

Review

## Circulating miR-18a: A Sensitive Cancer Screening Biomarker in Human Cancer

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**Abstract.** *MicroRNAs have been reported to be stably detectable in plasma/serum and to exhibit resistance to endogenous ribonuclease activity because of binding to proteins such as Argonaute-2 and high-density lipoprotein, or being packed by secretory particles such as exosomes. These secretory particles include specific microRNAs and can function as intercellular transmitters. These findings could open-up a new and promising field in the use of circulating microRNAs for cancer treatment. In particular, miR-18a, which is located in the potentially oncogenic miR-17-92 cluster, is a highly expressed microRNAs in several types of cancers. The concentration of miR-18a in plasma/serum of patients with cancer such as esophageal (AUC=0.944), pancreatic (AUC=0.936), hepatocellular (AUC=0.881), colorectal and other types of cancers is much higher than that of healthy volunteers. Such reports provide evidence that circulating miR-18 might be a next-generation biomarker and contribute to cancer screening in non-invasive liquid biopsy, to a clinically-satisfactory degree of sensitivity and specificity.*

MicroRNAs are a group of non-coding small RNAs which are involved in post-translational regulation of gene expression by inhibiting stability and translation of mRNAs (1). Since their discovery in 1993 (2), altered expression of microRNAs has been associated with several diseases, and tumor microRNAs are involved in tumorigenesis and development of various types

of cancers (3-6). Through research, microRNAs have emerged as integral components of the oncogenic and tumor suppressor network, regulating almost all cellular processes altered during tumor development.

Recently, microRNAs have been reported to be stably detectable in plasma and serum (7, 8) and to exhibit resistance to endogenous ribonuclease activity because of their binding to proteins such as Argonaute-2 protein and high-density lipoproteins (9-11), or their being packed by secretory particles including apoptotic bodies and exosomes, in plasma/serum (12, 13). Furthermore, these microRNA-containing secretory vesicles have been found to function as intercellular transmitters (12-14). Based on these findings, the measurement of microRNA expression in plasma/serum could be a reproducible and reliable biomarker. Indeed, accumulating representative reports have suggested the presence of circulating microRNAs and their potential use as novel biomarkers for prostate (7), leukemia (15), oral (16), esophageal (17, 18), pancreatic (19-21), colorectal (22), lung (23), breast (24), and gastric cancer (25, 26). In the present review, we summarize several reports concerning the diagnostic value of the circulating miR-18a, located in the potentially oncogenic miR-17-92 cluster, which is one of the highly expressed microRNAs in several types of cancers. These reports provide evidence that plasma miR-18a might contribute to next-generation cancer screening in non-invasive liquid biopsy to a clinically-satisfactory degree of sensitivity and specificity.

### miR-17-92 Cluster in Cancer

Human microRNA expression profiling studies were performed including cancer tissues in 2005 (5). Several microRNAs were found up-regulated and widespread down-regulation of microRNA expression has been found in human cancer (5, 27-29). In particular, the up-regulation of the miR-17-92 cluster in various types of cancers implied their oncogenic role in tumorigenesis and cancer development (30,

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31). In addition, recent reports have revealed oncogenic mechanisms and correlation between up-regulation and cell proliferation derived either from genome amplification or transcriptional activators such as MYC and E2Fs (4, 32, 33), as well as hypoxia-induced apoptosis caused by p53-mediated repression (34). These findings strongly prompted to execute further investigations under the assumption that circulating microRNAs located in the miR-17-92 cluster could be potentially useful biomarkers in several types of cancers.

### Overexpression of miR-18a in Cancer Tissues and its Significance in Cancer Development

The miR-17-92 cluster consists of seven microRNAs: miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a. miR-18a is one of the most highly expressed microRNAs in the miR-17-92 cluster. miR-18a has been found to be significantly up-regulated in head and neck squamous cell carcinoma (35), esophageal squamous cell carcinoma (ESCC) (18), gastric carcinoma (36), diffuse large B-cell lymphoma (37), urothelial carcinoma of the bladder (38), nasopharyngeal carcinoma (NPC) (39), hepatocellular carcinoma (40), pancreatic carcinoma (20), colorectal carcinoma (41, 42), breast cancer (43) and basal cell carcinoma (44) (Table I). In colorectal cancer (41) and serous ovarian carcinoma (45), patients with overexpression of miR-18a in the cancer tissue had a poorer clinical prognosis. Interestingly, miR-18a was reported to have a pro-proliferative effect on hepatocellular carcinoma cells, but an inhibitory effect on breast cancer cells *in vitro* (46). In breast cancer, the relationship with ER $\alpha$ , identified as a direct target of miR-18a, has been elucidated. miR-18a represses ER $\alpha$  translation directly by binding to its mRNA at the 3' untranslated region (40, 46). Conversely, ER $\alpha$  also binds to c-MYC, and in turn up-regulates the expression of pre-miR-18a in an estrogen-dependent manner (39, 47). In nasopharyngeal carcinoma, miR-18a was reported to negatively-regulate Dicer1 and promote the growth, migration and invasion of NPC cells by regulating Dicer1 expression, which consequently caused global down-regulation of microRNA expression (39). However, whether these miR-18a tumor effects *in vitro* on each cancer cell line is indeed associated with miR-18a function *in vivo* and the expression of primary cancer tissues and peripheral blood remains controversial and unclear.

