OncoTargets and Therapy

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REVIEW

Circulating microRNAs as promising diagnostic biomarkers for pancreatic cancer: a systematic review

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Abstract: Pancreatic cancer (PC) is one of the most common forms of malignant tumors and causes of tumor-related death worldwide. The current prognosis of PC still remains poor due to the lack of effective early detection method. Recently, there is strong support that circulating miRNAs can be used as biomarkers for early detection of various cancers, including PC. The purpose of this review is to provide an overview of previous published studies on circulating miRNAs in plasma/serum for early detection of PC and summarize their diagnostic value. PubMed, Embase and Web of Science were systematically searched for eligible studies on circulating miRNAs for PC detection. Overall, 29 studies published between 2009 and 2018 evaluating 51 individual miRNAs (no *P*-value exceeding 0.05) and 13 miRNAs panels were included. Generally, the diagnostic performance of circulating miRNAs for PC detection was strong, with both the sensitivity and specificity of 36% individual miRNAs and 40% miRNAs panels exceeding 80%. Moreover, two promising miRNA panels were discovered and verified externally with all AUC values exceeding 0.95. Therefore, circulating miRNAs may hold potential to be used as noninvasive diagnostic biomarkers for PC, but large-scale studies are still needed to validate the promising miRNAs and optimize the miRNA panels. Since, the tremendous heterogeneity of studies in this field hampers translating miRNA markers into clinical practice, miRNA analytical procedures are also needed to be standardized in the future.

Keywords: pancreatic cancer, early detection, circulating microRNAs

Introduction

Pancreatic cancer (PC) is one of the most malignant tumors worldwide. The morbidity is projected to grow at a rate of 3% per year in males in the United States,[1] and is predicted to become the second leading cause of total cancer-related death before 2030.[2] Currently, radical resection is always the most effective curative option for patients with localized and regional PCs.[3] However, most PC patients are diagnosed with major vascular invasion or distant metastasis when radical resection is usually not available.[4] Consequently, early diagnosis and effective screening of high-risk populations for PC is a valid approach to improve prognosis. Traditional PC imaging tests have drawbacks that are often not suitable for PC screening: computed tomography (CT) has radiation exposure and a high false positive rate;[5,6] magnetic resonance (MR) is expensive and prone to misdiagnosis because of its thicker scanning layer;[7,8] endoscopic ultrasound (EUS) is generally less tolerant, has certain risks, and is limited by technical difficulties.[9]

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DovePress
http://doi.org/10.2147/OTT.S207963

In clinical, several serological biomarkers are widely used for PC diagnosis and prognosis evaluation, such as CA199, CA50, CEA, and CA242, but are usually negative in smaller pancreatic tumors, and show poor specificity for PC detection due to being overexpressed in many other diseases, such as gastroenteric tumors, bile duct cancer, and pancreatitis.[10–13]

In recent years, liquid biopsy based on microRNAs (miRNAs) has become a popular research field for the early diagnosis of malignancies. MicroRNAs are highly conserved, small noncoding RNA species of 17–25 nucleotides in length[14] and remarkable stable in tissue, saliva, urine, serum, plasma, and exosomes.[15] Approximately 50% of miRNAs are located in tumor-related regions.[16] Aberrantly expressed miRNAs profiles were found in plasma/serum of PC patients and many PC-related circulating miRNA candidates/panels, have been identified for PC detection with high diagnostic efficiency. Several studies have even identified abnormally expressed exosomal miRNAs in plasma specimens of PC patients, suggesting that exosomal miRNA may also be useful for PC diagnosis.[17–19] Two recent prospective studies[20,21] demonstrated that the closer the recruitment time to PC occurrence, then the higher the diagnostic value of miRNAs, which offers evidence for circulating miRNAs as noninvasive diagnostic markers for early stage PC. The purpose of this systematic review is to provide an overview of published studies on circulating miRNAs for early detection of PC, and to summarize their diagnostic performance.

Methods

This review was implemented in accordance with a predefined protocol, and follows the PRISMA statement for systematic reviews and meta-analysis of priority reporting items.[22]

Literature search strategy

A systematic literature search was performed to identify studies assessing circulating miRNAs as biomarkers for detection of PC. We searched PubMed, ISI Web of Knowledge, and EMBASE databases for eligible articles until June 28, 2018. The combination keywords were as follows: ([pancreatic OR pancreas] AND [cancer OR carcinoma OR neoplasm OR tumor OR malignancy OR adenocarcinoma OR adenoma] AND [microRNA* OR miRNA* OR miR*] AND [detection OR diagnosis OR biomarker OR marker OR sensitivity OR specificity OR area under the curve] AND [blood OR serum OR plasma]). Duplicate publications were removed.

Eligibility criteria

Only articles written in English were included in this review. Non-original articles such as reviews and conference abstracts were excluded because of insufficient information reported regarding the diagnostic performance of miRNA markers. We required studies that reported relevant information on the diagnostic performance of miRNA markers for human PC detection as well as the sample sizes used in the studies. Studies using treated cases before sampling or disease controls were further excluded.

Data extraction and statistical analysis

Two investigators (EJ and HY) independently filtered the relevant studies against the above-mentioned criteria. Information on first author, publication year, country, sample size, mean or median age, male proportion, specimen type, PC stage, miRNA and/or miRNAs panels investigated, diagnostic related indicators (sensitivity, specificity, AUC), and *P*-value were extracted by the two investigators independently. MicroRNAs with *P*-value greater than 0.05 were ruled out. Any inconsistency was resolved by further review and discussion among the authors. MiRbase was used to check and unify the same miRNA with different names (http://www.mirbase.org/). Mean or median age, and male proportion of included studies were calculated using statistical software R (version 3.4.3, R Foundation, Vienna, Austria) if relevant information was not reported but raw data was available.

Quality assessment of the included studies

The two investigators independently assessed the quality of the included studies using QUADAS-2 (quality assessment tool for diagnostic accuracy studies)[23] included in the Review Manager software (version 5.3.5, Cochrane Collaboration, Copenhagen, Denmark) package.[24] QUADAS-2 is used to evaluate the risk level of bias, which mainly consists of four components: (1) patient selection; (2) index test; (3) reference standard; and (4) flow and timing. The first three components also evaluate clinical applicability. Based on the answers to signaling questions included in each component, the risk level of bias is judged as "low", "high" or "unclear", and the clinical applicability is judged as "low", "high" or "unclear". Any disagreement, such as inconsistent answers

to the questions, was settled by further discussion between the two investigators.

