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Review

Comparison of Prognostic MicroRNA Biomarkers in Blood and Tissues for Gastric Cancer

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Abstract

Gastric cancer (GC) still keeps up high mortality worldwide with poor prognosis. Efficient and non-invasive prognostic biomarkers are urgently needed. MicroRNAs are non-coding RNAs playing roles in post-transcriptional gene regulation, which contribute to various biological processes such as development, differentiation and carcinogenesis. MicroRNA expression profiles have been associated with the prognosis and outcome in GC. MicroRNA prognostic biomarkers have been identified from blood or tissues samples, but with different prognostic features. Understanding the various roles of microRNAs in different sample sources of GC will provide deep insights into GC progression. In this review, we highlight the distinct prognostic roles of microRNAs biomarkers in blood and tissue according to their relationships with prognostic parameters, survival rates and target pathways. This will be useful for non-invasive biomarker development and selection in prognosis of GC.

Key words: blood, gastric cancer, microRNA, prognostic biomarker, tissues

Introduction

Gastric cancer (GC) or stomach cancer (SC) is highly heterogeneous in histological pattern, biological behavior, outcome and biomarkers. It is the fourth common cancer and the second major contributor to cancer mortality worldwide [1, 2]. Although the incidence and mortality of GC declined in the last decades because of the improvement in surgical and adjuvant multimodal treatment approaches, the overall prognosis for advanced GC remains poor and the 5-year survival rate for advanced GC is still low between 10% and 25% [3, 4]. New prognostic biomarkers for GC are extremely needed.

MicroRNAs are small non-coding RNAs (18–25 nucleotides) and changes in the abundance of them reveal promising prognostic associations with major cancer outcome such as clinicopathological features and survival rates. Many works have demonstrated that microRNAs could be as potential biomarkers in different diseases, such as prostate cancer, clear cell renal cell carcinoma, sepsis, gastric cancer and so on [5-8]. In GC, several reports have reviewed the microRNAs as biomarkers for GC from different perspectives. Shrestha et al. and Wang et al. focused on the systematic summarizing microRNA expression profile from 6 studies and 14 studies in gastric cancer tissues, respectively [9, 10]. Li and her colleagues overviewed the epigenetic biomarkers including DNA methylation, histone modification and microRNAs in gastrointestinal cancers [11]. Another review also summarized the epigenetic biomarkers, DNA methylation and microRNAs, but only paid attention to their function as diagnostic markers in body fluids [12]. A meta-analysis was performed on circulating microRNAs in 22 studies and concluded

that miR-21 can be a biomarker for detection of GC with AUC = 0.91 and Q = 0.8466 [13]. Most of the comprehensive reviews summarized the microRNAs function and the role of microRNAs as markers for GC diagnosis, prognosis or therapeutic response [14-20]. But the different prognostic roles of microRNAs in blood and tissues remain poorly understood, which is much more important to the understanding of the clinical roles of these microRNAs in different sample sources.

In this review, we give an elaborate comparison of microRNAs as prognostic biomarkers in blood and tissues. MicroRNA biomarkers in tissues indicate the samples from tissues of patients while microRNA biomarkers in blood indicate the samples from serum, plasma, or blood. We selected the studies by the search criteria "(gastric cancer OR stomach cancer) AND (biomarker* OR marker*) AND (prognos*) AND (microRNA OR miRNA)" from PubMed. We considered only the researches which take the expression of microRNAs as prognostic biomarkers. Since we compared the prognostic features of microRNAs in human blood and tissues, articles about microRNA biomarkers in other body fluid such as gastric juice and other animal samples were excluded. Altogether, as prognostic markers, 14 microRNAs in blood and 36 microRNAs in tissues from 45 studies were compared according to their association with clinicopathological features of GC and survival analysis (See Tables 1 and 2). We also summarized the validated targets of given microRNAs in GC by searching databases, such as, TarBase [21], miR2Disease [22], and miRTarBase [23]. In each database, we just considered the terms which studied gastric cancer of Homo sapiens. In TarBase, the terms with prediction score larger than 0.8 were included. In miRTarBase, we only selected the reports based on 'strong evidence'. This review provides complementary to the previous reviews and essential information that will help discover non-invasive biomarkers in prognosis of GC.

Table 1. MicroRNA biomarkers in blood for gastric cancer.

