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Mini-review

MicroRNAs as potential biomarkers in human solid tumors

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ABSTRACT

MicroRNAs (miRNAs) regulate the expression of approximately 30% of protein-coding genes. Functions of miRNAs are essential to maintain a steady state of cellular machinery. Dysregulations of miRNAs play pivotal roles in the initiation and progression of malignancies. Abnormal miRNA expressions have been found in a variety of human solid tumors. Furthermore, extracellular miRNAs could circulate in body fluids, and hence show great promise for refining diagnosis and prognosis of cancer. Here we review the progress of analysis of microRNAs as a potential approach for diagnosis and prognosis of solid cancer. We will also discuss obstacles in developing miRNAs as circulating biomarkers.

1. Introduction

1.1. Discovery of circulating miRNAs

Cancer is a leading cause of death worldwide, mainly due to the lack of approaches for its early detection and the development of resistance to chemotherapy. Biomarkers that can be used for the early detection of cancer and precisely identifying early-stage solid tumors after surgery at high risk for recurrence could reduce the mortality [1]. To develop such biomarkers, particularly analyzing tumor-related molecular changes in body fluids, for cancer diagnosis has been investigated for decades. However, little progress has been made.

MicroRNAs (miRNAs) involve in the regulation of several key cellular processes, including cellular development, differentiation, proliferation, cell death, and metabolism. Because a single miRNA can target hundreds of mRNAs, function of dysregulated microRNAs could contribute to the initiation and progression of a variety of malignancies, including solid tumors [2,3]. Furthermore, miRNA expression profiles have been shown as potential signatures for the classification, diagnosis, and progression of cancer [4–7]. In addition, the tumor-specific miRNA expression profiles are more informative and discriminatory as compared with mRNA profiles, and hence have the potential to be developed as cancer biomarkers.

Various miRNA expression patterns in body fluids have been evaluated [8,9]. The resulting data implied that miRNAs could be present in the biological fluids, including blood, urine, tears, breast milk, bronchial lavage, colostrum, seminal, amniotic, pleural, peritoneal, and cerebrospinal fluids, et al. [8]. Furthermore, miRNAs ubiquitously existing in the biological fluids have functional roles associated with the surrounding tissues. For example, Kosaka et al. [10] found that human breast milk contain large amount of miRNAs capable of transfer to immune cells for the development of an infant's immune system. As presented in Fig. 1 and Tables 1 and 2, numerous studies, including our own, have shown the diagnostic and prognostic values of miRNAs in body fluids for a variety of solid cancers [11–13]. For instance, we [14–16] found that plasma miRNA expression profiles could be useful in discriminating non-small cell lung cancer (NSCLC) patients from healthy controls and patients with chronic obstructive pulmonary disease (COPD). Our findings strongly imply the potential of miRNAs as circulating biomarkers for lung cancer diagnosis. Furthermore, by

Abbreviations: miRNA, microRNA; NSCLC, non-small cell lung cancer; COPD, chronic obstructive pulmonary disease; SCC, squamous cell carcinoma; RNase, ribonuclease; MVBs, multivesicular bodies; HDL, high density lipoprotein; Ago2, Argonaute 2; NPM1, nucleophasmin 1; NGS, next-generation sequencing; qRT-PCR, quantitative real-time reverse-transcription polymerase chain reaction; RAS, reticular activating system; HmgA2, high-mobility group AT-hook 2; CRC, colorectal cancer; APC, adenomatous polyposis coli; MSS, microsatellite stability; AUC, area under curve; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; EGFR, epidermal growth factor receptor; PTEN, phosphatase and tensin homolog; TIMP, metalloproteinases; CT, computed tomography; ER, estrogen receptor; PDCD4, programmed cell death 4; AIB1, amplified in breast 1; SOX4, SRY-related HMG-box 4; PSA, prostate specific antigen; CDKN1, cyclin-dependent kinase inhibitor 1; AKT, protein kinase B; RECK, reversion-inducing cysteine-rich protein with Kazal motifs.

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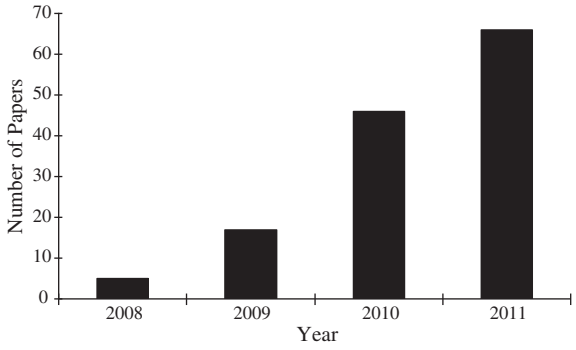

Fig. 1. The number of published papers about circulating miRNAs and solid tumors.

analyzing a panel of four miRNA in sputum of 67 patients with NSCLC and 55 healthy controls, we demonstrated that studying expressions of mir-205, mir-210, and mir-708 in sputum could distinguish lung squamous cell carcinoma (SCC) patients from normal controls [17]. In addition, Park et al. [18] found that mir-125a and mir-200a were present in significantly lower levels in saliva of patients with oral squamous-cell carcinoma, as compared with healthy controls. Moreover, measuring expression levels of mir-126 and mir-152 in urine could indicate the occurrence of bladder cancer with a specificity of 82% and a sensitivity of 72% [19]. Therefore, recent research work in the field of miRNA opened up new perspectives in the development of cancer biomarkers. In this review, we will summarize the development of miRNAs as potential biomarkers that can be tested in clinical specimens, particularly body fluids, for the early detection of solid tumors and predicting prognosis of the patients.

