR-101 is frequently downregulated in multiple types of cancer and acts as a tumor suppressor by repressing many critical oncogenes. In hepatocellular carcinoma, Wang et al found c-Myc collaborates with EZH2-containing PRC2 complex in silencing miRNA-101 during hepatocarcinogenesis and lower expression of miR-101 is positively correlated with poorer prognosis [38]. In CRC, it is reported that loss of miR-101 expression promotes Wnt/β-catenin signaling pathway activation and malignancy in colon cancer cells [39]. Similarly, in glioblastoma, Liu et al demonstrated that miRNA-101 inhibits proliferation, migration and invasion of glioblastoma by targeting SOX9 [40], Michiel et al also found that miRNA-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation.
migration, and angiogenesis [41]. Moreover, miRNA-101 reverses temozolomide resistance by inhibition of GSK3β in glioblastoma[42]. In nasopharyngeal carcinoma, MicroRNA-101 inhibits invasion and angiogenesis through targeting ITGA3 and its systemic delivery inhibits lung metastasis[43]. On the other hand, 175 targeted genes validated by experiment and 5206 targeted genes predicted by miRanda software can be found in GCBI website based on classical miRNA-3'-UTR pathway (https://www.gcbi.com.cn/gclib/html/dictSearchAct/MI0000103/miRNA), indicating miRNA-101 may play a complicated role in many gene ontology functions and pathways networks. Further experiments need to be conducted to elucidate the role of miR-101 in carcinogenesis. However, among all the studies referring to the relationship between miRNA-101 and OS/PFS, there were still some contradictory views requiring adequate attention, a comprehensive study is therefore in urgent.

To the best of our knowledge, few studies have systemically explored the possible prognostic role of miR-101 down-regulation in solid malignancies before. In order to get a more convincing outcome, HRs for both OS and PFS were calculated independently. On the whole, our quantitative results strongly supported the current mainstream viewpoint that an undesirable impact of miR-101 low expression was related with poor OS and PFS, taking no account of confounding factors. Among all the included studies, on the contrary, two studies [13, 14]
highlighted that obvious advantage on survival duration was obtained in miR-101 under-expression cases and no significant HR was found in other three studies [26–28, 35], whose HR value and 95%CI were extracted from K-M survival curve, indicating that this indirect method may impose slightly bias on the HR we calculated. It’s worth mentioning that 4 studies providing K-M curve was excluded due to the lack of clear categorization [9, 44–46]. The appropriate HRs can’t be obtained until the K-M survival curve and the exact number of each group are available simultaneously. Based on the outcome of subgroup analysis, significant HR was not found in all subgroup even significant HR was found in each studies, which may partly attribute to a relative small number of studies and high heterogeneity and need further elucidate.

Apart from the inspiring outcomes, limitations still exist in this quantitative meta-analysis. First of all, despite the usage of random-effects model and subgroup analysis, the heterogeneity across studies failed to be eliminated completely, which could result in bias of the outcome in certain extent. Secondly, due to lack of direct HR and 95%CI data, we merely extracted the data by using Engauge software indirectly, which may bring about slight error in HR and 95%
Cl. Thirdly, lack of abundant miR-101 expression data in the global population makes it difficult to set a standard cut-off value for measurement of miR-101 expression levels, categorization between studies did not get in consensus. Additionally, other parameters that may partially contribute to the heterogeneity were not explored, such as pathological grade and body mass index.

In spite of the limitations mentioned above, there are still numerous valuable implications in this comprehensive meta-analysis, which reveals that low expression of miR-101 is associated with unfavorable survival outcomes in patients with various types of carcinomas, particularly with regard to OS. Further large-scale, well-designed and multi-center prospective studies should be conducted to confirm these findings before the application of miR-101 for the prognosis of cancers.

