Role of miR-10b in breast cancer metastasis

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Abstract

Ninety percent of cancer-related mortality is caused by metastasis. Current cancer treatments can control many primary tumors but rarely stop the metastatic spread. Accumulating evidence demonstrates that miRNAs are involved in cancer initiation and progression. Furthermore, several miRNAs have been found to regulate metastasis. In particular, recent studies provide the first functional evidence that overexpression of a specific miRNA, miR-10b, can contribute to the development of metastasis, which can be exploited therapeutically in treating breast cancer metastasis in mice. Further in-depth analysis should provide more precise evaluation of the roles, mechanisms, and therapeutic utility of this miRNA in breast cancer.

Introduction

The ability of primary tumor cells to disseminate and metastasize depends on their genetic and epigenetic alterations as well as the microenvironmental cues they receive. New molecular technologies, such as DNA microarrays, have identified a variety of molecules that contribute to the development of metastasis. These molecules include growth factors, cytokines and chemokines, pro-angiogenic factors, extracellular matrix-remodeling molecules, several epithelial–mesenchymal transition (EMT)-inducing transcription factors, as well as certain microRNAs [1-3]. Understanding of the molecular and cellular determinants of metastasis, however, is still limited. Moreover, current prognostic markers of many cancers, including primary breast carcinomas, only poorly predict eventual metastatic progression [4]. For these reasons, critical regulators of the metastatic process that have implications for diagnosis, prognosis, and treatment – including proteins, and small and large noncoding RNAs – continue to be highly sought.

miR-10b expression correlates with high-grade malignancy and metastatic behaviors

miR-10b was first identified as a miRNA that is highly expressed specifically in metastatic breast cancer cell lines – cell lines that are capable of launching metastases when growing as primary mammary tumors in mice. When compared with normal human mammary epithelial cells, metastatic cell lines MDA-MB-231 and SUM1315 exhibit 50-fold higher miR-10b expression levels [6]; in contrast, nonmetastatic breast cancer cell lines SUM149, SUM159, and MCF-7 express lower miR-10b levels than human mammary epithelial cells [6]. This expression pattern was confirmed and further extended by an independent study [8]. In addition, miR-10b is among the most significantly upregulated miRNAs in the 4T1 metastatic mouse mammary tumor cell line relative to its nonmetastatic or poorly metastatic isogenic relatives (67NR, 168FARN, and 4TO7) [9].

miRNAs are small noncoding RNA molecules that bind to perfect or imperfect complementary sequences at the 3’ UTR of target mRNAs, leading to either mRNA degradation or inhibition of their translation, or both [5]. In an initial screen for miRNAs differentially expressed in human breast cancer cells, the three most significantly upregulated miRNAs miR-155, miR-9, and miR-10b were identified [6].

miR-10b is a particularly interesting candidate given its close correlation with metastatic behaviors. The subsequent functional studies of miR-10b validated its candidacy as a mechanistically important miRNA, as demonstrated by in vivo experiments showing that overexpression of miR-10b in otherwise nonmetastatic breast tumors triggered tumor invasion and distant metastasis in xenotransplantation models [6]. These findings provided the first evidence that overexpression of a specific miRNA can contribute to the development of metastasis. Conversely, therapeutic silencing of miR-10b with antagonirs suppressed metastasis in a mouse mammary tumor model [7]. Further studies are needed to address the remaining questions, including: Does miR-10b play a role in normal development and in progression of spontaneous breast cancer? At which stage and in which subset of tumor cells is miR-10b expression activated? Which clinical cancers would respond to inhibition of miR-10b?
In clinical breast cancers, miR-10b expression levels were first examined in 23 advance-stage breast cancer patients whose tumor samples were obtained at the time of mastectomy: relative to normal mammary tissues, the level of this miRNA is lower in primary breast tumors isolated from metastasis-free patients, while 50% of metastasis-positive patients show elevated miR-10b levels in their breast tumors [6]. On the other hand, miR-10b expression levels in unfractonated bulk cell populations of tumors removed from early-stage breast cancer patients do not predict future metastatic recurrence [10]. It may not be informative, however, to make a prognosis based on expression analysis performed on the heterogeneous cell populations within early-stage primary tumors, in which metastatic cells may not be present or may represent only a rare subset of the total tumor mass.