ID	Sample	Features	Poor Survival	Expression	Reference	Validated Targets
miR-122	96 GC 7 BGC 10 CG 36 HC	Distance metastases	Down	Down	Chen et al.[68]	-
miR-17-5p	79 PRE GC 30 POST GC 6 relapse GC	Differentiation TNM stages	Up	Up[1]	Wang et al.[30]	-
miR-18a	82 GC 65 HC	LNM Pathological grade	Up*	Up	Su et al. [66]	-
miR-20a	79 PRE GC 30 POST GC 6 relapse GC	Differentiation TNM stages	Up	Up[1]	Wang et al.[30]	-
miR-200c	67 GC 15 HC	LNM	Up	Up	Valladares-Ayerbes et al.[64]	BCL2, XIAP[99]
miR-203	154 GC 22 HC	Gender Lymphatic invasion Venous invasion Peritoneal metastasis Distance metastasis LNM Liver metastasis TNM stage	Down	Down	Imaoka et al. [28]	-
miR-21	69 GC	Venous invasion	Up*	-	Komatsu et al.[26]	RECK[92] PTEN[100] Serpini1[101]
miR-21	42 PRE GC 42 POST GC	Differentiation LNM	-	Up[1]	Ma et al.[27]	RECK[92] PTEN[100] Serpini1[101]
miR-218	68 GC 56 HC	Metastasis Tumor stage	Down	Down	Xin et al.[69]	ECOP[102]
miR-221	82 GC 46 dysplasia 128 SG or CAG	Differentiation	-	Up	Song et al.[31]	p27, p57 [103] PTEN[104]
miR-222	114 GC 36 CAG 56 HC	LNM	Up	Up	Fu et al.[65]	p27, p57 [103] PTEN[104] RECK[105]
miR-25	Tissue: 33 GC 33 HC Blood: 70 GC 70 HC	LNM TNM stage	Up	Up	Li et al.[70]	p57 [103] BCL2L11[106] FBXW7[46]
miR-27a	82 GC	Metastasis	Up	Up	Huang et al.[67]	Prohibitin [107]

		Recurrent				APC[108]
miR-376c	82 GC 46 dysplasia 128 SG or CAG	Differentiation	-	Up	Song et al.[31]	-
miR-744	82 GC 46 dysplasia 128 SG or CAG	Differentiation	-	Up	Song et al.[31]	-

Abbreviations and note: BGC: benign gastric ulcer; CAG: chronic atrophic gastritis; CG: chronic gastritis; GC: Gastric cancer; HC: healthy control; LNM: Lymph node metastasis; PRE: pre-operative; POST: post-operative; SG: superficial gastritis; * Disease-specific; [1] Pre-operation.

Table 2. MicroRNA biomarkers in tissues for gastric cancer.

ID	Sample	Features	Poor Survival	Expression	Reference	Validated Targets
miR-107	161 GC 161 ANTT	Invasion LNM Tumor stage	Up	Up	Inoue et al. [41]	CDK6[40] DICER1[41]
miR-1207-5p	23 GC with LNM 23 GC without LNM	LNM Lymphovascular invasion Stromal reaction type TNM stage	-	Down[1]	Huang et al. [53]	-
miR-125a-3p	70 GC 70 ANTT	Invasion LNM Liver metastasis Tumor stage Tumor size Peritoneal dissemination	Down	Down	Hashiguchi et al. [48]	-
miR-125a-5p	87 GC	Invasion depth Liver metastasis Tumor stage Tumor size	Down	Down[4]	Nishida et al. [47]	ERBB2[47]
miR-130a	41 GC 41 ANTT	Metastasis Invasion Proliferation	Up	Up	Jiang et al. [45]	RUNX3[45]
miR-141	36 GC 36 ANTT	Invasion Proliferation Metastasis	-	Down	Zuo et al. [54]	-
miR-142-5p	29 REGC 36 non-REGC	Recurrence	Up	Down[2]	Zhang et al. [76]	-
miR-143	138 GC 30 NTT	Tumor stage Scirrhous type	Up*	Up	Naito et al. [73]	-
miR-145	138 GC 30 NTT	Tumor stage Scirrhous type	Up*	Up	Naito et al. [74]	CDH2[109]
miR-148a	106 GC 106 ANTT	Distant metastasis Organ invasion Peritoneal invasion	Down	Down	Tseng et al. [50]	DNMT1[110] p27[111] ROCK1 [63]
miR-153	80 GC 80 ANTT	Invasion LNM Migration	Down	Down	Zhang et al [55]	-
miR-181c	103 GC	Differentiation Invasive depth Tumor stage	Up	Up[4]	Cui et al. [32]	NOTCH4, KRAS[112] BCL2[113]
miR-192	118 GC 118 ANTT	Tumor sizes Borrmann type	-	Down[3]	Chiang et al [61]	-
miR-192	38 GC 38 ANTT	LNM	-	Up	Xu et al. [62]	-
miR-193b	48 GC 48 ANTT	Differentiation Lauren type Tumor stage Invasion Metastasis	Down	Down	Mu et al. [35]	-
miR-195	45 GC	Recurrence	-	Up[2]	Brenner et al. [75]	-
miR-196a	109 GC 20 ANTT	Invasion depth Serosal invasion Lymphatic invasion LNM Distant metastasis TNM stage Peritoneal seeding Gross type Lauren subtype	Up	Up	Tsai et al. [44]	radixin[44]
miR-196a	48 GC 48 ANTT	Differentiation	Up	Up	Mu et al. [35]	-
miR-196a-5p	58 GC 58 ANTT	LNM TNM stage	Up	Up	Li et al. [58]	-
miR-199a-3p	45 GC	Recurrence	-	Up[2]	Brenner et al. [75]	SMARCA2 [114]
miR-199a-5p	28 GC	Metastasis	-	Up	Zhao et al. [60]	MAP3K11 [115]