1.2. Functional findings of identified miRNAs as tumor biomarkers

Basically, cancer-related miRNAs function as oncogene (oncomir), tumor suppressor gene (tumor suppressor miRNA), or both oncogene and tumor suppressor [1]. Furthermore, circulating miRNAs have numerous biological effects close by or at a certain distance to affect various types of cells [20]. Circulating miRNAs could be delivered either independent of cell contact or adhesion. Circulating miRNAs could also deliver multiple messages at once and regulate numerous target genes simultaneously [20].

Functional findings of circulating miRNAs in body fluids have recently been described. First, because substantial differences of miRNA expressions exist between platelets and peripheral blood mononuclear cells in healthy subjects [21], miRNAs could have an important function in regulation of hematopoiesis and cellular differentiation [21]. Second, miRNAs could be transported in microvesicles, and thus have important function in gene silencing in recipient cells [22]. Third, as cells could actively secrete endogenous miRNAs through microvesicle, the microvesicle-based miRNA release may play an important role in ceramide dependent secretory machinery [22]. Fourth, because exosomes play essential function in cell–cell communication by transporting miRNAs to surrounding cells, the circulating exosomes-contained miRNAs might have significant functions in transporting genetic information of tumor cells to surrounding cells and supporting tumor growth and progression [23,24]. Fifth, miRNAs might contribute to manipulating the microvesicles' target cells by regulating their RNA stability and translation [25]. Sixth, circulating miRNAs could involve in adjusting immune responses [26,27]. Nevertheless, with increased advances in molecular biology, we will have more functional findings of circulating miRNAs in tumorigenesis.

1.3. Mechanisms underlying stability of miRNAs in clinical samples

The development of molecules as biomarkers that can robustly be tested in human specimens heavily depends on their stability and resistance to storage handling. RNase presenting in body fluids will degrade molecules, particularly mRNAs, and thus affects their stability. For example, blood contains high levels of RNase activity that can rapidly degraded exogenously added mRNAs in just seconds [28]. Therefore, the development of molecular biomarkers has been a great challenge. Notably, endogenous serum miRNAs could remain stable after being subjected to boiling, very low or high pH levels, extended storage time, and multiple freeze–thaw cycles, in which, most RNAs will be degraded [28,29]. Furthermore, human breast milk miRNA was quite stable even treated with acidic (pH1) solution for one hour and RNase A/T [30]. In addition, miRNAs could be preserved in an archived 10-year-old human serum sample [31]. Moreover, miRNAs were also stable in unrefrigerated dried serum blots. Therefore, compared with mRNAs and other longer RNA molecules, miRNAs are fairly steady in fresh and archived clinical specimens [8]. The unique feature of miRNAs in body fluids shows great promise for the discovery of new and more useful biomarkers for cancer diagnosis and prognosis [29,32,33].

The mechanisms underlying the miRNA's stability can be first explained by the discovery of exosomes [34]. Exosomes contain miRNAs [35]. Inside cells, exosomes are formed through inward budding of endosomal membranes, and therefore, give rise to intracellular multivesicular bodies (MVBs). MVBs fuse with the plasma membrane, thus inducing the release of exosomes to the outer of cells [36]. In addition, exosomes containing miRNAs can be found in various types of body fluids [21,37]. Importantly, although normal cells within the peripheral circulation can also contribute to exosome population, tumor tissues might be the primary source of circulating exosomes in cancer patients [38]. For instance, the tumor-derived exosomes and an associated miRNA signature were found in peripheral blood of ovarian cancer patients [39]. Furthermore, a similar trend between miRNA signatures in circulating exosomal miRNA and primary lung tumor was found, whereas there was a significant difference in exosomal miRNA levels in peripheral blood between the lung cancer patients and controls [40]. In addition, miRNA containing tumor-derived exosomes can affect biological processes inside the recipient cells [4]. For example, exosomal miRNAs could promote gene silencing similar to cellular miRNA [4]. Furthermore, because messages delivered by the tumor-derived exosomes could be translated by recipient cells and stimulate proliferation of human glioma cells, the tumor-associated molecular changes could be detected in serum exosomes in 28% glioblastoma patients [41]. Interestingly, let-7, a well-known tumor suppressor miRNA family, is abundant in exosomes produced by a metastatic gastric cancer cells, because exosomal let-7 members might be related to the maintenance of an intercellular tumorigenic and metastatic state [41]. Altogether, exosomes may play a key role in circulating miRNA's stability. Nonetheless, the exosomes-based mechanisms underlying the miRNA's stability remain largely unknown.

Other possibilities also exist for explaining the existence of circulating miRNAs in body fluids. For instance, high density lipoprotein (HDL) transport system can be responsible for transporting circulating miRNAs [42]. For example, native HDL can readily associate with exogenous miRNAs and deliver genetic material to recipient cells with functional targeting capabilities, producing altered gene expression [42]. Furthermore, the RNA-binding protein nucleophosmin may also play an important role in exportation, packaging, and protection of extracellular miRNAs [43]. For instance, almost 90% of the plasma and serum miRNAs are cofractionated with protein complexes rather than encapsulated by vesicles