Clinica Chimica Acta 485 (2018) 33–41

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

MiR-139 in digestive system tumor diagnosis and detection: Bioinformatics and meta-analysis

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ARTICLE INFO

Keywords:
miR-139
Digestive system tumors
Bioinformatics
Meta-analysis

ABSTRACT

Background: Accumulating evidence has indicated that microRNAs play important roles in the initiation and progression of digestive system tumors. However, previous studies suggest that the accuracy of miRNA detection in digestive system tumors was inconsistent.

Methods: The candidate miRNAs were obtained from The Cancer Genome Atlas (TCGA). Meta-analysis was performed to evaluate the diagnostic value of these miRNAs in digestive system tumors. Furthermore, the potential target genes of the miRNAs were predicted and assessed with functional analysis.

Results: According to TCGA data, miR-139 was a common biomarker of digestive system tumors. It was markedly reduced in tumor tissues as compared with non-cancerous tissues in digestive system tumors. In the meta-analysis, the pooled diagnostic odds ratio (DOR) and AUC was 57.51 (95% CI: 14.25–232.04) and 0.96 (95% CI: 0.94–0.97), respectively. Furthermore, the overall sensitivity and specificity was 0.89 (95% CI: 0.73–0.96) and 0.91 (95% CI: 0.75–0.97), respectively. The diagnostic value of tissue miR-139 was higher than the diagnostic value of blood miR-139. In particular, miR-139 was a superior marker for distinguishing colorectal cancer.

Conclusion: miR-139 could be a potential biomarker for diagnosis of digestive system tumors especially colorectal cancer.

1. Introduction

Digestive system tumors, including colorectal cancer, gastric cancer, liver cancer, esophagus cancer and pancreatic cancer, are common malignancies causing cancer-related deaths worldwide [1]. According to the latest statistics, digestive system tumors are the most frequently diagnosed cancers. Colorectal carcinoma, stomach cancer, liver cancer and esophageal cancer rank the third, fourth, fifth and seventh in new cases of male malignancies, respectively, while they rank the second, fifth and ninth new cases of female cancers, respectively [2]. Despite the advances in diagnostic and therapeutic approaches, the optimal treatment for digestive system tumors is still surgical resection. In addition, the difficulty in early diagnosis and the high recurrence of digestive system tumors are responsible for their poor prognosis [3–5]. Therefore, accurate early diagnosis of digestive system tumors is in urgent demand.

MicroRNAs (miRNAs) are small single-stranded RNA molecules with ~22 nucleotides in length. They act in a combinatorial manner via binding to the 3′ untranslated regions (UTRs) of mRNA transcripts to negatively regulate target gene expression. Thus, they play an important role in the initiation and progression of cancers [6–8]. miRNAs are considered to be involved in tumorigenesis through modulating cell cycle, DNA repair, proliferation, apoptosis, self-renewal and differentiation of tumor cells [9–11]. For example, miR-21 is a classic research hotspot in multiple cancers [12, 13]. miR-221 and miR-222 act as oncogenic miRNAs by targeting and suppressing PTEN in various solid tumors [14]. Meanwhile, accumulating evidence demonstrates that SNP occurring in miR-196a2 and miR-499 potentially increases the risk of urological cancers [11]. Using miRNAs as novel diagnostic biomarkers and therapeutic targets will provide new opportunities for the diagnosis and therapy of digestive system tumors [7, 15].

Microarray and high-throughput sequencing are emerging technologies that can extensively analyze genomic information. They are broadly used to identify the relationship between microRNA and cancers [16–18]. The information globalization makes it possible for researchers to search for significant differential gene expression in

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https://doi.org/10.1016/j.cca.2018.06.006
Received 19 February 2018; Received in revised form 2 June 2018; Accepted 4 June 2018
Available online 05 June 2018
0009-8981/

diseases through data mining from public databases such as The Cancer Genome Atlas (TCGA) [19]. Subsequently, it is feasible to use bioinformatics analysis to predict dysregulation of miRNAs, potential target genes and signal pathways in various disorders. Therefore, bioinformatics analysis can be a novel tool to identify crucial miRNAs to direct early diagnosis, prognosis and therapeutic design for cancer patients. It is also powerful for understanding the molecular mechanism of miRNAs in cancers [20].

In our study, a systematic expression profiling analysis was conducted based on the gene expression data from TCGA on digestive system tumors. miR-139, which was differentially expressed between the tumor tissues and adjacent normal tissues, stood out to be a common cancer biomarker. To further substantiate the significance of miR-139, we performed an integrated meta-analysis to quantitatively evaluate the effect of miR-139 on the diagnosis of digestive system tumors. Additionally, bioinformatics analysis was also used to predict the target genes of miR-139 as well as their potential roles in several signal pathways. Furthermore, GO enrichment analysis and KEGG analysis of target genes were conducted. Our results suggest that miR-139 is a potential tumor biomarker for the screening and diagnosis of digestive system tumors.