	48 GC LNM 25 NTT					Smad4[116] SMARCA2 [114]
miR-196b	109 GC 20 ANTT	Invasion depth Serosal invasion Lymphatic invasion LNM Distant metastasis TNM stage Peritoneal seeding Gross type	Up	Up	Tsai et al. [44]	-
miR-206	98 GC 98 ANTT	Venous invasion LNM Hematogenous recurrence pStage	Down	Down	Yang et al. [51]	CCND2[117]
miR-20b	102 GC 102 ANTT	LNM Distance metastasis TNM stage	Up	Up	Xue et al. [59]	-
miR-21	56 GC without LNM 30 GC with LNM 72 ANTT	Differentiation LNM	Up	Up	Xu et al. [33]	RECK[92] PTEN[100] Serpini1[101]
miR-215	118 GC 118 ANTT	Borrmann type Tumor sizes pT stage	-	Down[3]	Chiang et al [61]	-
miR-215	38 GC 38 ANTT	-	-	Up	Xu et al. [62]	-
miR-217	83 GC 83 ANTT	Differentiation Distant metastasis Invasion Tumor size TNM stag	Down	Down	Chen et al. [36]	-
miR-22	98 GC 98 ANTT	LNM Distant metastasis pStage	Down	Down	Yang et al. [51]	SP1[118]
miR-23a/b	160 GC 160 ANTT	Invasion LNM TNM stage	Up	Up	Ma et al. [43]	IL6R[119]
miR-25	40 GC 40ANTT	Invasion Proliferation LNM Migration	Up	Up	Gong et al [46]	p57 [103] BCL2L11[106] FBXW7[46]
miR-29c	115 GC 115 ANTT	Venous invasion TNM stage	-	Down	Gong et al. [52]	-
miR-335	31 REGC 43 non-REGC	Recurrence	Up	Up[2]	Yan et al. [77]	-
miR-34a	137 GC 137	Lymph node involvement TNM stage Differentiation Tumor recurrence	Down	Down	Zhang et al. [34]	BCL2[120]
miR-375	29 REGC 36 non-REGC	Recurrence	Up	Up[2]	Zhang et al. [76]	PDK1, YWHAZ[121] JAK2[122]
miR-451	45 GC	Recurrence	Up*	Up[2]	Brenner et al. [75]	MIF [123]
miR-520d-3p	120 GC 120 ANTT	Invasion depth LNM Tumor stage	Down	Down	Li et al. [49]	-
miR-630	236 GC 236 ANTT	Invasion LNM Distant metastasis TNM stage.	Up	Up	Chu et al. [42]	-
miR-92a	97 GC	Tumor growth	Up	-	Wu et al. [78]	-

ANTT: adjacent non-tumor tissues; GC: gastric cancer; LNM: Lymph node metastasis; NTT: non-tumor tissues; REGC: gastric cancer with recurrence; non-REGC: GC without recurrence;

* Disease-specific; [1] LNM samples; [2] Recurrence; [3] GC cell line; [4] advanced GC

Clinicopathological features

We summarized and discussed the association between GC clinicopathological features and microRNAs biomarkers in blood and tissues. The clinicopathological features were generally classified into five groups including differentiation, invasion, metastasis, tumor stages and others, these are related to the important cancer 2hallmarks [24, 25] (See Figure 1).

We first compared the number of clinicopathological factors that correlated with the microRNAs in blood and tissues. As shown in Figure 2(a), 42.86% of the blood microRNAs significantly correlated with one and two clinicopathological factors. Only two microRNAs (14.29%) significantly correlated with

three or more than three factors: miR-21strongly correlated with venous invasion, differentiation and lymph node metastasis [26, 27] and miR-203 correlated with gender, invasion, metastasis, TNM stage [28]. Conversely, tissues-based microRNAs tend to correlated with more clinicopathological factors. Nearly 60% microRNAs in tissues were significantly associated with more than three clinicopathological factors and only 25 % microRNAs were associated with one factor.

Differentiation

In histology, tumor was classified as three degrees of differentiation: well, moderate and poor differentiation according to WHO classification [29]. Patients with well-differentiated tumors usually carry a better prognosis whereas patients with poorly differentiated tumors carry a worse prognosis. There are six circulating microRNA biomarkers associated with differentiation and all of them were up-regulated in poor differentiation group, including miR-17-5p, miR-20a, miR-21, miR-221, miR-376c and miR-744 [27, 30, 31], whereas in tissue samples, miR-181c, miR-196a and miR-21, were up-regulated and miR-193b, miR-217 and miR-34a, were down-regulated in poor differential groups [32-36].