Results

Literature search result

The initial literature search yielded 903 articles according to the aforementioned retrieval strategy (Figure 1). After removing 294 duplicates, we looked through the titles and abstracts of the remaining 609 articles and further excluded 557 articles based on the exclusion criteria. The remaining 52 articles went through full-text reading, of which 23 articles were excluded for the following reasons: 13 using disease controls, three recruiting treated cases before specimen collection, and seven studies not reporting sensitivity, specificity or AUC values. Finally, 29 studies[17-21,25–48] published between 2009 and 2018 were eligible for inclusion in

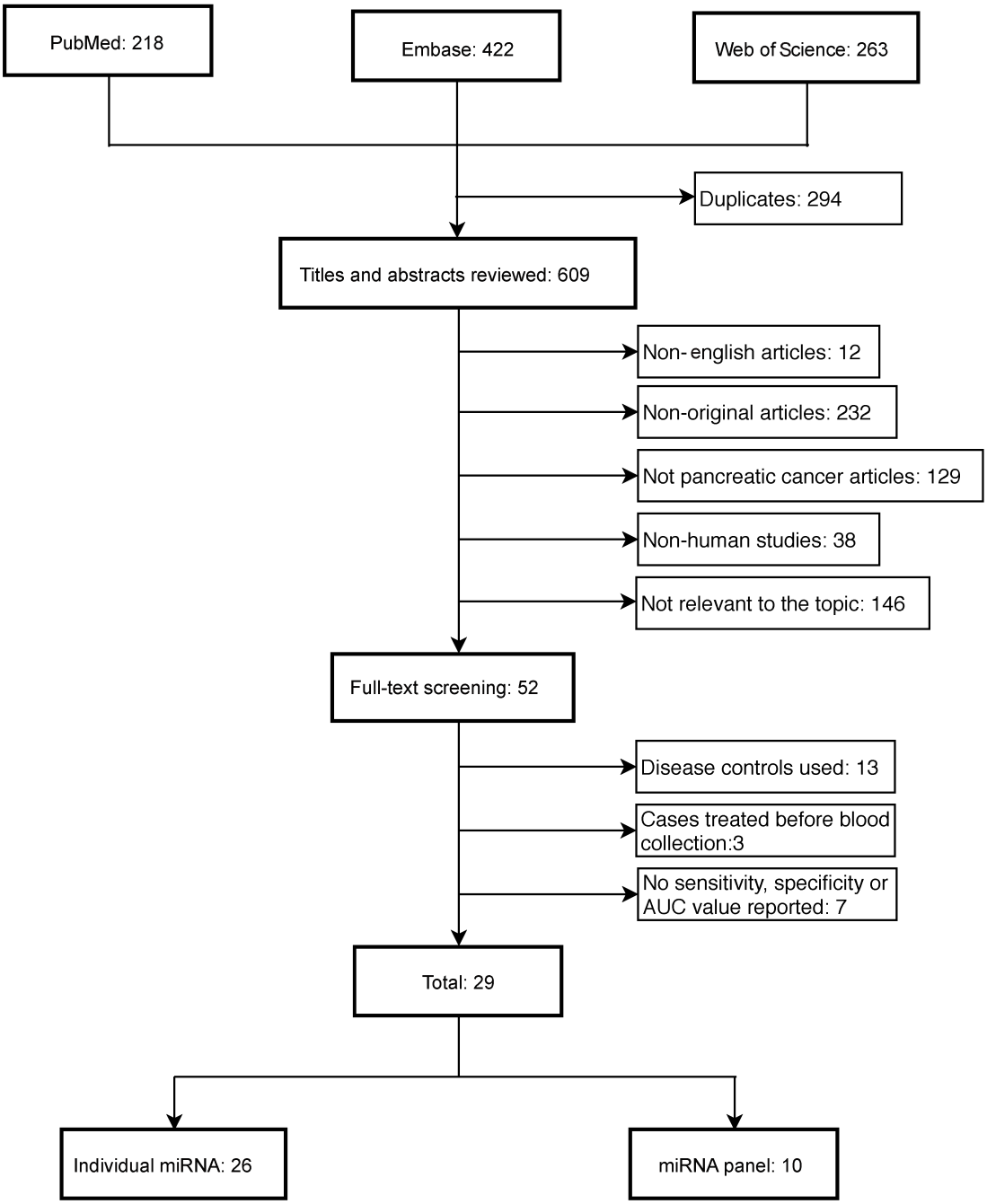

Figure 1 Overview of the literature search process (up to 28th of June 2018).

this systematic review, and used to evaluate the diagnostic performance of circulating miRNAs for PC.

Study quality and characteristics

QUADAS-2 was carried out for the 29 included studies for quality assessment (Figures S1 and S2). High risk bias was found in seven studies (24%) in the patient selection domain, and unclear risk bias was found in 13 studies (45%) in the index test domain. For applicability concerns, 18 studies (62%) displayed high concerns in patient selection domain, and 13 studies (45%) displayed unclear concerns in the index test domain.

Of the 29 included studies, 18 were from East Asia,[17,25–28,30–33,35,37–40,42,44,46,47] nine studies were from Europe and North America,[18–21,34,41,43,45,48] one from Africa,[29] and one from South America.[36] The majority of the included studies were cross-sectional studies, and only two were nested case-control studies[20,21] in which blood samples were taken before diagnosis. The median number (range) of included cases and controls was 56 (9–303) and 30 (6–600), respectively. Among the 29 included studies, four cross-sectional studies[17,18,34,43] reported the diagnostic value of miRNAs for early stage (stage I and II) PC, and two nested case-control studies reported the predicted value of miRNAs for PC risk (Tables 1 and 2).

Fifteen studies analyzed plasma samples for miRNA,[17,19–21,25,27–29,31,32,37–39,43,47] 12 studies analyzed serum samples for miRNA,[26,30,33–36,40–42,44–46] and two studies additionally analyzed exosomes samples for miRNA.[17,18] Twenty-six studies reported 51 individual miRNAs, [17–20,25–33,35–45,47,48] among which six studies carried out external validation.[28,31,32,37,43,45] Ten studies reported 13 miRNAs panels,[20,21,34,36,41–43,46–48] of which three studies performed external validation (Tables 1 and 2).[34,43,46] All included studies used quantitative real-time polymerase chain reaction (qRT-PCR) to detect miRNAs concentrations. The miRNAs were isolated by different extraction kits among the included studies; six studies[21,25,29,31,41,42] used miRNeasy Kit which has been proven to have a higher extraction efficiency.[49] The normalization methods for the expression of miRNAs were not uniform, with cel-miR-39, U6 snRNA, miR-16 being the three most common reference standards for data normalization (Table S1).