Supporting information

S1 Fig. Forest plot to assess the association between miR-101 under-expression and OS in subgroups based on test method.
(TIF)

S2 Fig. Forest plot to assess the association between miR-101 under-expression and OS in subgroups based on extracting method.
(TIF)

S3 Fig. Forest plot to assess the association between miR-101 under-expression and OS in subgroups based on country origin.
(TIF)
S4 Fig. Forest plot to assess the association between miR-101 under-expression and OS in subgroups based on multivariate method.
(TIF)

S5 Fig. Forest plot to assess the association between miR-101 under-expression and OS in subgroups based on tumor type.
(TIF)

S6 Fig. Forest plot to assess the association between miR-101 under-expression and OS in subgroups based on cutoff method.
(TIF)

S1 Table. PRISMA 2009 checklist.
(DOC)

Acknowledgments
This study was generously supported by the National Natural Science Foundation of China (No. 81572413) and Scientific and Technological Application Foundation Project of Wuhan (No. 2015060101010044)

Author Contributions
Conceptualization: Xianxiong Ma.
Data curation: Jie Bai, Gengchen Xie, Yulin Liu, Xiaoming Shuai, Kaixiong Tao.
Formal analysis: Xianxiong Ma, Jie Bai.
Funding acquisition: Xiaoming Shuai, Kaixiong Tao.
Investigation: Xianxiong Ma.
Methodology: Xianxiong Ma, Jie Bai.
Project administration: Kaixiong Tao.
Software: Xianxiong Ma, Jie Bai.
Supervision: Gengchen Xie, Yulin Liu, Xiaoming Shuai, Kaixiong Tao.
Visualization: Xianxiong Ma.
Writing – original draft: Xianxiong Ma.
Writing – review & editing: Jie Bai, Gengchen Xie, Yulin Liu, Xiaoming Shuai, Kaixiong Tao.

References
1. Izaurralde E. GENE REGULATION. Breakers and blockers-miRNAs at work. Science. 2015; 349(6246):380–2. https://doi.org/10.1126/science.1260969 PMID: 26206919.
2. Thomson DW, Dinger ME. Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet. 2016; 17(5):272–83. https://doi.org/10.1038/nrg.2016.20 PMID: 27040487.
3. Yoshimura A, Numakawa T, Odaka H, Adachi N, Tamai Y, Kunugi H. Negative regulation of microRNA-132 in expression of synaptic proteins in neuronal differentiation of embryonic neural stem cells. Neurochem Int. 2016; 97:26–33. https://doi.org/10.1016/j.neuint.2016.04.013 PMID: 27131735.
4. Guan B, Li Q, Shen L, Rao Q, Wang Y, Zhu Y, et al. MicroRNA-205 directly targets Kruppel-like factor 12 and is involved in invasion and apoptosis in basal-like breast carcinoma. Int J Oncol. 2016; 49(2):720–34. https://doi.org/10.3892/ijo.2016.3573 PMID: 27278159.
5. Zhang Y, Xue C, Zhu X, Zhu X, Xian H, Huang Z. Suppression of microRNA-125a-5p upregulates the TAZ-EGFR signaling pathway and promotes retinoblastoma proliferation. Cell Signal. 2016; 28(8):850–60. https://doi.org/10.1016/j.cellsig.2016.04.002 PMID: 27094723.

6. Yamamoto H, Mori M. MicroRNAs as Therapeutic Targets and Colorectal Cancer Therapeutics. Adv Exp Med Biol. 2016; 937:239–47. https://doi.org/10.1007/978-3-319-42059-2_13 PMID: 27573904.

7. Xie Y, Yao Q, Butt AM, Guo J, Tian Z, Bao X, et al. Expression profiling of serum microRNA-101 in HBV-associated chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Cancer Biol Ther. 2014; 15(9):1248–55. https://doi.org/10.4161/cbt.29688 PMID: 24971953; PubMed Central PMCID: PMC31428867.

8. Li X, Shi Y, Yin Z, Xue X, Zhou B. An eight-miRNA signature as a potential biomarker for predicting survival in lung adenocarcinoma. J Transl Med. 2014; 12:159. https://doi.org/10.1186/1479-5876-12-159 PMID: 24893932; PubMed Central PMCID: PMCPMC4062505.

