Review

The role of microRNAs in metastasis and epithelial-mesenchymal transition

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Abstract. For a tumour cell to metastasise it must successfully negotiate a number of events, requiring a series of coordinated changes in the expression of many genes. MicroRNAs are small non-coding RNA molecules that post-transcriptionally control gene expression. As microRNAs are now recognised as master regulators of gene networks and play important roles in tumorigenesis, it is no surprise that microRNAs have recently been demonstrated to have central roles during metastasis. Recent work has also demonstrated critical roles for microRNAs in epithelial-mesenchymal transition, a phenotypic change underlain by altered gene expression patterns that is believed to mirror events in metastatic progression. These findings offer new potential for improved prognostics through expression profiling and may represent novel molecular treatment targets for future therapy. In this review, we summarise the multistep processes of metastasis and epithelial-mesenchymal transition and describe the recent discoveries of microRNAs that participate in controlling these processes.

Keywords. Micro-RNA, metastasis, cancer, epithelial-mesenchymal transition, ZEB, gene regulation.

Introduction

The spread and growth of cells from a primary tumour site (known as metastasis) is the most common cause of death for cancer patients and may occur through organ damage caused by growing lesions, paraneoplastic syndromes or treatment complications. Although primary cancers are often treatable by radiation therapy or surgery, metastasis indicates a wider systemic disease and strongly correlates with poor prognosis (reviewed in [1, 2]). At the cellular level, early stages of metastasis are characterised by the loss of contact with neighbouring cells and an increase in invasive capacity. It is hypothesised these same changes are a recapitulation of the developmental process known as epithelial-mesenchymal transition (EMT), in which epithelial cells acquire a fibroblast-like morphology, increased motility and gene expression patterns characteristic of mesenchymal cells. MicroRNAs (miRNAs) are endogenous small RNA molecules that act as master regulators of gene expression (reviewed in [3]). Over the past few years, characteristic miRNA expression profiles have been identified that enable tumour classification and prognostication and various individual miRNAs have...
been described as oncogenes or tumour suppressors (reviewed in [4]). Several recent studies have identified specific miRNAs that are central to metastatic progression and EMT. This review will discuss the importance of metastasis in tumour progression, the hypothesised role that EMT plays in cancer and the findings of recent studies that identify roles for miRNAs in these processes.

Metastasis

For a metastatic lesion to arise, tumour cells must disseminate by intravasating into the blood or lymphatic system. This requires the breaking of local cell-cell contacts and invasion into the surrounding stroma and may be enhanced by neo-angiogenesis into the primary tumour site which is a pre-requisite for continued tumour growth. After intravasation and surviving mechanical stresses associated with circulation, tumour cells then become trapped in capillary beds of distant organs, adhering to endothelial cell surfaces or to an exposed sub endothelial basement membrane. Extravasation then occurs into tissue at this secondary site. To survive and grow beyond a size otherwise limited by the diffusion of oxygen, nutrients and growth factors, these so-called “micrometastases” must continue evading the immune system and induce neo-angiogenesis to develop a vasculature. This new vasculature may then provide access to the circulation for tumour cells from the secondary site to produce additional metastases [1].

Invasion, which is the initial step in the metastatic process, requires tumour cells to break contact with neighbouring cells and migrate through the extracellular matrix (ECM) and basement membrane. One of the most fundamental features of invasion is therefore the breaking of cell-cell and cell-matrix adhesion. Cell-cell adhesion is mediated by various molecules including cadherins which form interactions between cells via their extracellular domains, whilst their intracellular domains mediate signalling to the actin cytoskeleton [5]. A hallmark of metastatic invasion is a change in cadherin expression from E (epithelial)-cadherin, which is expressed on epithelial cells and inhibits invasion by promoting contact between tumour cells, to N (neural)-cadherin, which is expressed in mesenchymal cells and facilitates tumour cell binding to the stroma [6]. The adherence of cells to the ECM is largely mediated by integrins, a diverse family of heterodimeric receptors which also undergo changes in expression in malignant compared to non-malignant cells [7, 8]. For example, elevated expression of the integrin α6β1 promotes the growth and spread of melanoma cells and, together with decreased α6β1 levels, correlates with metastasis [8]. Invasion through the ECM is further enhanced by ECM-degrading proteases, including matrix metalloproteinases and urokinase plasminogen activator, which are frequently upregulated in malignant tumours and are associated with poor prognosis [9]. During the invasive process, cells establish a leading edge from which pseudopod protrusions form, driven by actin polymerisation and assembly into filaments. This pushes the cell membrane forward and establishes new interactions with the ECM. Matrix metalloproteinases and other ECM-degrading enzymes are upregulated and recruited to the leading edge, breaking down pericellular ECM and creating a path for the advancing cell. Contraction of membrane-anchored myosin-actin filaments propels the cell body forward, coupled with the simultaneous breaking of existing ECM contacts at the trailing edge [10, 11].

Following detachment from the tumour mass, the tumour cell must avoid anoikis, an apoptotic program activated in anchorage-dependent cells after detachment from the extracellular matrix [12]. Anoikis is believed to contribute to the inefficiency of metastasis, as are other mechanisms including immune surveillance within the stroma and velocity-induced shear forces after intravasation into the bloodstream [13]. For secondary tumours to arise, the tumour cells that survive must arrest within the bloodstream and extravasate into new sites. This arrest is likely to involve size restriction within narrowing capillaries and both specific and non-specific interactions with various molecules expressed on endothelial surfaces. Certain tumours preferentially metastasise to certain organs [14]. For example, melanoma cells intravenously injected into mice metastasize to the lung or to lung tissue implanted into muscle, but do not form metastases in the kidney or similarly implanted renal tissue [15]. Whilst the characteristics of the tumour cell will contribute in large part to this, with gene expression signatures that mediate metastasis to different sites having been identified [16, 17, 18, 19, 20], the microenvironment at the secondary site also plays a major role. This is probably due both to a distinct repertoire of surface proteins expressed on the endothelial cells of different organs and to the nature of local growth factors. The importance of the tumour microenvironment has been demonstrated recently by comparing mRNA profiles of tumour-associated and normal breast stroma [21]. Here, a tumour-associated stromal signature capable of predicting outcome was identified, which may in future be useful for identifying patients who require aggressive therapy. This provides further demonstration of the “soil and seed” hypothesis initially postulated in 1889 [22], whereby the ability of a tumour cell (the seed) to grow at a