Diagnostic efficiency of miRNAs

The 29 included studies reported a total number of 68 miRNAs with the diagnostic potential for PC, of which, 21 miRNAs were reported in more than two studies. The reported miRNA panels for PC diagnosis contained the number of miRNAs from 2 to 15, with 10 miRNAs appearing in at least two panels (Table S2). Among studies with reported sensitivity and specificity, both exceeded 80% among 14 individual miRNAs (36%) and 4 miRNAs panels (40%) (Figure 2). Twenty-three individual miRNAs and four miRNA panels were externally validated, and diagnostic performance with ≥0.70 AUC was observed in 18 miRNAs and all the four miRNA panels (Figure 3). MiR-21 is the most frequently reported miRNA (Table 3), whose sensitivity ranged from 46% to 100% (median sensitivity 78%), the specificity ranged from 78% to 100% (median specificity 86%), and the AUC values ranged from 0.62 to 1.00 (median AUC value =0.83). In the study by Lai et al.,[19] the sensitivity and specificity of miR-21, miR-10b, miR-30c, miR-181a, and miR-let7a in exosomes all reached 100% (Table 1). Several miRNAs panels showed excellent diagnostic performance for PC;[41,46] the AUC values of 7-miRNA panel (miR-20a, −21, −24, −25, −99a, −185, and −191) in Liu R's study and 2-miRNA panel (miR-196a and −196b) in Slater's study were 0.99, and 1.00, respectively (Table 2).

For early stage of PC, miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, and miR-191 were significantly dysregulated in serum samples of stage I (26 cases) and II (48 cases) PC patients compared to healthy controls in Liu R et al.'s study,[46] with positive detection rates of 96% and 91.7%, respectively. Johansen et al.[34] evaluated the diagnostic efficiency of four miRNAs panels for stage I and II PC (Table 2), and the results showed AUC values of 0.87, 0.86, 0.77, and 0.83. Ganepola et al.[43] investigated a 3-miRNA panel (miR-22, −642b, and −885–5p) for stage II PC and found the sensitivity, specificity, and AUC value were 91%, 91%, and 0.97, respectively. A nested case-control study by Duell et al.[20] explored the risk prediction value of a 7-miRNA panel (miR-10a, −10b, −21, −30c, −106b, −155, and −212) in plasma for PC occurring in ≤5 years, ≤8 years, and ≤12 years, and the results of which showed that the AUC values were 0.73, 0.70, and 0.69, respectively. More recently, Franklin et al.[21] conducted a study which contained both prospective and cross-sectional (PC stage: I-II) parts. The prospective part indicated that the AUC values of a 15-miRNA panel (miR-106b, −574, −34a, −451a, −130b, −26a, −144, −423, −101, −122, −24, −22-5p, let-7d-3p, −197, and −885-5p) for predicting PC occurring in ≤5 years, 5–10 years, and >10 years were 0.60, 0.55, and 0.65, respectively. The cross-sectional part

Table 1 Diagnostic performance of miRNA markers in pancreatic cancer

Study	Country	Cases vs Controls			Specimen	Stage	miRNA	SEN	SPE	AUC	*P*-value
		Number	Age(y)	Male (%)							
Goto, 2018[17]	Japan	32/22	64/58	53/64	Exosomes	I-IV I-IIa II-IV	miR-191 miR-21 miR-451a miR-191 miR-21 miR-451a miR-191 miR-21 miR-451a	72 81 66 67 67 67 79 86 70	84 81 86 84 81 86 79 81 81	0.79 0.83 0.76 0.75 0.74 0.74 0.80 0.86 0.77	0.001 <0.001 0.002 0.032 0.004 0.044 0.001 <0.001 0.002
Hua, 2017[30]	China	103/50	/	60/NA	Serum	I-IV	miR-373	81	84	0.85	/
Imamura, 2017[28]	Japan	100/80	/	52/NA	Plasma	/	miR-107	**82**	**69**	**0.85**	<0.0001[a]
Xu, Y, 2017[18]	USA	15/15	67/48	53/28	Exosomes	I-IIa	miR-1246 miR-196a miR-196b	/ / /	/ / /	0.73 0.81 0.71	0.019 <0.001 0.033
Yu, 2017[25]	China	31/28	49/45	65/64	Plasma	/	miR-21 miR-210 miR-155 miR-196a miR-20a miR-25	/ / / / / /	/ / / / / /	0.85 0.69 0.82 0.79 0.88 0.76	0.000[a] 0.013[a] 0.002[a] 0.000[a] 0.000[a] 0.000[a]
Qu, 2017[26]	China	56/15	52/NA	61/NA	Serum	I-IV	miR-21	77	80	0.78	/
Li, F, 2017[27]	China	87/48	/	58/NA	Plasma	I-IV	miR-221	/	/	0.69	/

(*Continued*)

Table 1 (Continued).

Study	Country	Cases vs Controls			Specimen	Stage	miRNA	SEN	SPE	AUC	*P*-value
		Number	Age(y)	Male (%)							
Lai, 2017[19]	USA	29/6	67/NA	52/NA	Plasma	/	miR-10b	100	100	1.00	<0.001
							miR-21	86	100	0.95	<0.001
							miR-30c	100	100	1.00	<0.001
							miR-106b	97	100	0.98	<0.001
							miR-20a	93	100	0.99	<0.001
							miR-181a	97	100	0.97	<0.001
							miR-let7a	93	100	0.99	<0.001
							miR-122	100	67	0.89	0.003
					Exosomes		miR-10b	100	100	1.00	<0.001
							miR-21	100	100	1.00	<0.001
							miR-30c	100	100	1.00	<0.001
							miR-106b	62	100	0.85	0.007
							miR-20a	83	100	0.95	<0.001
							miR-181a	100	100	1.00	<0.001
							miR-let7a	100	100	1.00	<0.001
							miR-122	93	100	0.99	<0.001
Hussein, 2017[29]	Egypt	35/15	57/41	40/27	Plasma	Ib-IV	miR-22	97	93	0.94	<0.001
							miR-642b	100	100	1.00	<0.001
							miR-885-5p	100	100	1.00	<0.001
Duell, 2017[b20]	Europe[c]	29/29	/	/	Plasma	/	miR-10a	/	/	0.75	/
							miR-10b	/	/	0.76	/
							miR-21-3p	/	/	0.74	/
							miR-21	/	/	0.79	/
							miR-30c	/	/	0.77	/
							miR-106b	/	/	0.74	/
							miR-155	/	/	0.74	/
							miR-212	/	/	0.73	/
Yuan, 2016[31]	China	82/88	59/59	57/49	Plasma	I-IV	miR-21	/	/	**0.81**	<0.001[a]
							miR-25	/	/	**0.66**	<0.001[a]
Sun, 2016[33]	China	126/47	60/61	/	Serum	I-IV	miR-124	/	/	0.98	<0.001[a]

(*Continued*)

Table 1 (Continued).