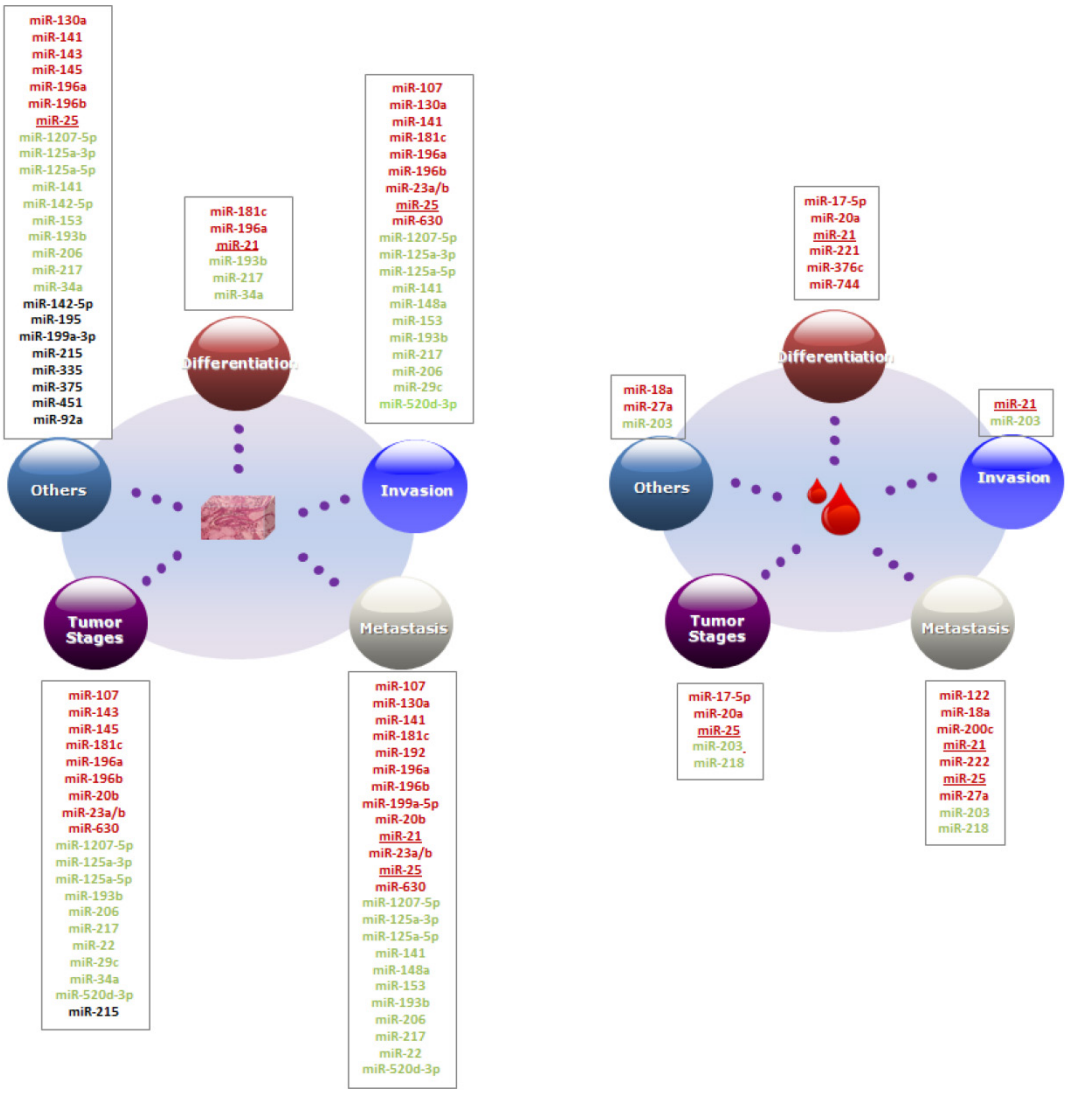

Figure 1. Association between clinicopathological features and microRNA biomarkers. MicroRNAs in red and green denote the up-regulated and down-regulated expression in GC. MicroRNAs in black denote that the microRNAs were differentially expressed between two-sample groups other than GC patient and healthy controls, e.g. between recurrence and non-recurrence groups. microRNAs marked with underline present the microRNAs could be prognostic markers both in tissues and blood.