9. Liu HT, Xing AY, Chen X, Ma RR, Wang YW, Shi DB, et al. MicroRNA-27b, microRNA-101 and microRNA-128 inhibit angiogenesis by down-regulating vascular endothelial growth factor C expression in gastric cancers. Oncotarget. 2015; 6(35):37458–70. https://doi.org/10.18632/oncotarget.6059 PMID: 26460960.

10. Chakravarthi BVSK, Goswami MT, Pathi SS, Robinson AD, Ciesliński M, Chandrasekar DS, et al. MicroRNA-101 regulated transcriptional modulator SUB1 plays a role in prostate cancer. Oncogene. 2016; 35(49):6330–40. https://doi.org/10.1038/onc.2016.164 PMID: 27270442.

11. Su H, Yang JR, Xu T, Huang J, Xu L, Yuan Y, et al. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenesis. Cancer Res. 2009; 69(3):1135–42. https://doi.org/10.1158/0008-5472.CAN-08-2886 PMID: 19155302.

12. Scheel K, Bokas P, Kappenberg TW, Thomas J, Brandt K. Clinical relevance of microRNA miR-21, miR-31, miR-92a, miR-101, miR-106a and miR-145 in colorectal cancer. BMC Cancer. 2012; 12. https://doi.org/10.1186/1471-2407-12-505 PMID: 23121918.

13. Slattery ML, Herrick JS, Pellatt DF, Mullaney LE, Stevens JR, Wolff E, et al. Site-specific associations between miRNA expression and survival in colorectal cancer cases. Oncotarget. 2016; 7(37):60193–205. https://doi.org/10.18632/oncotarget.11173 PMID: 27517623; PubMed Central PMCID: PMCPMC5312378.

14. Lv X, Li J, Yang B. Clinical effects of MIR-101 on prognosis of hepatocellular carcinoma and carcinogenic mechanism of anti-MIR-101. Oncol Rep. 2016; 36(4):2184–92. https://doi.org/10.3892/or.2016.4980 PMID: 27496785.

15. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol. 2010; 25(9):603–5. https://doi.org/10.1007/s10654-010-9491-z PMID: 20652370.

16. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials. 2007; 8:16. https://doi.org/10.1186/1745-6215-8-16 PMID: 17555558; PubMed Central PMCID: PMCPMC1920534.

17. Cochran WG. The comparison of percentages in matched samples. Biometrika. 1950; 37(3–4):256–66. PMID: 14801052.

18. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997; 315(7109):629–34. PMID: 9310563; PubMed Central PMCID: PMCPMC2127453.

19. Zheng F, Liao YJ, Cai MY, Liu TH, Chen SP, Wu PH, et al. Systemic Delivery of MicroRNA-101 Potently Inhibits Hepatocellular Carcinoma In Vivo by Repressing Multiple Targets. Plos Genet. 2015; 11(2):1–21. https://doi.org/10.1371/journal.pgen.1004873 PMID: 25693145.

20. Zhang Y, Guo X, Xiong L, Kong X, Xu Y, Liu C, et al. MicroRNA-101 suppresses SOX9-dependent tumorigenicity and promotes favorable prognosis of human hepatocellular carcinoma. Febs Lett. 2012; 586(24):4362–70. https://doi.org/10.1016/j.febslet.2012.10.053 PMID: 23178713.

21. Zhang H, Qi F, Cao Y, Chen M, Zu X. Down-regulated microRNA-101 in bladder transitional cell carcinoma is associated with poor prognosis. Med Sci Monitor. 2014; 20:812–7. https://doi.org/10.12659/MSM.890300 PMID: 24834983.

22. Ye Z, Yin S, Su Z, Bai M, Zhang H, Hei Z, et al. Downregulation of miR-101 contributes to epithelial-mesenchymal transition in cisplatin resistance of NSCLC cells by targeting ROCK2. Oncotarget. 2016; 7(25):37524–35. https://doi.org/10.18632/oncotarget.6852 PMID: 27229528; PubMed Central PMCID: PMCPMC5122329.