Study	Country	Cases vs Controls			Specimen	Stage	miRNA	SEN	SPE	AUC	*P*-value
		Number	Age(y)	Male (%)							
Xu, J, 2016[32]	China	156/65	/	61/NA	Plasma	I-IV	miR-938 miR-126 miR-486	**62** **62** **75**	**74** **60** **88**	**0.69** **0.62** **0.86**	<0.0001 0.0044 <0.0001
Deng, 2016[35]	China	303/600	62/49	62/60	Serum	I-IV	miR-25	76	93	0.92	/
Alemar, 2016[36]	Brazil	24/9	62/NA	50/NA	Serum	Ia-IV	miR-21 miR-34a	83 91	78 78	0.89 0.87	0.001 0.002
Miyamae, 2015[37]	Japan	94/68	/	55/NA	Plasma	0-IV	miR-744	**59**	**90**	**0.83**	<0.0001[a]
Komatsu, 2015[38]	Japan	71/67	/	58/NA	Plasma	/	miR-223	62	94	0.83	<0.001[a]
Abue, 2015[39]	Japan	32/30	71/45	69/37	Plasma	I-IV	miR-483 miR-21	/ /	/ /	0.75 0.79	<0.0006[a] <0.0001[a]
Zhang, 2014[40]	China	70/40	/	/	Serum	/	miR-194	54	58	0.57	/
Slater, 2014[41]	Germany	9/10	/	NA/30	Serum	I-IV	miR-196b miR-196a	100 90	78 89	0.86 0.97	/ /
Lin, 2014[42]	China	49/27	62/61	55/56	Serum	I-IV	miR-663a miR-492	86 76	80 70	0.87 0.79	<0.05[a] <0.05[a]
Ganepola, 2014[43]	USA	11/11	68/46	54/54	Plasma	IIa- IIb	miR-22 miR-642b miR-885-5p	**82** **82** **82**	**82** **55** **73**	**0.86** **0.79** **0.84**	0.004 0.02 0.006
Zhao, 2013[44]	China	70/40	/	60/NA	Serum	I-IV	miR-192	76	55	0.63	/

(*Continued*)

Table 1 (Continued).

Study	Country	Cases vs Controls			Specimen	Stage	miRNA	SEN	SPE	AUC	*P*-value
		Number	Age(y)	Male (%)							
Li, A., 2013[45]	USA	41/19	65/44	61/90	Serum	I-III	miR-1290	**88**	**84**	**0.96**	<0.001[a]
							miR-744*	**68**	**53**	**0.69**	0.0187[a]
							miR-628	**75**	**84**	**0.82**	<0.001[a]
							miR-550	**73**	**58**	**0.74**	0.0022[a]
							miR-1825	**63**	**79**	**0.70**	0.0012[a]
							miR-24	**73**	**68**	**0.79**	0.0003[a]
							miR-134	**73**	**68**	**0.80**	0.0002[a]
							miR-146a	**78**	**79**	**0.82**	<0.001[a]
							miR-378	**76**	**79**	**0.81**	0.0002[a]
							miR-210	**73**	**58**	**0.73**	0.0052[a]
							miR-22	**71**	**79**	**0.73**	0.005[a]
							miR-625	**63**	**53**	**0.66**	0.0175[a]
							miR-484	**76**	**63**	**0.78**	0.0005[a]
Liu, J., 2012[47]	China	138/68	62/61	64/66	Plasma	I-IV	miR-16	/	/	0.77	0.000
							miR-21	/	/	0.83	0.000
							miR-155	/	/	0.80	0.000
							miR-181a	/	/	0.86	0.000
							miR-181b	/	/	0.84	0.000
							miR-196a	/	/	0.88	0.000
							miR-210	/	/	0.80	0.000
Wang, 2009[48]	USA	28/19	/	/	Plasma	/	miR-21	46	89	0.62	/
							miR-210	42	73	0.65	/
							miR-155	53	78	0.67	/
							miR-196a	43	84	0.69	/

Note: [a]*P*-value represents the difference of miRNA levels between cases and controls (all other *P*-values represent the statistical significance of AUC values; [b]represents the incidence of Pancreatic Cancer by follow-up 5 years); [c]10 European countries including Denmark (Aarhus, Copenhagen), France, Germany (Heidelberg, Potsdam), Greece, Italy (Florence, Turin, Varese, Naples, Ragusa), The Netherlands (Bilthoven, Utrecht), Norway, Spain (Asturias, Granada, Murcia, Navarra, Guipuzcoa), Sweden (Malmo, Umeå) and the United Kingdom (Oxford, Cambridge); SENs, SPEs, and AUCs in bold fonts represent results from validation set (non-bold fonts represent results without validation).
Abbreviations: SEN, sensitivity; SPE, specificity; AUC, area under the curve; NA, not available.

Table 2 Diagnostic performance of miRNA panels in pancreatic cancer

Study	County	Cases vs Controls			Specimen	Stage	miRNA	SEN	SPE	AUC
		Number	Age(y)	Male (%)						
Franklin, 2018[a21]	Sweden	23/22	64/62	52/55	Plasma	I-II	Panel A	/	/	0.96
Duell, 2017[b20]	Europe[c]	29/29	/	/	Plasma	/	Panel B	/	/	0.73
Johansen, 2016[34]	Denmark	86/44	67/55	57/50	Serum	I-IV	Panel C Panel D	**85** **85**	**67** **71**	**0.84** **0.72**
	Denmark, Germany	153/247	/	/	Serum	I-II	Panel E Panel F Panel C Panel D	86 83 82 86	68 73 60 73	0.83 0.86 0.77 0.87
	Denmark, Germany	11/247	/	/	Serum	I	Panel E Panel F Panel C Panel D	73 55 82 64	68 73 60 73	0.70 0.76 0.74 0.78
	Denmark, Germany	142/247	/	/	Serum	II	Panel E Panel F Panel C Panel D	87 85 82 88	68 73 60 73	0.84 0.87 0.77 0.88
Alemar, 2016[36]	Brazil	24/10	62/NA	50/NA	Serum	I-IV	−21, −34a	/	/	0.89
Slater, 2014[41]	Germany	9/10	/	/	Serum	I-IV	−196b, −196a	100	100	1.00
Lin, 2014[42]	China	49/27	62/61	55/56	Serum	I-IV	−663a, −492	86	80	0.87
Ganepola, 2014[43]	USA	11/11	68/46	54/54	Plasma	II	Panel G	**91**	**91**	**0.97**
Liu, R, 2012[46]	China	95/81	/	/	Serum	I-IV	Panel H	**94**	**93**	**0.99**
Liu, J, 2012[47]	China	138/68	62/61	64/66	Plasma	I-IV	−16, −196a	87	74	0.90
Wang, 2009[48]	USA	28/19	/	/	Plasma	/	Panel I	64	89	0.82