2. Materials and methods

2.1. TCGA data downloading and analysis

High throughput miRNA sequencing data in the project LIHC, PAAD, ESCA, CHOL, STAD, COAD and READ were downloaded from The Cancer Genome Atlas (TCGA) database. miRNA expression profiling was conducted on 375 liver cancer tissues and 50 normal liver tissues, 179 pancreatic cancer tissues and 4 normal pancreatic tissues, 187 esophageal cancer tissues and 13 normal esophageal tissues, 36 cholangiocarcinoma tissues and 9 normal bile ducts tissues, 446 stomach cancer tissues and 45 normal stomach tissues, 619 colorectal carcinoma tissues and 11 normal colorectal tissues, respectively. Because the sample size of project CHOL is limited, we pooled data from project CHOL and LIHC for analysis. The differential miRNA expression between each cancer and corresponding normal tissue was determined using R package “edgeR” [21]. Benjamini-Hochberg method was used to control the false discovery rate (FDR). Differentially expressed miRNAs (DEmiRNAs) were identified using a threshold of $|\text{LogFoldChange}| > 1.5$ and FDR < 0.05.

2.2. Literature search strategy

PubMed, Embase, Web of Science, the Cochrane Library, CNKI and Wanfang database were thoroughly searched for studies (in English or Chinese) related to the role of miR-139 in human digestive system tumors until May 4, 2018. The keywords used in the search were “cancer” or “tumor” or “neoplasm” together with “miR-139” or“microRNA-139” or “miRNA-139” or “hsa-miR-139”. After the search, the published papers were carefully reviewed and additional relevant references were also included.

2.3. Screening criteria

Published papers were selected based on the following criteria: (1) studies are about the diagnostic potential of miR-139 for digestive system tumors; (2) cancer cases are confirmed by histological examination; (3) studies provide diagnostic indices such as sensitivity and specificity, or sufficient information to calculate. Several published papers were excluded because: (1) they are unrelated to the role of miR-139 in the diagnosis of digestive system tumors; (2) there are no available diagnostic test indices; (3) they are not human research; (4) they are reviews, case reports, meta-analysis and letters; (5) they have duplicate references.

2.4. Data extraction and quality assessment

Two independent researchers (Yu-Hui Wang and Hong Weng) extracted the data from each eligible study for analysis, including the first author, published papers date, country, cancer type, sample size, controls, specimens, sensitivity and specificity of the detection. The third reviewer (Jia Ji) joined the discussion to resolve differences. Methodological quality of selected diagnostic studies was assessed by the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies 2) [22].

2.5. Prediction and functional analysis of target genes

Target genes of miR-139 were predicted using two prediction databases including Pictar [23] (Available online: http://pictar.mdc-berlin.de/) and miRDB [24] (Available online: http://www.mirdb.org/), and were further validated in DIANA-TarBase v7.0 [25] database with strong evidence including luciferase reporter assay, WB and RT-PCR (Available online: http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index). Only genes that were confirmed by all three databases were considered target genes. To comprehensively study miR-139, GO [26] enrichment analysis and KEGG [27] analysis were conducted on the target genes using an online tool DAVID (https://david.ncifcrf.gov/, version 6.8). P-value < 0.05 was set as the cut-off criterion.

2.6. Statistical analysis

R package “edgeR” of R language (version 3.2.5) was used to analyze the high-throughput data to determine the differential gene expression. Statistical analysis was performed using the STATA software 12.0 (Stata Corp, College Station, TX, USA) to calculate two-sided P-values. In the meta-analysis, P-value < 0.05 was considered significant. We used the bivariate meta-analysis model to estimate the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odd ratio (DOR) with the 95% confidence intervals (95% CIs). Meanwhile, we plotted the summary receiver operator characteristic (SROC) curve and calculated the area under the curve (AUC) to evaluate the differential diagnosis power. The heterogeneity of multiple studies was assessed by the Cochran's Q test and the inconsistency index (I^2) test [28]. P-value > 0.10 and I^2 value $< 50\%$ suggest acceptable heterogeneity, and a fixed effects model was adopted. In contrast, a random-effects model was adopted to estimate the pooled results. To evaluate the sources of heterogeneity, we further performed subgroup analysis and meta-regression. Furthermore, the publication bias analysis was conducted using Deeks' test.

3. Results

3.1. Hsa-miR-139 as a potential biomarker for the diagnosis of digestive system tumors

Following the screening criteria, 183 DEmiRNAs between liver cancer tissues and normal liver tissues, 6 DEmiRNAs between pancreatic cancer tissues and normal pancreatic tissues, 93 DEmiRNAs between esophageal cancer tissues and normal esophageal tissues, 190 DEmiRNAs between stomach cancer tissues and normal stomach tissues, 448 DEmiRNAs between colorectal carcinoma tissues and normal colorectal tissues were obtained. Among these DEmiRNAs, miR-139 was the only common DEmiRNA in all five digestive system tumors. Meanwhile, further analysis of TCGA data indicated that miR-139 was markedly reduced in digestive system tumor tissues in comparison with adjacent non-cancerous tissues (Fig. 1).