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Review

Regulation of cancer metastasis by cell-free miRNAs

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ABSTRACT

MicroRNAs (miRNAs) are integral molecules in the regulation of numerous physiological cellular processes that have emerged as critical players in cancer initiation and metastatic progression, both by promoting and suppressing metastasis. Recently, cell-free miRNAs shed from cancer cells into circulation have been reported in cancer patients, raising hope for development of novel biomarkers that can be routinely measured in easily accessible samples. In fact, establishing miRNA expression in the circulation likely has advantages over determination in primary tumor tissue, further augmenting the potential applications of miRNA detection in oncological practice. In addition, secretion of miRNAs impacting distant cell signaling or promoting the formation of a niche that sustains a distant tumor microenvironment allows for new treatment approaches to thwart cancer progression.

Contents

Abbreviations: cfNA, cell-free nucleic acid; CSC, cancer stem cell; CTC, circulating tumor cell; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; MET, mesenchymal–epithelial transition; miR, microRNA; miRNA, microRNA; NGS, next-generation sequencing; RISC, RNA-induced silencing complex; qRT-PCR, quantitative real-time polymerase chain reaction; TAM, tumor-associated macrophage; UTR, untranslated region

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1. Introduction

Cancer metastasis is a complex, multi-step process accounting for the vast majority of cancer-related deaths [1]. Each step of the metastatic cascade involves co-evolution of the tumor and its microenvironment [2,3]. Despite being the driving force of metastasis, cancer cells have to actively recruit stromal components to the primary and metastatic sites in order for tumors to thrive and progress. The tumor microenvironment at the primary and secondary sites is thus formed by the combined action of cancer and stromal cells. The establishment of a supportive tumor microenvironment is highly dependent on extracellular signaling between cancer and stromal cells [4], which involves soluble factors, signaling molecules, extracellular matrix (ECM) components, and mechanical cues. In addition, membrane vesicles derived from both cancer and stromal cells play important roles in the promotion of tumor growth and metastasis [4]. For the majority of solid cancers, tumor-secreted factors play crucial roles in orchestrating the establishment and instructing the dynamic evolution of the microenvironment at primary and distant sites [2–4]. For example, in bone metastasis, cancer cells secret factors such as sVACM-1, sICAM-1 and RANKL to activate the differentiation of osteoclasts [5–8], which in turn break down the bone matrix and release growth factors, such as TGFβ, to further enhance expression of bone metastasis genes and promote metastatic tumor growth [9–11]. Understanding mechanisms of tumor–stroma interaction by secreted factors has lead to the development of novel therapeutics such as RANKL blocking antibody denosumab.

While the role of secreted proteins has been intensively investigated during metastatic progression [12–16], cell-free nucleic acids (cfNAs), such as DNA, mRNA and miRNA, have only recently attracted interest as mediators of cancer metastasis. While cfNA levels are higher in patients with malignant lesions than in patients without tumors [17–24], increased levels have also been quantified in patients with benign lesions, inflammatory disease and tissue trauma. Although the physiological events leading to the increase of cfNAs during cancer development and progression are not well understood, specific cfNAs present in serum and other body fluids may represent potential biomarkers that could be used as a non-invasive, rapid, sensitive and accurate method of diagnosis and prognosis of cancer and/or metastasis [25–27]. In addition to their potential application as prognostic markers, cfNAs may also guide treatment decisions, as a rise or decline in circulating levels may predict therapy response earlier than conventional evaluation [22,28]. Furthermore, cfNAs with a functional role in caner or metastatic progression could serve as new therapeutic targets.

Cell-free miRNAs are of particular interest due to their pleiotropic roles in modulating many physiological and pathological processes, including cancer metastasis [14,29–36]. MiRNAs are small non-coding RNAs with diverse functions that regulate gene expression at the post-translational level by binding to the 3′ untranslated-region (UTR) of their target mRNAs [37–39]. A single miRNA can influence multiple genes thereby altering the expression profile of a cell. Moreover, recent discoveries of secreted miRNAs in virtually all body fluids [40,41] have provided evidence that miRNAs can influence remote cells and function at a long distance [42], a feature that is critical for metastatic spread of cancer cells. Tumor-secreted miRNAs were first discovered in the serum of patients with diffuse large B-cell lymphoma where high levels of miR-21 correlated with improved relapse-free survival [43]. Using a mouse model, Mitchell et al. demonstrated that tumor-derived miRNAs enter the circulation even when originating from epithelial cancer types [44]. They also showed that circulating miRNA-141 levels are elevated in metastatic prostate cancer compared to healthy controls. Their presence in body fluids, combined with their lower complexity compared to other macromolecules, the absence of known post-processing modifications, simple detection and amplification methods, tissue-restricted expression profiles, and sequence conservation between humans and model organisms make extracellular miRNAs ideal candidates for non-invasive biomarkers to reflect and study various physiopathological conditions.

In addition, miRNAs have been identified in tumor-secreted microvesicles called exosomes which can direct intercellular communication under physiological and pathological conditions [4,45–48]. Accumulating evidence supports that horizontal transfer of exosomal factors can functionally influence stromal cells at distant sites [45–49], thereby facilitating tumor–stroma interactions and promoting the formation of a supporting metastatic niche in distant organs. Here we review the recent discoveries of cell-free miRNAs in metastatic progression as well as their diagnostic, prognostic and therapeutic potential in cancer patients.

2. Metastatic progression

Cancer metastasis is a complex process by which cancer cells spread from a primary tumor to other organs and tissues, forming viable secondary deposits of cancer. Cancer cells often initiate metastasis by breaking away from their neighbor cells and invading adjacent tissues after having undergone an epithelial–mesenchymal transition (EMT) that changes their characteristics to acquire motility and invasiveness. The motile cancer cells then enter into lymphatic and blood vessels. Circulating cancer cells (CTCs) that survive in the vasculature arrest in capillaries distant from the primary tumor site and extravasate into the foreign microenvironment. At this point, the cancer cells are speculated to revert to an epithelial phenotype via a mesenchymal–epithelial transition (MET) and either stay dormant or proliferate into macroscopic secondary tumors [50,51]. These consecutive steps require close interplay between cancer cells and their microenvironment. Among the multiple factors underlying metastasis, the adaptation of the primary tumor microenvironment and pre-metastatic or metastatic niches by the tumor to facilitate cancer cell dissemination and distant engraftment plays an important pro-metastatic role that is starting to be recognized [3,8,15,52–54]. The recent discovery of miRNAs and their extracellular presence suggest a potential role of these regulatory molecules in defining the metastatic potential of cancer cells and mediating the tumor–stroma communication.

Despite metastasis being the leading cause of mortality in cancer patients [1], the mechanism underlying metastatic spread and growth in secondary sites is poorly understood. Moreover, specific and sensitive markers that can detect metastatic spread at very early stages or can predict which patients are more likely to progress to metastasis are lacking. Therefore, there is a great and urgent need to develop predictive or early diagnostic markers for metastasis and to elucidate the molecular mechanisms of metastasis to allow the development of efficient treatment options.

3. Biogenesis and function of miRNAs

MiRNAs encompass a large family of non-coding small RNA molecules which occur as single-stranded RNAs of 21–23 nucleotides in length and play important roles in regulating gene expression [38]. In fact, miRNAs are estimated to regulate about 50% of all protein-coding