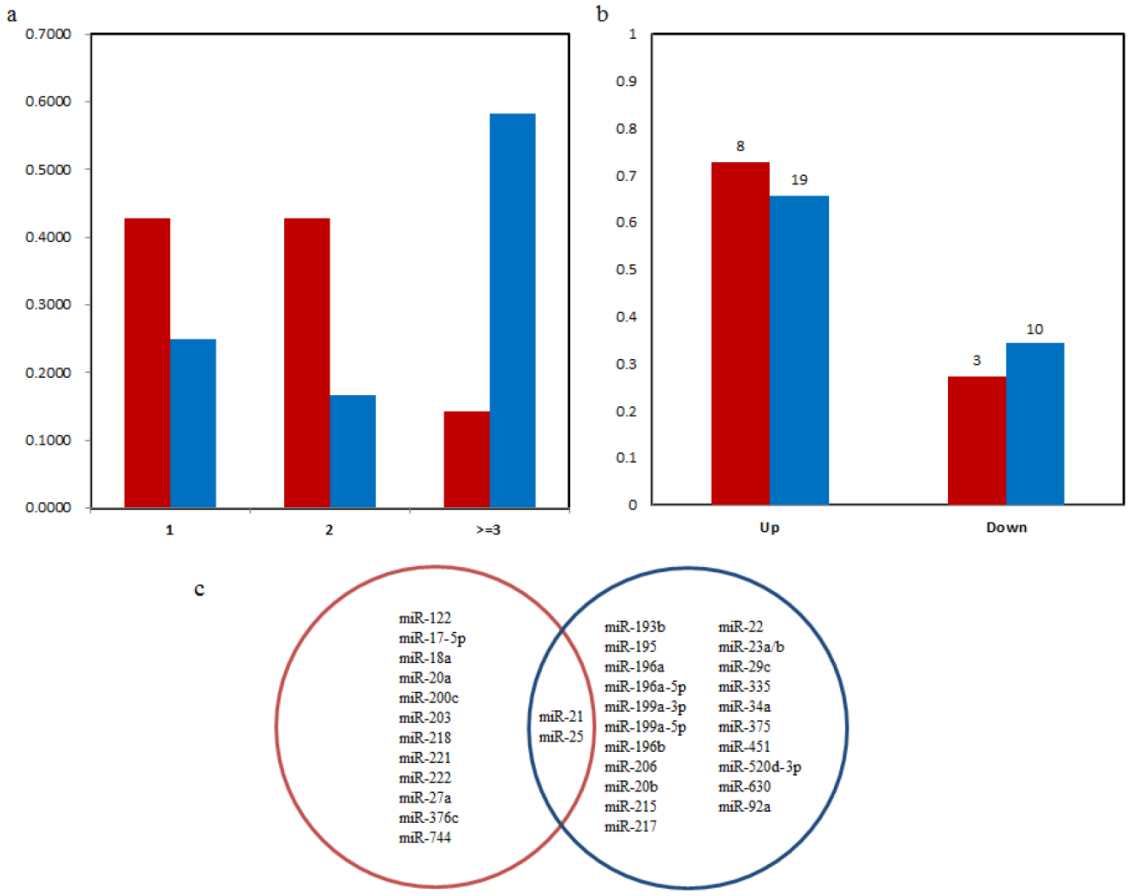

Figure 2. **(a)** Distribution of the number of clinicopathological features correlated with microRNAs. X axis is the number of clinicopathological features. Y axis is the percent of microRNAs in blood or tissues that correlated with different number of features. **(b)** Distribution of expression pattern of microRNA biomarkers from blood and tissues with poor survival of GC patients. Red is the microRNAs from blood and blue is from tissues. Numbers above the bars are the number of microRNA biomarkers in corresponding group. (**c**) The Venn diagram for microRNA prognostic biomarkers in blood and tissue. Blue and red circles represent microRNAs in tissue and blood respectively.

Invasion

GC invasion is a process when tumor cells invade the tumor nearby tissues and vasculature. It is an elementary factor that affects patient survival rate and the most important step of tumor cells dissemination and metastasis in different types of cancer [37-39]. Zhao et al. had reviewed the role of microRNAs in the GC invasion and metastasis [39]. Some of the microRNAs could be as prognostic biomarkers. miR-107, for example, is a potential prognostic biomarker in tissue and inhibits the GC cells invasion by directly targeting the cyclin-dependent kinase 6 (CDK6) [40, 41]. In tissue, expression levels of miR-181c, miR-630 and co-expression of miR-23a and miR-23b were strongly associated with invasion depth [32, 42, 43]. Increased miR-196a/b expression was significantly correlated with serosal, vascular, lymphatic and depth of invasion but in another study miR-196a doesn't have such significantly association with depth of invasion one-way analysis of variance [35, 44] . High expression of miR-130a and miR-25 promoted the migration, invasion and proliferation of gastric cancer cells by targeting RUNX3 and FBXW7, respectively [45, 46].

Decreased expression of eight microRNAs were associated with different types of invasion, e.g. miR-125-3p/-5p and miR-520d-3p with depth of invasion [47-49], miR-148a with organ invasion and peritoneal invasion [50], miR-29c and miR-206 with venous invasion [51, 52]. Patients with down-regulated miR-1207- 5p had more lymphovascular invasion [53]. Down regulation of miR-141 promoted cell proliferation, invasion and migration in AGS GC cell lines [54]. Suppression of miR-153 also promoted GC cell migration and invasion by inhibiting SNAI1-induced epithelial-mesenchymal transition (EMT) [55]. In blood, however, only two microRNAs miR-21 and miR-203 in blood was associated with invasion [26, 28].

Metastasis

Metastasis, a complex and multi-step process, is a primary clinicopathological feature of advanced GC. In metastasis, cancer cells migrate from the primary neoplasm to a distant location and proliferate to form

anther macroscopic tumors [56, 57]. However, the mechanisms that regulate metastasis remain poorly understood.

In tissues, lymph node metastasis (LNM) was significantly related with higher expression of miR-107, miR-181c, miR-196a/b, miR-20b, miR-23a/b, miR-25 and miR-630 [32, 41-44, 46, 58, 59]. Increased miR-196a/b, miR-20b and miR-630 expression were also more detected in GC with distant metastasis. miR-196a/b simulates cell metastasis through direct negative regulation of radixin in GC [42, 44]. High-level of miR-199a-5p expression could promote cell metastasis in GC cells since suppression of miR-199a-5p decreased the metastatic ability in GC cells in vitro and in vivo [60]. Moreover, there are controversial results about miR-196a, -215 and -192. As mentioned above, miR-196a was reported significantly up-regulated in distance metastasis [44] and correlated with lymph node metastasis [58] while no such correlation was found in the subsequent study.[35] . Chiang et al. found there were no significant difference in the expression levels of these miR-215 and -192 between GC tissue and non-tumor tissue and both of them were decreased in the GC cell lines [61]. But Xu and Fan found that miR-215 and -192 levels were increased in GC tissue and related with lymph node metastasis [62].