Notes: [a]Represents the study concluded prospective and cross-sectional study and the data extracted from the part of cross-sectional study; [b]represents the incidence of Pancreatic Cancer by follow-up 5 years); [c]10 European countries including Denmark (Aarhus, Copenhagen), France, Germany (Heidelberg, Potsdam), Greece, Italy (Florence, Turin, Varese, Naples, Ragusa), The Netherlands (Bilthoven, Utrecht), Norway, Spain (Asturias, Granada, Murcia, Navarra, Guipuzcoa), Sweden (Malmo, Umeå) and the United Kingdom (Oxford, Cambridge); SENs, SPEs, and AUCs in bold fonts represent results from validation set (non-bold fonts represent results without validation). **Panel A** (15 miRs): −106b, −574, −34a, −451a, −130b, −26a, −144, −423, −101, −122,-24, −22-5p, let-7d-3p, −197, −885-5p; **Panel B:** −10a, −10b, −21, −30c, −106b, −155, −212; **Panel C**: −16, −27a, −25, −29c, −483-5p; **Panel D** (12 miRs): −16, −18a, −24, −27a, −30a, −323, −20a, −25, −29c,-191, −345, −483-5p; **Panel E**: −16, −27a, −30a, −323, −20a, −29c, −483-5p; **Panel F**: −16, −24, −27a, −30a, −323, −20a, −25, −29c, −483-5p; **Panel G**: −22, −642b, −885-5p; **Panel H**: −20a, −21, −24, −25, −99a, −185, −191; **Panel I**: −21, −210, −155, 196a.

Abbreviations: SEN, sensitivity; SPE, specificity; AUC, area under the curve; NA: not available.

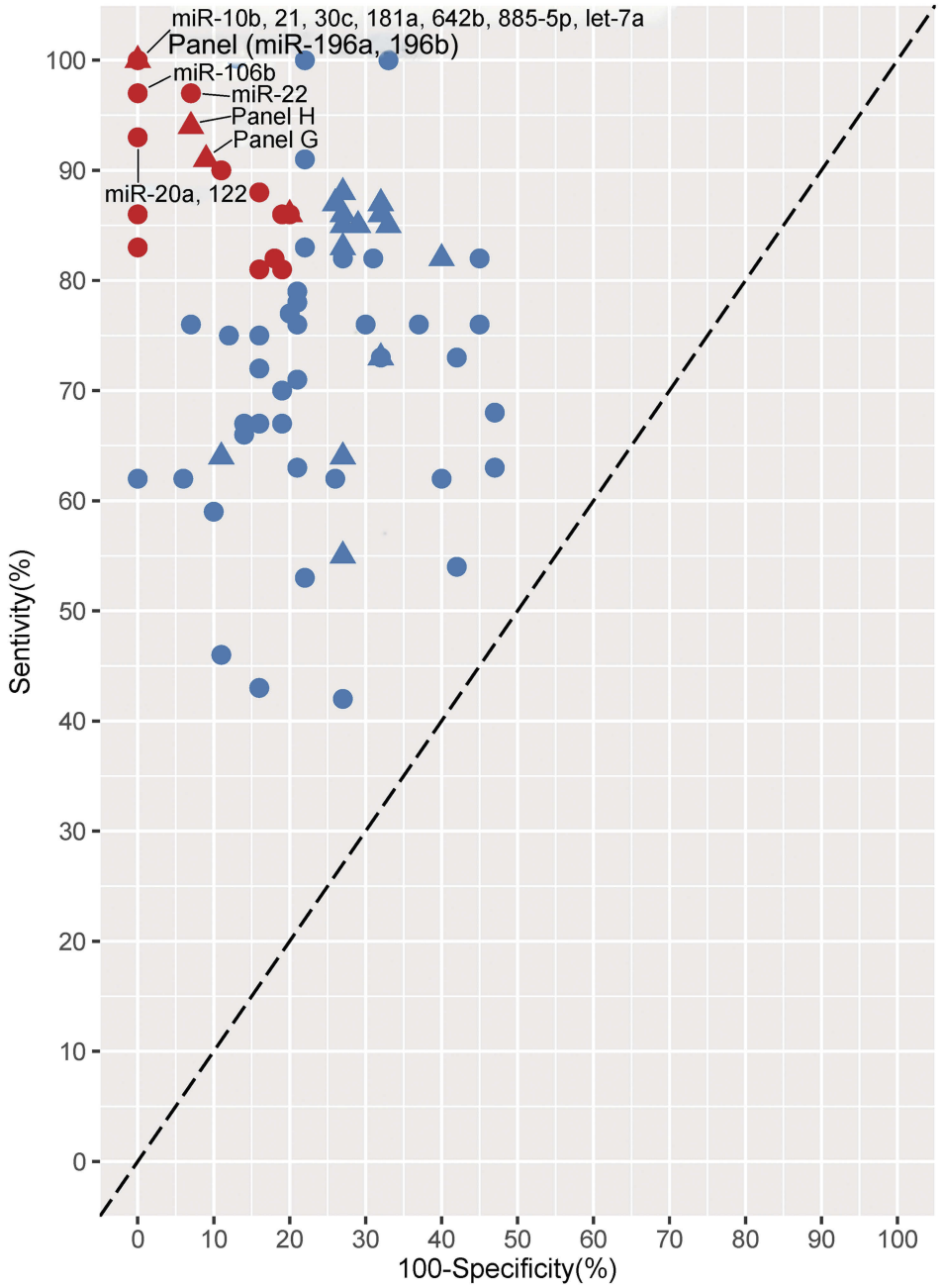

Figure 2 Graphical representation of sensitivity vs specificity of analyzed miRNAs. Sensitivity is plotted on the y-axis while on the x-axis the false-positive rate is presented (100-Specificity). ○ miRNA individual; △ miRNA panel. Plots in red color represent miRNAs or miRNA panels with ≥80% sensitivity and ≥80 specificity. (G): −22, −642b, −885-5p; (H): −20a, −21, −24, −25, −99a, −185, −191.

reported that the AUC value of the above-mentioned miRNA panel for PC at diagnosis was 0.96.