In advance-stage tumors, miR-10b expression indeed correlates with high-grade malignancy in various cancer types. Higher levels of miR-10b were observed in metstatic samples relative to matched primary tumors [11]. Another study reported that miR-10b is upregulated in hepatocellular carcinomas from metastasis-positive patients compared with hepatocellular carcinomas from metastasis-free patients [12]. In addition, miR-10b is one of the top upregulated miRNAs in human pancreatic adenocarcinomas [13] and glioblastomas [14,15], two types of highly metastatic and/or invasive cancers. In human gliomas, miR-10b levels correlate with tumor grade, invasiveness, and levels of the tumor invasive factors urokinase plasminogen activator receptor and RhoC [16]. Compared with normal Schwann cells, miR-10b is markedly upregulated in tumor tissues from malignant peripheral nerve sheath tumors and in Schwann cells isolated from neurofibromatosis type 1 (NF1) neurofibromas [17].

**miR-10b functionally contributes to tumor invasion and metastasis**

Overexpression of miR-10b can endow cancer cells with invasive and metastatic abilities *in vivo*. The first evidence came from overexpression analyses in two xenograft models. In both cases, human breast carcinoma cells were implanted into the mammary fat pads of NOD-SCID mice. In the SUM149 model, at 6 weeks post implantation, the control tumors were non-invasive, as evidenced by their confinement within fibrotic capsules; in contrast, the miR-10b-expressing SUM149 tumors displayed substantial invasion, with islands of carcinoma cells that invaded the stroma [6]. From 9 weeks, the lungs of mice bearing miR-10b-expressing tumors, but not the control tumors, showed clusters of micrometastatic cells, as evidenced by cytokeratin immunostaining. In another otherwise nonmetastatic human breast cancer cell line, the SUM159 line, ectopic expression of miR-10b led to more visible signs of lung metastases as well as macroscopic peritoneal metastases [6]. These results suggest that miR-10b is a metastasis-promoting miRNA *in vivo* in breast cancer.

miR-10b is also involved in the progression of other types of cancer. Antisense silencing of miR-10b in NF1 malignant peripheral nerve sheath tumor cells reduced cell proliferation, migration and invasion [17]. In esophageal cancer cells, ectopic expression of miR-10b increased cell motility and invasiveness, whereas inhibition of miR-10b reduced cell invasiveness [18]. When miR-10b was overexpressed in nasopharyngeal carcinoma cells, it markedly induced the cells’ *in vitro* migration and invasion, as well as *in vivo* metastasis formation in nude mice [19]. Although the role of miR-10b in pancreatic cancer has not yet been reported, miR-10a – which shares the same seed sequence as miR-10b, and differs from miR-10b by a single nucleotide – has been reported to be a metastasis-promoting miRNA in pancreatic cancer cells [20].

**Regulation of expression and molecular mechanisms of miR-10b action**

In breast cancer cells, miR-10b levels correlate not only with metastatic potential, but also with expression levels of the EMT-inducing, metastasis-promoting transcription factor Twist [6]. This association has also been extended in other types of cancer. For instance, in a collection of 46 head and neck squamous cell carcinomas, miR-10b and Twist were found to be highly correlated (*P* = 0.006) [21].

Twist is a pleiotropic transcription factor [22]. Among its multiple targets, Twist activates the transcription of the *mir-10b* gene (Figure 1) by binding directly to an E-box sequence proximal to its putative promoter [6]. Although the miR-10b miRNA does not trigger an EMT by itself, it appears to be required for Twist-induced cell motility and invasiveness in human mammary epithelial cells [6], suggesting that miR-10b is a mediator of some elements of the multicomponent, Twist-induced EMT program, but not an inducer of EMT on its own.