With regard to down-regulated microRNAs in GC tissues, reduced expression of miR-125-3p, miR-153, miR-206, miR-22 and miR-520d-3p were strongly correlated with lymph node metastasis [48, 49, 51, 55]. MiR-1207-5p was significantly down-regulated in samples with LNM compared with those without LNM [53]. Additionally, expression levels of miR-125-3p, miR-5p, miR-148a and miR-22 were associated with distance metastasis, especially the correlation between liver metastasis and miR-125-3p/-5p [47, 48, 50, 51]. miR-217 was significantly down-regulated in patients with liver metastasis and lung metastasis and promoted tumor progression and metastasis in *vivo* experiment [36]. miR-148a inhabits the GC cell metastasis by reducing the mRNA and protein levels of ROCK1 in GC [63]. miR-141expression level was found to be decreased in primary tumors that subsequently metastasized compared with those that did not metastasize [54].

Several circulating microRNA biomarkers also displayed significantly correlation with metastasis. The expression level of miR-18a, miR-203, miR-200c and miR-222 was significantly correlated with the number of lymph node metastases [28, 64-66]. Increased expression levels of miR-27a in plasma were significantly correlated with poor overall survival for metastatic or recurrent GC [67]. miR-122 was significantly lower in GC with distant metastasis than healthy controls and GC with no distant metastasis [68]. miR-218 was found to be associated with tumor metastasis and decreased in metastasis than non-metastasis and normal serum [69].

Two microRNAs, miR-25 and miR-21 have the same expression pattern in tissue and blood. The miR-25 expression was elevated both in plasma and tissues of GC patients with tumor node metastasis stage or lymph node metastasis [70]. Although miR-21 in plasma of Japan GC patients was not associated with metastasis [26], its expression in plasma of post-operative patients in China was highly associated with lymph node metastasis rate [27] and was higher in tissues of GC patients with lymph node metastasis than those without lymph node metastasis [33].

Tumor Stages

The TNM (tumor-node-metastasis) classification is a widely used cancer staging systems based on the size and extension of the primary tumor (T), nearby lymph nodes involvement (N), and the presence of or otherwise of distant metastatic spread (M). Recently, the seventh edition of the TNM classification was published which introduced many changes for gastric cancer, especially the N stage reclassification [71, 72].

In tissues, high expression of miR-107, -181c, -196a/b, -20b, -23a/b and -630 was more frequently to be detected in GC with advanced tumor stage [32, 41-44, 59]. In particular, as a potential prognostic biomarker of scirrhous type GC miR-143 and -145 expression levels were higher in scirrhous type GC than non- scirrhous type GC and strongly correlated with tumor stage and scirrhous type histology [73, 74]. GC patients with low expression of miR-125-3p, -125-5p, -193b, -206, -217, -22,-29c, -34a, -520d-3p were more often found at advanced tumor stage [34-36, 47-49, 51, 52].

In blood, miR-17-5p, -20a, -203, -25 and -28 were significantly associated with TNM staging classification system. Expression levels of miR-17-5p and miR-20a were only significantly higher in TNM III stage group than I and II group [30] and the level of miR-25 was higher both in TNM III and IV than I and II [70], while miR-218 and miR-203 were decreased in the TNM later stages III and IV [28, 69].

Other clinicopathological features

Beyond the above four main clinicopathological features, microRNA biomarkers are also related with other clinicopathological features, such as GC histological classification, recurrence, tumor growth, tumor size, etc. miR-143 and miR-145 were associated with scirrhous type histology [73, 74]. miR-196a was more frequently detected in diffuse and infiltrative

GC subtype [44]. Brenner et al. found that miR-451, -199a-3p and -195 expression were increased in GC patients with recurrence than patients without recurrence after all the patients received tumor resected surgery [75]. Zhang and colleagues identified that miR-375 and miR-142-5p were differentially expressed between recurrence groups and non-recurrence groups and the combination of these two microRNAs could recognize the above groups both in the training and test samples as a classifier [76]. Recently, high expression level of miR-335 was also detected in high recurrence groups and it was involved in several oncogenic pathways such as TP53, TGF-β and Wnt [77]. Improving miR-90a expression promoted tumor growth in vitro and in vivo [78].

Survival analysis

Prediction of survival is one of the main functions of the prognostic biomarkers. As shown in Figure 2(b), Table 1 and Table 2, we summarize the correlation between patients' survival and expression levels of microRNA biomarkers from blood and tissues. Up-regulated microRNAs were more significantly correlated with poor survival than down-regulated ones, both in blood and tissues. In blood, high concentration of 6 microRNAs (miR-17-5p, -20a, -200c, -222, -25 and -27a) and low concentration of 3 microRNAs (miR-122, miR-203 and -218) were significantly associated with worse overall survival [28, 30, 64, 65, 67-70]. High expression of miR-18a in plasma was associated with shorter both disease-free and disease-specific survival of GC patients [66]. Post-operative patients with increased miR-21 levels had a significantly worse prognosis (disease-specific survival) than those with decreased expression levels [26]. In tissues, increased levels of 13 (miR-107, -130a, -142-5p, -181c, -196a, -196b, -20b, -21, -25, -335, -375, -630 and -92a) and 3 (miR-143, -145 and -451) microRNAs were correlated with overall poor survival and disease specific survival rates separately while decreased expression of 10 microRNAs (miR-125a-3p, -125a-5p, -153, -193b, -148a, -206, -217, -22, -34a and -520d-3p) were correlated significantly with overall poorer survival rates [32-36, 41, 42, 44-51, 55, 59, 73-78] .