23. Chandra-Mangalhara K, Manvati S, Saini SK, Ponnusamy K, Agarwal G, Abraham SK, et al. ERK2-ZEB1-miR-101-1 axis contributes to epithelial-mesenchymal transition and cell migration in cancer. Cancer Lett. 2017; 391:59–73. https://doi.org/10.1016/j.canlet.2017.01.016 PMID: 28109909.
Bao RF, Shu YJ, Hu YP, Wang XA, Zhang F, Liang HB, et al. miR-101 targeting ZFX suppresses tumor proliferation and metastasis by regulating the MAPK/Erk and smad pathways in gallbladder carcinoma. Oncotarget. 2016; 7(16):22339–54. https://doi.org/10.18632/oncotarget.7970 PMID: 26968949

Shen Q, Bae HJ, Eun JW, Kim HS, Park SJ, Shin WC, et al. MiR-101 functions as a tumor suppressor by directly targeting nemo-like kinase in liver cancer. Cancer Lett. 2014; 344(2):204–11. https://doi.org/10.1016/j.canlet.2013.10.030 PMID: 24189458

Li JT, Jia LT, Liu NN, Zhu XS, Liu QQ, Wang XL, et al. MiRNA-101 inhibits breast cancer growth and metastasis by targeting CX chemokine receptor 7. Oncotarget. 2015; 6(31):30818–30. https://doi.org/10.18632/oncotarget.5067 PMID: 26360786; PubMed Central PMCID: PMCPMC4741570.

Li MH, Tian LL, Ren H, Chen XX, Wang Y, Ge JC, et al. MicroRNA-101 is a potential prognostic indicator of laryngeal squamous cell carcinoma and modulates CDK8. J Transl Med. 2015; 13(1). https://doi.org/10.1186/1471-2407-12-505 PMID: 23121918; PubMed Central PMCID: PMCPMC3124376.

Tian T, Mingyi M, Qiu X, Qiu Y. MicroRNA-101 reverses temozolomide resistance by inhibition of GSK3β in glioblastoma. Oncotarget. 2016; 7(48):79584–95. https://doi.org/10.18632/oncotarget.12861 PMID: 27792996

30. Gao X, Zhang W, Yuan J, Xu X, He J, Fu C. Association of microRNA101 expression with clinicopathologic features and prognosis in colorectal cancer. Zhonghua wei chang wai ke za zhi = Chinese journal of gastrointestinal surgery. 2015; 18(4):365–9. PMID: 25940181

31. Hiroki E, Akahira JI, Suzuki F, Nagase S, Ito K, Suzuki T, et al. Changes in microRNA expression levels correlate with clinicopathological features and prognoses in endometrial serous adenocarcinomas. Cancer Sci. 2010; 101(1):241–9. https://doi.org/10.1111/j.1349-7006.2009.01385.x PMID: 19891660

32. Schee K, Boye K, Abrahamsen TW, Fodstad O, Flatmark K. Clinical relevance of microRNA miR-21, miR-26a, and miR-26b in colorectal cancer. BMC Cancer. 2012; 12:505. https://doi.org/10.1186/1471-2407-12-505 PMID: 23121918; PubMed Central PMCID: PMCPMC3519622.

33. Maftouh M, Avan A, Funel N, Paolicchi E, Vasile E, Pacetti P, et al. A polymorphism in the promoter is associated with EZH2 expression but not with outcome in advanced pancreatic cancer patients. Pharmacogenomics. 2014; 15(5):609–18. https://doi.org/10.2217/pgs.13.225 PMID: 24798718.

34. Liu C, Sun Y, She X, Tu C, Cheng X, Wang L, et al. CASC2 as an unfavorable prognosis factor interacts with miR-101 to mediate astrocytoma tumorigenesis. Cell Death Dis. 2017; 8(3):e2639. https://doi.org/10.1038/cddis.2017.11 PMID: 28252647.

