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Circulating MicroRNA in Digestive Tract Cancers

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For many decades, cell-free nucleic acids have been known to be present in peripheral blood. Several studies have identified tumor-specific and/or tumor-associated alterations in the circulating nucleic acids of patients with various cancers. In recent years, cell-free microRNA (miRNA) have been stably detected in the plasma and serum, like other molecules; their presence in the blood has attracted the attention of researchers due to their potential use as valuable blood biomarkers.[1]

MiRNAs are short, noncoding RNAs that play important roles in various physiologic and developmental processes. The mature miRNAs are produced from long primary transcripts through 2 sequential cleavage steps. The long primary miRNA transcript is cleaved by the Drosha complex in the nucleus, generating intermediate precursor miRNA. Precursor miRNA is transported by exportin-5 from the nucleus into the cytoplasm, and then subjected to further cleavage by a Dicer RNAase III enzyme, generating a short double-strand miRNA. One strand (guided strand) of mature miRNA is then incorporated into the RNA-induced silencing complex and subsequently hybridize to the 3′-untranslated region of their target mRNAs to repress translation or degrade these mRNAs. Thus, a single miRNA can influence the expression of hundreds of genes and allow them to function in a coordinated manner. Therefore, miRNAs have been implicated as key molecules in all cellular processes. Numerous studies have shown that alterations in miRNA expression correlate with various diseases, including the development and progression of cancer, and some miRNAs can function as oncogenes or tumor suppressors. These findings have opened up a new and interesting field in the diagnosis of cancer and the treatments of cancer patients.

Mitchell et al[2] first demonstrated that circulating miRNAs had the potential to be new biomarkers in patients with solid cancers. In recent years, several papers have demonstrated that circulating miRNAs can also be detected in the peripheral blood of patients with digestive tract cancers. Although the origins and physiologic functions of cell-free miRNAs in the blood remain to be fully elucidated, a noninvasive assay for miRNAs should be developed to exploit these molecules as potential diagnostic and prognostic biomarkers. This assay undoubtedly contributes to an improvement in the clinical outcomes of cancer patients. In this article, we review the current state of biological and clinical research regarding circulating miRNAs of digestive tract cancer patients and discuss the future perspectives.

The Biology of Circulating MiRNAs

It has been theorized that the necrosis and the apoptosis of tumor cells are the main sources of cell-free nucleic acids in the plasma and serum. However, several recent studies have demonstrated that extracellular nucleic acids, especially miRNAs, occur not only through cell lysis but also through active secretion.[1,3–5] Cell-derived endogenous miRNAs are present in the blood in a remarkably stable form that is protected from endogenous RNase activity. In contrast, synthetic exogenous miRNAs are rapidly degraded when added directly to the plasma.[2] Kosaka et al[4] clearly demonstrated that a subset of miRNAs was packaged into exosome vesicles and released through a ceramide-dependent secretory mechanism. Arroyo et al[6] systematically investigated circulating miRNAs in the plasma and serum using differential centrifugation and size-exclusion chromatography techniques. This group demonstrated ≥2 populations of circulating miRNAs in the plasma and serum and discovered agonaute-2, a key effector protein involved in miRNA-mediated silencing as an miRNA carrier in the blood.[6] In addition, high-density lipoprotein has been described as an alternative transporter of extracellular miRNAs in human plasma.[7] All circulating miRNAs, regardless of whether they are incorporated into protein complexes and/or cell-derived microvesicles, seem to be adequately protected against the degradation caused by the abundant RNases in human plasma and serum. Indeed, the extracellular miRNAs in the plasma and serum are extremely stable under severe conditions, such as extended storage and exposure to multiple freeze–thaw cycles.[2]

Regarding the composition of circulating miRNAs, Pigati et al[5] investigated the difference between extracellular and cellular miRNAs using epithelial cell lines and

Abbreviations used in this paper: AUC, area under the receiver operating curve; CP, chronic pancreatitis; CRC, colorectal cancer; ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; HCC, hepatocellular cancer; miRNA, microRNA; PC, pancreatic cancer; PCR, polymerase chain reaction; RT, reverse transcriptase; RT-qPCR, reverse transcriptase-quantitative polymerase chain reaction.

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concluded that the release of miRNAs did not necessarily reflect the abundance of miRNAs in the cell of origin. Kosaka et al[4] also demonstrated that some specific miRNAs were expressed to a greater extent in cell-derived exosomes compared with their donor cells.[4] Moreover, other groups demonstrated that the non–vesicle-associated miRNA profiles within protein complexes were distinctly different from the purified, exosomes-associated miRNA profiles.[6] These findings indicate that intracellular miRNAs are exported to the extracellular environment through a selective secretion mechanism.