Enrichment analysis of targets of prognostic microRNA biomarkers

To further explore different functions of the above microRNA biomarkers in tissue and blood, we performed enrichment analysis of their target genes by Ingenuity Pathways Analysis (IPA®). The targets of microRNAs were collected from experimentally validated database miRecords [79], TarBase [21], miR2Disease [22], and miRTarBase [23] or predicted by computational software tools HOCTAR [80], ExprTargetDB [81], and starBase [82] as reported in our previous research [5]. For the 36 microRNA biomarkers in GC tissue, 3735 target genes were predicted and 199 pathways were significantly enriched by their targets ($p < 0.01$). 2093 targets were predicted for 14 microRNA biomarkers in blood and significantly enriched in 134 pathways ($p < 0.01$). More than half of the enriched pathways by targets of microRNAs in tissue and in blood are overlapped, as see in Figure 3 (a). As shown in Table 3 and Figure 3 (b) and (c), the top 10 significantly enriched pathways by the targets of the given microRNAs in blood and tissue were listed. Among the top 10 enriched pathways, four of them are same as molecular mechanisms of cancer, p53 signaling, chronic myeloid leukemia signaling and Wnt/β-catenin Signaling. In the case of tissue, several in the top ten pathways are related with epithelial cell or tissue such as regulation of the epithelial-mesenchymal transition pathway [83], epithelial adherens junction signaling and germ cell-sertoli cell junction signaling. The function is different for the top 10 pathways that enriched by target genes of blood based microRNA markers. There are not so many pathways related to epithelial cell or tissue, but most of them play important roles in GC such as AMPK signaling, which was reported to induce apoptosis through the mitochondrial apoptotic pathway [84, 85], Wnt signaling, the one is well-known for promoting the development of hematoendothelial cell [86-89], and IGF-1 Signaling which induces epithelial-mesenchymal transition in gastric cancer [90, 91].

We then calculated the percentage of mapped targets of the microRNA in the top ten pathways and listed the top three microRNAs in Table 3. In the case of blood, miR-200c and miR-21 are in the top three in every pathway whereas in tissue, miR-21 is in the top three in eight pathways. This indicates that miR-21 plays a pivotal role in the development of gastric cancer [92, 93]. Additionally, in each of the four overlapped pathways, the top three microRNAs are not the same. Take molecular mechanisms of cancer as an example, miR-200c and miR-23b are in the top three ones in blood and tissue respectively besides miR-21 and miR-25.

Conclusions

In this review, we made a comparison of the prognostic abilities for microRNA in blood and tissues. There are almost twice as many prognostic microRNA biomarkers in tissues as in blood. This may be due to more studies investigating microRNAs from tissues. Another important reason is that microRNAs may be released into the blood selectively [94, 95]. Although some microRNAs display the same expres-

sion pattern in blood and tissues, they correlate with different clinicopathological features. miR-21 for example, is associated with invasion in blood but not in tissue. Most of microRNAs in blood were significantly correlated with no more than two clinicopathological features while microRNAs in tissues were associated with more features. Both in blood and tissues, microRNAs could be strongly associated with survival and most of the microRNAs with high expression level were detected in the poor survival groups.

Table 3. Top 10 significantly enriched pathways by targets of microRNA biomarker from GC blood and tissue.

Source	Ingenuity Canonical Pathways	p-value	Ratio	miRNA
Blood	Molecular Mechanisms of Cancer	1.00E-11	0.39	miR-200c, miR-21, miR-25
	Glucocorticoid Receptor Signaling	6.76E-09	0.37	miR-200c, miR-21, miR-25
	IGF-1 Signaling	2.95E-08	0.48	miR-200c, miR-21, miR-221
	Wnt/β-catenin Signaling	6.46E-08	0.43	miR-25, miR-21, miR-200c
	Pancreatic Adenocarcinoma Signaling	1.12E-07	0.48	miR-21, miR-200c, miR-20a
	AMPK Signaling	1.35E-07	0.40	miR-200c, miR-21, miR-25
	p53 Signaling	2.82E-07	0.46	miR-21, miR-200c, miR-20a
	14-3-3-mediated Signaling	3.09E-07	0.44	miR-200c, miR-221, miR-21
	Chronic Myeloid Leukemia Signaling	5.13E-07	0.46	miR-20a, miR-200c, miR-21
	Myc Mediated Apoptosis Signaling	5.50E-07	0.56	miR-20a, miR-200c, miR-21
Tissue	Molecular Mechanisms of Cancer	3.98E-16	0.59	miR-21, miR-25, miR-23b
	Germ Cell-Sertoli Cell Junction Signaling	7.94E-14	0.69	miR-141, miR-23b, miR-21
	PI3K/AKT Signaling	2.51E-11	0.70	miR-22, miR-23b, miR-195
	Epithelial Adherens Junction Signaling	7.94E-11	0.65	miR-23b, miR-21, miR-141
	p53 Signaling	3.89E-10	0.70	miR-23b, miR-21, miR-141
	Mouse Embryonic Stem Cell Pluripotency	4.17E-10	0.73	miR-143, miR-451a, miR-21
	HGF Signaling	8.32E-10	0.68	miR-21, miR-23b, miR-196b
	Wnt/β-catenin Signaling	2.14E-09	0.61	miR-141, miR-25, miR-34a
	Chronic Myeloid Leukemia Signaling	2.29E-09	0.69	miR-21, miR-34a, miR-22
	Regulation of the Epithelial-Mesenchymal Transition Pathway	2.88E-09	0.62	miR-141, miR-21, miR-22