Regulation direction of PC-related miRNA

Of the 21 miRNAs reported more than twice, the dysregulation direction of most miRNAs was consistently upregulated, but the dysregulation direction of three miRNAs (miR-106b, miR-122, and miR-451a) was inconsistent (Table 3). Of which, miR-106b was found to be upregulated in two studies[19,20] and downregulated in one study;[21] miR-122 and miR-451a were reported upregulated in one study[17,21] and downregulated in another study,[19,21] respectively. The inconsistent dysregulation direction of the above-mentioned miRNAs was not found to be significantly related to the specimen types or the stage of PC.[17,19–21]

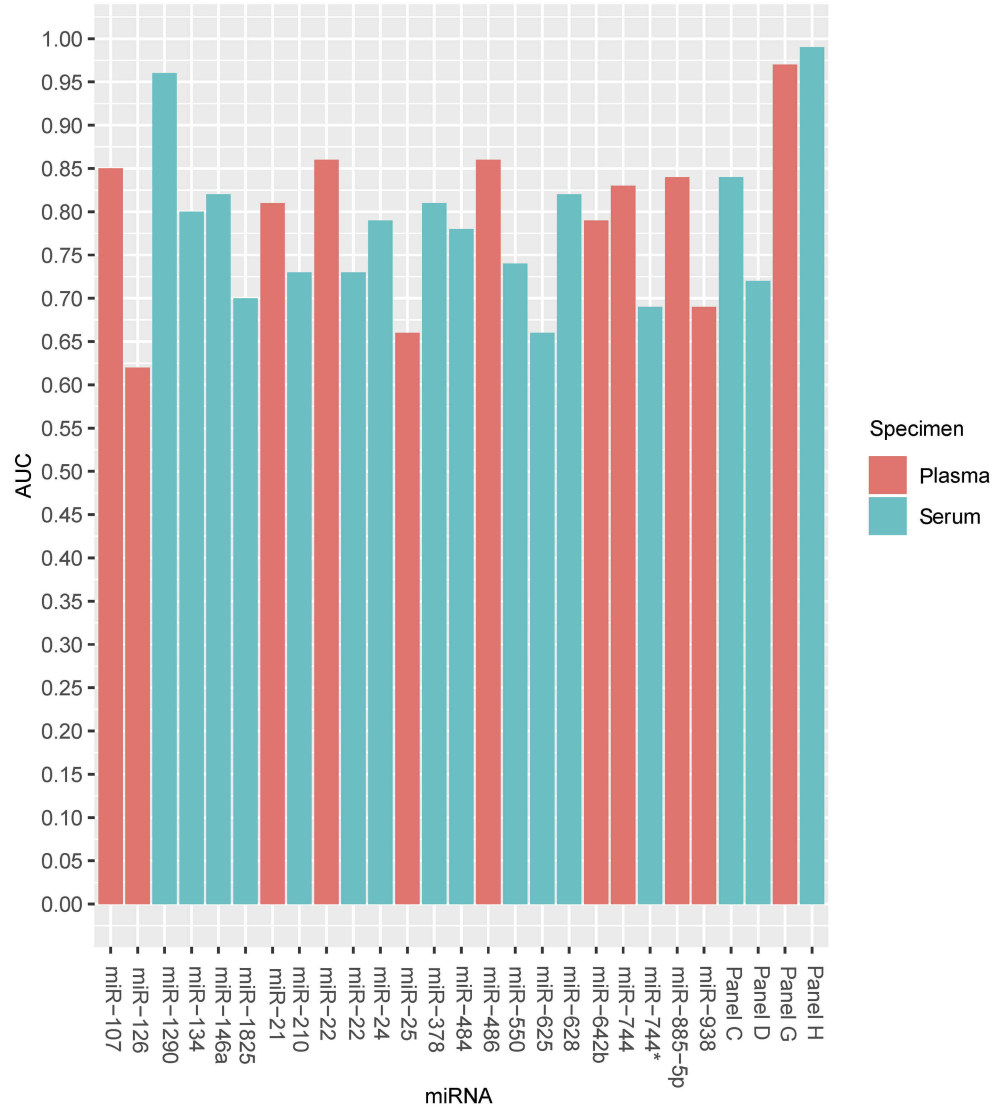

Figure 3 Graphical representation of diagnostic performance of the externally validated miRNAs and miRNA panels in PC. (**C**): −16, −27a, −25, −29c, −483-5p; (**D**) (12 miRs): −16, −18a, −24, −27a, −30a, −323, −20a, −25, −29c,-191, −345, −483-5p; (**G**): −22, −642b, −885-5p; (**H**): −20a, −21, −24, −25, −99a, −185, −191.
Abbreviations: AUC, area under the curve; PC, pancreatic cancer.

Discussion

Our systematic review identified a total number of 68 miRNAs from 29 eligible studies evaluating the diagnostic performance of circulating miRNA for PC detection. Ten studies integrated individual miRNAs into miRNA panels (2–15 miRNAs for each panel) (Table 2). Two promising miRNA panels were discovered and verified in two cross-sectional studies,[43,46] with AUC values all exceeding 0.95. Only two studies[17,34] conducted PC stage subgroup analysis for the diagnostic performance of miRNA. However, due to the lack of sufficient data, stage-specific miRNA for PC is still elusive.

Overall, circulating miRNAs present strong diagnostic value for PC with the sum of sensitivity and specificity of all reported miRNAs or miRNA panels being greater than one (Figure 2). Sensitivity and specificity both exceeded 80% in 36% of individual miRNAs and 40% of miRNA panels (Figure 2). Eleven miRNAs and three panels marked in Figure 2 showed even better diagnostic performance for PC with ≥90% sensitivity and ≥90% specificity. Ganepola et al.[43] used a panel composed of miR-22, miR-642b-3p, and miR-885-5 in plasma for the diagnosis of stage II PC, and the AUC value reached 0.97. Another study by Liu R et al.[46] used a panel consisting of miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, and miR-

Table 3 Summary of studies reporting diagnostic performance of miRNAs for pancreatic cancer (Only miRNAs that have been reported in ≥2 studies)

miRNA	Goto, 2018[17]	Xu, Y., 2017[18]	Yu, 2017[25]	Qu, 2017[26]	Lai, 2017[19]	Hussein, 2017[29]	Franklin, 2018[21]	Duell, 2017[20]	Yuan, 2016[31]	Johansen, 2016[34]	Deng, 2016[35]	Alemar, 2016[36]	Abue, 2015[39]	Slater, 2014[41]	Ganepola, 2014[43]	Li, A, 2013[45]	Liu, R, 2012[46]	Liu, J, 2012[47]	Wang, 2009[48]	Number Of Studies
miR-21	△↑		△↑	△	△↑			△↑	△↑			○↑	△↑				○↑	△↑	○↑	11
miR-196a		△↑	△↑									△		○↑				○↑	○↑	6
miR-25			△↑						△↑	○-	△↑						○↑			5
miR-155			△↑					△↑				△						△	○↑	5
miR-24							○↑			○-						△↑	○↑			4
miR-210			△↑													△↑		△↑	○↑	4
miR-20a			△↑		△↑					○-							○↑			4
miR-885-5p						△↑	○↑								○↑					3
miR-106b					△↑		○↓	△↑												3
miR-22						△↑									○↑	△↑				3
miR-191	△↑									○		○↑					○↑			3
miR-34a							○↑													2
miR-642b						△↑									○↑					2
miR-483					△								△↑							2
miR-181a					△↑			△↑										△↑		2
miR-30c					△↑			△↑												2
miR-10b					△↑															2
miR-122					△↓		○↑			○-										2
miR-16																		○↑		2
miR-451a	△↑						○↓													2
miR-196b		△												○↑						2

Notes: ○ represents miRNAs which are part of a panel; △ represents miRNAs which have only been analyzed individually and not as a part of a miRNA panel; ↑ represents upregulation; ↓ represents down-regulation; - represents no difference in overall study population.