35. Luo L, Zhang T, Liu H, Lv T, Yuan D, Yao Y, et al. MiR-101 and Mcl-1 in non-small-cell lung cancer: expression profile and clinical significance. Med Oncol. 2012; 29(3):1681–6. https://doi.org/10.1007/s12032-011-0085-8 PMID: 21993632.

36. Zhang S, Yuan W, Tang W, Xu C, Ma J. [Expression of microRNA-100 and its relation with prognosis of colorectal cancer]. Zhonghua Zhong Liu Za Zhi. 2015; 37(8):603–8. 26714601. PMID: 26714601

37. Jansen MP, Reijm EA, Sieuwerts AM, Ruigrok-Ristiti K, Look MP, Rodriguez-Gonzalez FG, et al. High miR-26a and low CDC2 levels associate with decreased EZH2 expression and with favorable outcome on tamoxifen in metastatic breast cancer. Breast Cancer Res Treat. 2012; 133(3):937–47. https://doi.org/10.1007/s10549-011-1877-4 PMID: 22094936; PubMed Central PMCID: PMCPMC387494.

38. Wang L, Zhang X, Jia LT, Hu SJ, Zhao J, Yang JD, et al. c-Myc-mediated epigenetic silencing of MicroRNA-101 contributes to dysregulation of multiple pathways in hepatocellular carcinoma. Hepatology. 2014; 59(5):1850–63. https://doi.org/10.1002/hep.26720 PMID: 24002871.

39. Stiriliacci A, Valeri MC, Sansone P, Caggiano C, Sgromo A, Vittori L, et al. Loss of miR-101 expression promotes Wnt/beta-catenin signalling pathway activation and malignancy in colon cancer cells. J Pathol. 2013; 229(3):379–89. https://doi.org/10.1002/path.4097 PMID: 22930392.

40. Liu N, Zhang L, Wang Z, Cheng Y, Zhang P, Wang X, et al. MicroRNA-101 inhibits proliferation, migration and invasion of human glioblastoma by targeting SOX9. Oncotarget. 2016. https://doi.org/10.18632/oncotarget.13706 PMID: 27911279.

41. Smits M, Nilsson J, Mir SE, van der Stoop PM, Hullemen E, Niers JM, et al. miR-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation, migration, and angiogenesis. Oncotarget. 2010; 1(8):710–20. https://doi.org/10.18632/oncotarget.205 PMID: 21321380; PubMed Central PMCID: PMCPMC3124376.

42. Tian T, Ma MY, Xia Q, Yang Q. MicroRNA-101 reverses temozolomide resistance by inhibition of GSK3 beta in glioblastoma. Oncotarget. 2016; 7(48):79570–81. doi: 10.18632/oncotarget.12861 PMID: 27792996
43. Tang XR, Wen X, He QM, Li YQ, Ren XY, Yang XJ, et al. MicroRNA-101 inhibits invasion and angiogenesis through targeting ITGA3 and its systemic delivery inhibits lung metastasis in nasopharyngeal carcinoma. Cell Death Dis. 2017; 8(1):e2566. https://doi.org/10.1038/cddis.2016.486 PMID: 28102841.

44. Wu B, Lei D, Wang L, Yang X, Jia S, Yang Z, et al. MiRNA-101 inhibits oral squamous-cell carcinoma growth and metastasis by targeting zinc finger E-box binding homeobox 1. Am J Cancer Res. 2016; 6(6):1396–407. PMID: 27429852

45. Zhang X, Schulz R, Edmunds S, Krüger E, Markert E, Gaedcke J, et al. MicroRNA-101 Suppresses Tumor Cell Proliferation by Acting as an Endogenous Proteasome Inhibitor via Targeting the Proteasome Assembly Factor POMP. Mol Cell. 2015; 59(2):243–58. https://doi.org/10.1016/j.molcel.2015.05.036 PMID: 26145175

46. Zhao H, Tang H, Huang Q, Qiu B, Liu X, Fan D, et al. MiR-101 targets USP22 to inhibit the tumorigenesis of papillary thyroid carcinoma. Am J Cancer Res. 2016; 6(11):2575–86. PMID: 27904772