The overlapped pathways are marked by underline. The ratio is the percentage of the mapped genes divided by the number of total genes in the pathway.

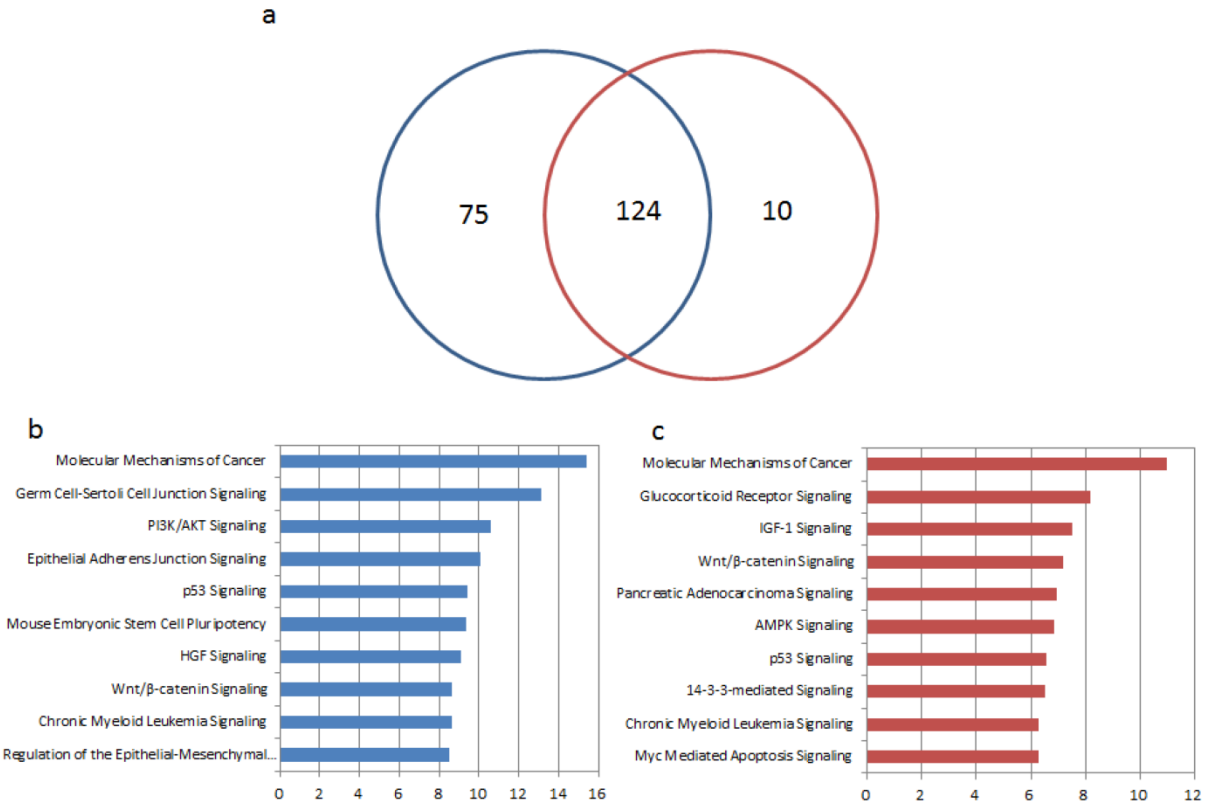

Figure 3 Enrichment analyses of target genes of microRNA biomarkers in tissue and blood. **(a)** The Venn diagram for numbers of significantly enriched pathways. The blue and red circles represent pathways enriched by targets of microRNAs in tissue and blood respectively. **(b)** Top 10 significantly enriched pathways by targets of microRNA biomarker from GC tissue. **(c)** Top 10 significantly enriched pathways by targets of microRNA biomarker from GC blood.

Gastric cancer with a very poor prognosis remains to account for considerable amount of morbidity and mortality in the world. MicroRNAs in blood are promising biomarkers since they are non-invasive and could have a possible clinical application in GC. As well-known that GC is heterogeneous and personalized, the understanding of the roles of microRNAs in GC progression needs further exploration at systems biological level [96-98].

Competing Interests

The authors have declared that no competing interest exists.

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