191 in serum for the diagnosis of stage I-IV PCs, and the AUC value reached 0.99. Moreover, the abovementioned results of the two studies have been externally verified (Figure 3). Two nested case-control studies[20,21] showed that circulating miRNAs had certain predictive value for PC occurring in 5 years before diagnosis, but the performance in the PC-free participants is significantly lower compared to the participants being diagnosed with PC. The sample sizes were small in most included studies, and few studies conducted external validation, so the possibility of overestimation cannot be ruled out. Hence, further validation is still indispensable, especially based on large scale PC screening studies.

Some benign diseases and treatment measures may affect the identification of circulating miRNAs. Expression profiles of circulating miRNAs in chronic pancreatitis are different from that of PC, but approximately 4% of chronic pancreatitis cases can develop PC within 20 years.[50] Some studies[51-53] have demonstrated that antineoplastic drugs and chemical regulators could regulate cell proliferation, apoptosis, and angiogenesis, all of which may impact miRNAs expression profiles. Therefore, in order to avoid the effect of disease and treatment on miRNA concentration, we only included healthy controls and PC cases sampled before any therapy.[54–56]

The overlap rates of PC-specific miRNAs are low in the current literature reports, and sometimes the regulation expression of the same miRNA in different studies was inconsistent.[17,19–21] Consequently, screening of circulating miRNAs for PC detection requires attention. Circulating miRNAs concentration could be influenced by many factors, including: (1) population differences;[57] (2) specimen types and volume;[58–60] (3) specimen preservation methods and time;[61] (4) centrifugation steps;[58] (5) miRNA extraction kits;[62] (6) normalization methods.[58,63] The concentration of intracellular miRNAs is higher than that of extracellular miRNAs in blood, so hemolysis can cause a release of intracellular miRNAs, which may contaminate extracellular miRNAs, and affect the identification of PC-specific miRNAs.[64–66] In addition, the blood samples in some studies[18,19,34] were processed with only one-step centrifugation (Table S1), so the residual cell debris, containing high concentration miRNAs, may remain in the supernatant and contribute to the total miRNA content. At present, two-step centrifugation procedure is recommended, and the second step requires high-speed with a centrifugal force of >15000 g to remove maximal cell debris to reduce their effect on the quantification of miRNAs in plasma and serum.[58,59,67] The miRNeasy kit is recommended as it has a higher miRNAs extraction efficiency compared to other kits,[49] but not all studies have applied this extraction kit (Table S1). Different normalization methods could also influence the final quantitative results of circulating miRNAs and could even affect miRNAs expression regulation.[58,68–70] Currently, qPCR quantitative standardization methods of miRNAs concentration are not uniform; cel-miR-39, U6 snRNA, and miR-16 are the most used standardization references in the included studies. The concentration of molecules used as the reference should be very stable among individuals, but there are still some references whose concentration varies between cancer cases and healthy controls, and result in a detection bias of miRNA concentration.[58,69,71–74]

Compared with other types of blood-based markers for PC detection, circulating miRNAs have the following advantages: (1) miRNAs are relatively stable and are insensitive to ribonuclease, acid or alkali environment, long-term room temperature preservation, and repeated freeze-thaw;[68,75] (2) it can be repeatedly used as a non-invasive detection method;[76,77] (3) it has certain predictive value for high PC risk population;[20,21] and (4) the detection of miRNAs is relatively cheap. Other blood markers currently being used to diagnose PC - eg CA199, CA50, and CA242 - are often used to monitor the disease progression,[78,79] but their diagnostic value is relatively low (whose sensitivity and specificity are generally lower than 81% and 80%, respectively).[80–82] In recent years, circulating tumor DNA (ctDNA), as a novel diagnostic marker for PC, has also shown pretty good diagnostic value, the specificity of which can reach 92.6% or even exceed 99.9% in some studies,[83,84] but the sensitivity is usually lower than 75%.[84] In addition to identifying more circulating miRNAs for the formation of diagnostically superior miRNA panels for PC, future research should also focus on exploring possibilities of enhancing diagnostic power by combining miRNA makers with other novel laboratory markers, such as ctDNA markers, in a diagnostic model for early detection of PC.

Conclusion

This review indicates that circulating miRNAs hold the potential of being applied as diagnostic markers for PC. Future studies should pay more attention to the standardization of samples processing procedures and miRNA detection protocol. It is also necessary to verify these PC-specific miRNAs in

larger scale screening studies, and examine the diagnostic efficiency of circulating miRNA for early stage PC.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Protocols of blood miRNA detection

Ref	Specimen	Centrifugation	Extraction	Normalization
Yu, 2017[1]	Plasma	1200g for 10min, 12000g for 10min	miRNeasy Serum/Plasma Kit	miRNeasy Serum/Plasma Spike-In Control (miR-39)
Qu, 2017[2]	Plasma	NA	TRIzol LS Reagent	cel-miR-39
Li, 2017[3]	Plasma	3500rpm for 10min	mirVana PARIS Kit	U6 snRNA
Lai, 2017[4]	Plasma	1000g for 30min	NA	NA
	Exosomes	1000g for 30min, 10000g for 30min, (thaw) 10000g for 30min, 11000g for 2h	Trizol-LS and Direct-zol RNA MiniPrep kit	miR-425-5p
Hussein, 2017[5]	Plasma	NA	miRNeasy serum/plasma Kit	miR-3196
Franklin, 2017[6]	Plasma	NA	miRNeasy Serum/Plasma Kit	NA
Duell, 2017[7]	Plasma	NA	Trizol-LS and Direct-zol RNA MiniPrep kit	miR-425-5p
Yuan, 2016[8]	Plasma	NA	miRNeasy Serum/Plasma Kit	cel-miR-39
Xu, 2016[9]	Plasma	3000rpm for 10min	mirVana PARIS kit	U6 snRNA
Sun, 2016[10]	Serum	NA	NA	U6 snRNA
Johansen, 2016[11]	Serum	2500 g for 10 min	TRI Reagent BD	NA
Deng, 2016[12]	Serum	NA	NA	normalized to the serum volume
Alemar, 2016[13]	Serum	1500rpm for 10 min	mirVana PARIS kit	cel-miR-39
Miyamae, 2015[14]	Plasma	1500rpm for 30min, 3000rpm for 5min, 4500rpm for 5min	mirVana PARIS Kit	cel-miR-39
Komatsu, 2015[15]	Plasma	1500rpm for 30min, 3000rpm for 5min, 4500rpm for 5min	mirVana PARIS Kit	cel-miR-39
Abue, 2015[16]	Plasma	3500rpm for 10min	mirVana PARIS kit	miR-16
Zhang, 2014[17]	Serum	NA	mirVana PARIS kit	U6 snRNA
Slater, 2014[18]	Serum	NA	miRNeasy Kit	miR-24
Lin, 2014[19]	Serum	1500g for 10 min	miRNeasy Kit	cel-miR-39
Ganepola, 2014[20]	Plasma	NA	TRI Reagent BD	miR-3196
Zhao, 2013[21]	Serum	NA	mirVana PARIS kit	U6 snRNA
Li, 2013[22]	Serum	NA	mirVana PARIS kit	miR-16
Liu, R., 2012[23]	Serum	800g for 10min, 10000g for 15min, 12000g for 10min, 16000g for 20min	TRIzol Reagent	normalized to the serum volume