Similar to other pleiotropically acting factors (for example, transcription factors), each miRNA can function through regulating the expression of many target mRNAs, particularly through one or a few key target mRNAs. In mammary epithelial cells and breast carcinoma cells, miR-10b can directly suppress the translation of *HOXD10*, an mRNA encoding a transcriptional repressor that inhibits expression of several genes involved in cell migration and extracellular matrix remodeling, such as RhoC, urokinase plasminogen activator receptor, α3-integrin, and MT1-MMP (Figure 1) [6]. Interestingly, *HOXD10* is not only targeted by the miR-10b miRNA, but also targeted by a long noncoding RNA termed *HOTAIR*, which has also been shown to promote breast...
cancer metastasis (Figure 1) [23]. Ostensibly, *HOTAIR* reprograms the chromatin state, causing increased Polycomb repressive complex-2 occupancy on promoters of genes that inhibit breast cancer progression, including *HOXD10* [23]. On the other hand, miR-10b could also suppress the expression of T-lymphoma invasion and metastasis-1, a guanidine exchange factor for Rac, in the SUM159 breast cancer cell line [24]. The effect of miR-10b on metastatic behaviors of cancer cells is thus most likely to be a consequence of regulating multiple target mRNAs expressed in these cells. This is analogous to other cancer genes that regulate the expression of numerous target genes with either similar or opposing functions, such as the oncogene MYC, which can activate both pro-survival and pro-apoptotic genes [25].

Several independent groups have further confirmed the Twist–miR-10b–HOXD10–RhoC pathway and demonstrated that this pathway is regulated by known cancer gene products (Figure 1). One study revealed that ectopic expression of the breast cancer metastasis suppressor-1 gene, a negative regulator of Twist expression, leads to downregulation of miR-10b and RhoC, as well as upregulation of HOXD10, in highly metastatic breast tumor cells [8]. Another group reported that this pathway is positively regulated by CD44 and Src: binding of hyaluronan to CD44 leads to c-Src kinase activation, which in turn activates Twist through phosphorylation and nuclear translocation. Further analyses demonstrated that miR-10b is controlled by the Twist binding site in its promoter region, and that induction of miR-10b expression by hyaluronan/CD44-activated c-Src in breast cancer cells is Twist dependent. This leads to downregulation of HOXD10, RhoA/RhoC upregulation, Rho-kinase activation and breast cancer cell invasion [26]. A third study showed that miR-10b is highly expressed in Epstein–Barr virus-positive, latent membrane protein-1 (LMP1)-expressing, metastatic nasopharyngeal carcinoma cells relative to LMP1-negative, nonmetastatic nasopharyngeal carcinoma cells, and is downregulated in response to silencing either LMP1 or Twist. Moreover, LMP1 expression leads to induction of miR-10b, which is Twist dependent [19].

Other targets of miR-10b have been identified in various tumor cell types. For instance, miR-10b can directly target the mRNA of the tumor suppressor neurofibromin in NF1 malignant peripheral nerve sheath tumor cells, indicating that this miRNA might play an important role in NF1 tumor formation and progression through silencing neurofibromin and activating RAS signaling in these cells (Figure 1) [17]. This also illustrated a new mechanism (in addition to NF1 loss of heterozygosity, unequal expression of NF1 alleles, NF1 mRNA editing, and so forth) for downregulating the NF1 tumor suppressor in tumors suffering from NF1 heterozygosity. Moreover, KLF4 – a transcription factor with context-dependent oncogenic or tumor-suppressor functions [27,28] that has been reported to inhibit esophageal cancer cell migration and invasion – has been identified as a direct target of miR-10b in esophageal squamous cell carcinoma cells (Figure 1) [18]. Similar to other miRNAs, the precise function of miR-10b may be tissue specific, which at least in part depends on the expression pattern of its target mRNAs in a given cell type.

**miR-10b is a potential target for anti-metastasis therapeutic approaches**

Targeting metastasis-promoting miRNAs may represent a novel therapeutic strategy for breast cancer treatment. Among several types of *in vivo* miRNA antagonists being developed are antagonirs – a type of chemically engineered, cholesterol-conjugated antisense RNA oligonucleotide [29,30]. The effect of the miR-10b antagonir (termed antagonir-10b) was tested in a 4T1 mouse mammary tumor metastasis model: systemic delivery of antagonir-10b had a potent and highly specific...
metastasis-suppressing effect on these malignant breast cancer cells without affecting their ability to grow as primary tumors – specifically, antagonir-10b blocked dissemination of cancer cells from the primary tumor, but did not affect late stages of the metastatic process after tumor cells had already disseminated. Furthermore, delivery of antagonir-10b to normal tissues did not have after tumor cells had already disseminated. Furthermore, delivery of antagonir-10b to normal tissues did not have substantial toxicity [7]. This work is the first report showing proof-of-principle that antagonirs can be efficiently delivered to rapidly growing metastatic tumor cells in vivo, can specifically silence the miRNA being targeted, and can prevent metastasis formation by otherwise highly malignant cells. The differential effects of antagonir-10b on primary mammary tumor growth, dissemination, and metastatic colonization could be explained by the previous findings that miR-10b specifically promoted breast cancer cell migration and invasion but did not affect proliferation of these cells [6]. This antagonir result also resembles the effect of shRNA-mediated knockdown of Twist, which blocked invasion of 4T1 tumor cells but did not alter primary tumor growth [31].