Interestingly, recent studies have demonstrated that extracellular miRNAs not only circulate in stable forms, but can also be incorporated into other surrounding and distant recipient cells in which they fulfill distinctive functions.[8–13] Rechavi et al[9] demonstrated that functional signals spread across cell boundaries between immune cells in a contact-dependent manner. Pegtel et al[12] reported that Epstein–Barr virus miRNAs were secreted from infected B cells and were present in both the circulation and noninfected non-B cells. This group also demonstrated that miRNAs were transferred from infected to noninfected cells in vivo and were functional (upon transfer via exosomes) in primary monocyte-derived dendritic cells. Other groups have shown that miR-126 in apoptotic bodies derived from atherosclerotic endothelial cells induces the CXCL-12–dependent vascular protection process in recipient vascular cells.[10]

There have also been some reports regarding miRNA-mediated intercellular communication in a neoplastic environment. Skog et al[8] reported that microvesicles that housed miRNAs derived from glioblastomas were taken up by and fulfilled functions in human brain microvascular endothelial cells in culture. Kosaka et al[4] also demonstrated that miR-146a, which is a tumor-suppressive miRNA in prostate cancer, significantly knocked down the target ROCK1 protein expression and decreased cell proliferation in a recipient prostate cancer cell line.[4] Their subsequent paper demonstrated that a variety of tumor-suppressive miRNAs were secreted by a normal adult prostatic epithelial cell line, and among these secretory miRNAs, miR-143 could inhibit growth exclusively in cancer cells both in vitro and in vivo.[13] Other groups found that the let-7 miRNA family was abundant in the extracellular fractions derived from a metastatic gastric cancer (GC) cell line, but not those derived from a low metastatic parental cell line, and it has been speculated that some cancer cells maintain their oncogenesis via specific extracellular miRNAs.[14] On the other hand, exosomes released from neoplastic cells have been reported to suppress immune surveillance, and cell-free miRNAs contained within the exosomes may be responsible for the immunosuppression systems.[15] These findings support the presence of miRNA-mediated intercellular communication in the normal cellular environment and the tumor environment (Figure 1).

Challenges for Clinical Blood Samples

Several methods can be used for extracting miRNAs; however, efficient protocols with high reproducibility should be utilized for the extraction of circulating miRNAs owing to the small amounts present in the plasma and serum. Commercial extraction kits that utilize glass fiber filters in the purification process have been widely used for clinical blood samples, and there are several methods for quantification. A polymerase chain reaction (PCR)-based technique using a stem-loop reverse-transcriptase (RT) primer has been widely used for determining quantity. A microarray assay, which can analyze hundreds of miRNAs simultaneously, has also been utilized for the identification of a specific marker among many circulating miRNAs. Recent advances in technology allow for the use of an oligonucleotide array to quantify the amount of circulating miRNAs without the need for PCR. Most recently, researchers have identified circulating miRNAs as new diagnostic markers in patients with cancer using direct sequencing methods[16] (Table 1[17–41]).

Esophageal Cancers

Zhang et al[17] have investigated the serum miRNA profiles of patients with esophageal squamous cell carcinoma (ESCC) using Solexa sequencing. Among 25 candidate miRNAs selected using direct sequencing, this group identified 7 serum miRNAs (miR-10a, 22, 100, 148b, 223, 133a, and 127-3p) as ESCC biomarkers. The areas under the receiver operating curves (AUCs) for the selected miRNAs were higher than for conventional serum tumor markers, and patients in the early stages of the disease could be distinguished from controls using the miRNA assay.[17] Using plasma samples from ESCC patients, our group also analyzed the plasma expression levels of candidate miRNAs that were thought to be associated with the development of ESCC based on previous reports. We found that the plasma levels of miR-21 tended to be higher in ESCC patients than in the controls, and the levels were significantly reduced in postoperative samples compared with preoperative samples. A re-elevation of the miR-21 concentration in plasma was confirmed at recurrence after surgery despite the lack of an increase in conventional serum tumor markers, which suggests that circulating miRNAs may be useful for diagnosis of recurrence in ESCC.[18] However, there have been no reports regarding circulating miRNAs for the other histologic type, adenocarcinoma.

Gastric Cancers

Our group first reported the usefulness of circulating miRNAs as biomarkers in patients with GC. We selected four miRNAs (miR-17-5p, 21, 106a, and 106b), which have been reported to be up-regulated in GC as candidate miRNAs and analyzed their levels in plasma using RT-quantitative PCR (RT-qPCR). In most cases, the plasma levels of these miRNAs reflected the tumor miRNA levels and were significantly