(*Continued*)

Table S1 (Continued).

Ref	Specimen	Centrifugation	Extraction	Normalization
Liu, J., 2012[24]	Plasma	1200g for 10min, 12000g for 10min	TRI Reagent BD	cel-miR-39
Wang, 2009[25]	Plasma	1300g for 10min, 12000g for 30min	Trizol LS reagent	miR-16
Goto, 2018[26]	Exosomes	5000g for 10min	Trizol kit	normalized to the serum volume
Hua, 2017[27]	Serum	3500rpm for 10min	mirVana PARIS kit	U6 snRNA
Imamura, 2017[28]	Plasma	1500rpm for 30min, 3000rpm for 5min, 4500rpm for 5min	mirVana PARIS kit	cel-miR-39
Xu, Y., 2017[29]	Exosomes	2000rpm for 15min, 10000g for 30min, 10000g for 1h	Trizol reagent	cel-miR-54

Table S2 Summary of studies reporting diagnostic performance of miRNAs in miRNA panels with pancreatic cancer (only miRNAs that have been reported in ≥2 panels)

miRNA	Franklin, 2018 [6]	Duell, 2017 [7]	Johansen, 2016 [11]	Alemar, 2016 [13]	Slater, 2014 [18]	Ganepola, 2014 [20]	Liu.R, 2012 [23]	Liu.J.Q, 2012 [24]	Wang, 2009 [25]	Number Of Studies
miR-21		○↑		○↑			○↑		○↑	4
miR-24	○↑		○-				○↑			3
miR-196a					○↑			○↑	○↑	3
miR-106b	○↓	○↑								2
miR-25			○-				○↑			2
miR-155		○↑							○↑	2
miR-34a	○↑			○↑						2
miR-191			○				○↑			2
miR-20a			○-				○↑			2
miR-885-5p	○↑					○↑				2

Notes: ○ represents miRNAs which are part of a panel; ↑ represents upregulation; ↓ represents down-regulation; - represents no difference in overall study population.

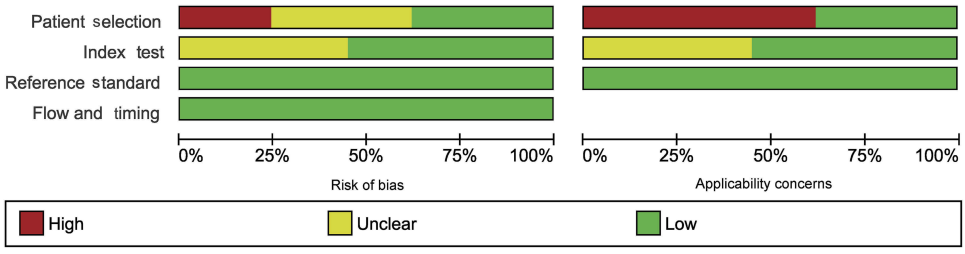

Figure S1 Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies.

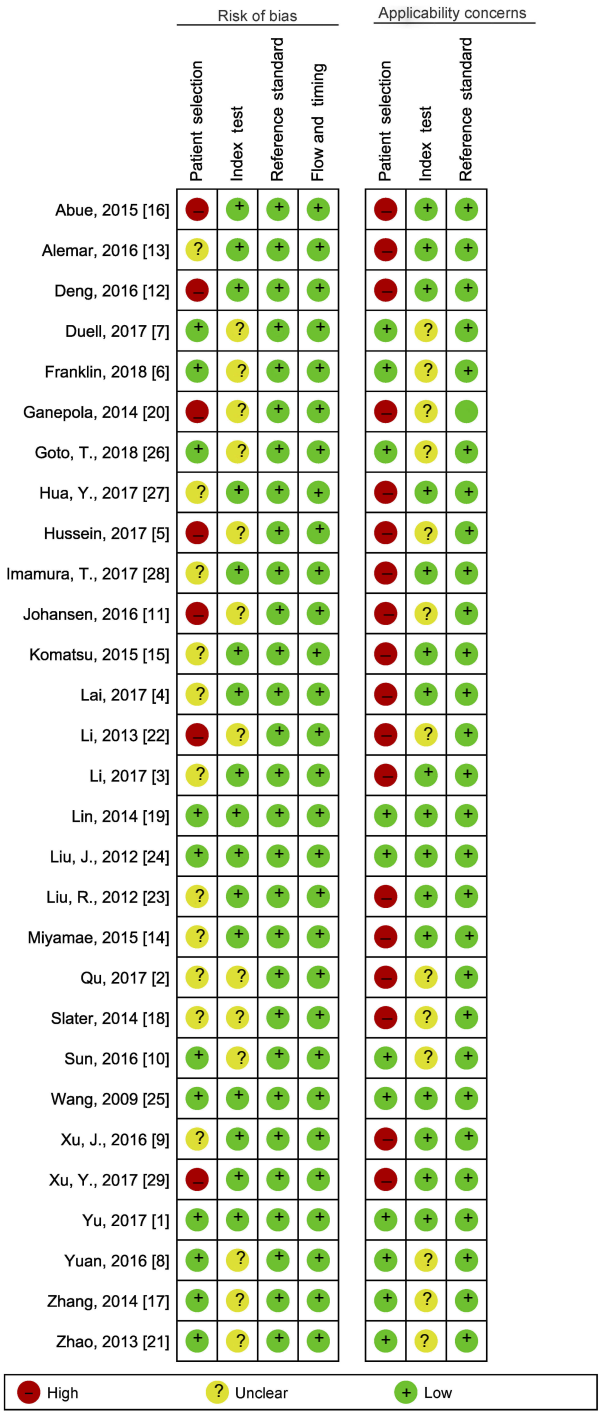

Figure S2 Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study.

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