In many breast cancer patients, disseminated, circulating tumor cells are readily detectable before surgery [32]. These disseminated tumor cells can later emerge at a secondary site where they grow into a macroscopic tumor (called metastatic recurrence). The current neoadjuvant therapies used in the breast cancer clinic are mainly intended to shrink the primary disease in order to make the subsequent surgery more complete; however, these existing neoadjuvant therapies may not be effective in blocking metastatic dissemination (RJ Lee and TA Ince, personal communication).

Because the miR-10b antagonir prevents metastatic dissemination but does not affect the late stages of the metastatic process after tumor cells have already disseminated, the main promise for developing an agent such as antagonir-10b as a potential therapy would be whether it can be added during treatment starting in the early stages as a prophylactic therapy against future metastasis formation. Since antagonir-10b does not shrink a primary tumor, it should be combined with other anti-tumor drugs and/or surgical resection of the primary tumor as a neoadjuvant therapeutic strategy, which can be first tested in preclinical models.

Preclinical studies are different from clinical settings. The 4T1 cell line used in the antagonir-10b study was derived from a subset of cancer cells present in the original tumor that are highly metastatic and express high levels of Twist and miR-10b, and thus is expected to respond to silencing of Twist or miR-10b. At the clinical level, as aforementioned, our current knowledge is that miR-10b is upregulated in some metastatic breast tumors. In contrast, miR-10b is expressed at very low levels in early-stage or nonmetastatic breast tumors [6,10]. Similarly, Twist has been found to be over-expressed in advanced tumors that are metastatic and/or invasive, including invasive lobular breast carcinoma [31], infiltrative gastric cancer [33], metastatic melanoma [34], glioblastoma [35], the aggressive subtype of neuroblastoma [36], and spindle cell carcinoma of the head and neck [37]. The obvious explanation is that upregulation of Twist and resulting activation of miR-10b expression occur late during primary tumor progression. What, then, is the rationale for giving the miR-10b antagonir to early-stage or nonmetastatic breast tumors, which express low levels of miR-10b?

It should be noted that all these expression analyses were carried out by northern blot or quantitative PCR on whole-tumor specimens. Only a small subset of primary tumor cells is probably responsive to stromal signals by upregulating Twist, resulting in activated expression of miR-10b. Whether a minor subpopulation of cancer cells present in some of the early-stage or nonmetastatic breast tumors express high levels of Twist and miR-10b, utilizing in situ hybridization techniques, remains to be seen. This expression could provide the rationale and selection criteria for treating early-stage or nonmetastatic breast tumors with the miR-10b antagonir, as part of the neoadjuvant regimen.

Conclusions

The present review summarizes evidence for the growing implication of miR-10b miRNA in cancer progression, particularly metastatic progression of breast cancer. The pro-metastatic function of miR-10b has been demonstrated in different xenograft models. Whether this Twist-induced miRNA plays a role in normal development, and whether it is required for metastasis formation in mouse models of spontaneous breast cancer, remain to be determined.

The miR-10b antagonir appears to be a starting point for the development of miRNA-based, anti-metastasis agents, and extensive analyses will be required to determine the efficacy and safety of such agents by using multiple model systems. Because antagonir-10b does not shrink a primary tumor but instead stops its metastatic ability, it would be of interest to use the antagonir in combination with surgical resection to treat breast tumor-bearing mice and to determine whether this combination treatment can lead to both primary tumor removal and prevention from future metastatic relapse. Finally, it is important to develop selection criteria to identify clinical breast tumors that are expected to respond to silencing of miR-10b.

Abbreviations

EMT, epithelial–mesenchymal transition; LMP1, latent membrane protein-1; miRNA, microRNA; MMP, matrix metalloproteinase; NF1, neurofibromatosis
type 1; NOD-SCID, nonobese diabetic, severe combined immunodeficient; PCR, polymerase chain reaction; UTR, untranslated region.

Competing interests

The author declares that she has no competing interests.

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