### Sensitive Cancer Screening with Circulating miR-18a in Human Cancer

Several studies have identified tumor-specific alterations in the plasma/serum nucleic acids of cancer patients, and demonstrated the possibility of using circulating nucleic acids as biomarkers in patients with various types of cancer (48, 49). Some microRNAs have been shown to exist highly stably in

plasma and serum (7, 50). Arroyo *et al.* reported that most microRNAs, including miR-18a, were stable in the plasma by binding the Argonaute-2 protein (9). In the clinical setting, our group was the first to report that circulating miR-18a was stably detectable in plasma of patients with pancreatic cancer (20). In detail, the expression of miR-18a was significantly higher in pancreatic cancer tissues ( $p=0.012$ ) and pancreatic cancer cell lines ( $p=0.015$ ) than in normal tissues and fibroblasts. Plasma concentrations of miR-18a were significantly higher in patients with pancreatic cancer patients than in controls ( $p<0.0001$ ). The value of the area under the receiver-operating characteristic curve (ROC) (AUC) was 0.9369. Moreover, plasma levels of miR-18a were significantly lower in postoperative samples than in pre-operative ones ( $p=0.0077$ ) (20). By a similar strategy, Hirajima *et al.* also reported that circulating miR-18a contributed to cancer diagnosis and monitoring in ESCC (18). Namely, plasma concentrations of miR-18a were significantly higher in patients with ESCC than in healthy volunteers ( $p<0.0001$ ; ESCC patients vs. healthy volunteers (mean $\pm$ s.d.)=11.77 $\pm$ 13.45 vs. 0.73 $\pm$ 0.54 atom mol/ $\mu$ l). The AUC was 0.9449. Surprisingly, the ROC curves to detect early ESCC such as pTis-1 and pStage 0-I showed great AUCs of 0.9479 and 0.9642, respectively. In hepatocellular carcinoma screening, serum miR-18a was significantly higher in patients with HBV-related hepatocellular carcinoma than in healthy controls ( $p<0.01$ ). The AUC was 0.881 with 86.1% sensitivity and 75.0% specificity in discriminating HBV-related hepatocellular carcinoma from healthy controls (51). In colorectal cancer, serum levels of miR-18a in patients with stage III colorectal cancer (52) and plasma level of miR-18a in patients with colorectal cancer (53) was significantly higher than in controls. In gastric cancer, we identified that plasma levels of miR-18a were significantly higher in patients than in controls (data not shown). In patients with retinoblastoma (54) or non-small cell lung cancer (55), significantly higher miR-18a expression in peripheral blood was detected in comparison to controls (Table I). Circulating miR-18a has so far been demonstrated to be a useful biomarker for cancer screening in these types of cancer, particularly, pancreatic cancer and ESCC, with an extremely high AUC (18, 20). However, larger prospective cohort studies might be needed to validate the utility of circulating miR-18a.

### Why Circulating MicroRNAs Are Promising Targets for Cancer Research and Clinical Application?

There have been some reports related to microRNA-mediated intercellular communication because some microRNAs are released into blood from tumor cells. Skog *et al.* reported that tumor-derived microvesicles which contained mRNA, microRNA and angiogenic proteins served as a means of

Table I. Reported miR-18a expression of cancer tissue and peripheral blood in human cancer.

Cancer type	Cancer tissue	Peripheral blood (plasma/serum)	Reference
Head and neck squamous cell carcinoma	Overexpression	Unknown	35
Non-small cell lung cancer	Unknown	Up-regulation	55
Esophageal squamous cell carcinoma	Overexpression	Up-regulation	18
Pancreatic cancer	Overexpression	Up-regulation	20
Hepatocellular carcinoma	Overexpression	Up-regulation	51
Gastric cancer	Overexpression	Unknown	36
Colon cancer	Overexpression	Up-regulation	41, 42, 52, 53
Breast cancer	Overexpression	Up-regulation	43
Nasopharyngeal carcinoma	Overexpression	Unknown	39
Urothelial carcinoma of the bladder	Overexpression	Unknown	38
Basal cell carcinoma	Overexpression	Unknown	44
Retinoblastoma	Overexpression	Up-regulation	54

delivering genetic information, as well as proteins to recipient cells in the tumor environment (56). Kosaka *et al.* also showed that miR-146a, which is a tumor-suppressive microRNA in prostate cancer, significantly knocked-down the target ROCK1 protein expression and reduced cell proliferation in a recipient prostate cancer cell line (12). Their recent report suggested that a variety of tumor-suppressive microRNAs were secreted by a normal adult prostatic epithelial cell line, and among these secretory microRNAs, miR-143 inhibited growth exclusively in cancer cells, both *in vitro* and *in vivo* (57). Regarding miR-18a, the abundance of plasma circulating miR-18a in patients with cancer and its detailed meaning remain unclear. These issues are currently under evaluation and will be reported in the near future.

### Important Issues to Be Resolved for Clinical Application of Circulating MicroRNAs

Several cautionary notes regarding blood-based microRNA research were made that circulating microRNAs could be derived from hematocytes in the peripheral blood of both patients with cancer and healthy individuals (58-60). In a previous study, we evaluated the correlation between plasma miR-18a concentrations and hematocytes of peripheral blood in patients with ESCC (18). As a result, there was no significant association between plasma miR-18a concentrations and any type of peripheral hematocyte data. To apply this theory to other types of microRNAs, we performed further analyses and identified several candidate microRNAs which might be derived from hematocytes in peripheral blood and affected by hemolysis (data not shown). miR-18a was not included in these candidates. In future clinical applications of each microRNA, the correlation of hematocytes with peripheral blood should be investigated in order to avoid the effect of hemolysis on the results.

In conclusion, this review clearly demonstrates that plasma miR-18a could be a useful blood-based biomarker for screening patients with cancer and monitoring tumor dynamics. The abundance and stability of plasma miR-18a indicate that this blood-based biomarker has great potential for clinical use in liquid biopsies.

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