Am J Digest Dis 2017;4(1):1-5
www.ajdd.us /ISSN:2329-6992/AJDD0040112

*Review Article*

# Effect of miR-200b on metastasis of gastric cancer

Leyu Chiu[1], Brock Humphries[2,3]

*[1]College of Human Medicine, [2]Department of Physiology, [3]Cellular and Molecular Biology Graduate Program, Michigan State University, East Lansing, MI, USA*

Received September 16, 2016; Accepted November 28, 2016; Epub January 15, 2017; Published January 30, 2017

**Abstract:** It has been well documented that the microRNA 200 (miR-200) family functions as an important regulator of cancer metastasis. Although many studies have implied the involvement of miR-200b in the metastatic pathway, the functional details of miR-200b in the metastatic pathway has yet to be fully elucidated. In gastric cancer, miR-200b is believed to be involved in cancer cell transformation, migration, and invasion. In this review, we present the current understanding of the impacts of miR-200b on the metastatic pathway and discuss the therapeutic potential of using miR-200b as targets for the inhibition of gastric cancer.

**Keywords:** microRNA, miR-200b, cancer metastasis, gastric cancer

## Introduction

Although there has been a decrease in its incidence globally, gastric cancer remains the second leading cause of cancer related deaths and the fourth most diagnosed cancer worldwide [1]. There is increasing evidence that down-regulated expression of the miR-200 family is inversely correlated with the WHO grades of gastric adenocarcinoma, with the most severe stages expressing the least amount of miR-200 family [2]. Many studies have also shown that miR-200b is often dysregulated in malignant metastatic cancers, including breast, colorectal, pancreatic, and gastric cancers. Furthermore, recent research has shown that miR-200b has suppressive effects not only on gastric cancer metastasis, but also on growth and cell transformation. In addition, the loss of miR-200b expression is being investigated as a marker for poor prognosis. The miR-200 family, which consists of 5 members (miR-200a, -200b, -200c, -141, -429), is a family of non-coding RNA that regulates the expression of many target genes associated with cancer progression and metastasis by binding to the 3'UTRs of their target mRNAs and has the potential to be important in cancer treatments. This family forms two clusters on two chromosomes, with miR-200b, -200a, and -429 forming cluster I on chromosome 1 and miR-200c and -141 forming cluster II on chromosome 12 [3]. Additionally, the miR-200 family can also be organized into two functional groups by the similarities in their seed sequences. The seed sequences for miR-200b, -200c, and -429 form functional group I (AAUACUG) while miR-200a and -141 form functional group II (AACACUG), with the two groups differing by one nucleotide in their seed sequence. The binding specificity of the miRNA is based on its seed sequence located in the second to eighth nucleotide region of the 5' end of the miRNA. When bound to its target mRNA, a variety of biological processes take place, including inhibition of translation, destabilization, and degradation of the mRNA [4].

One of the initial steps of cancer metastasis is epithelial to mesenchymal transition (EMT), the ability of the tumor cells to undergo a change from epithelial-like cells to mesenchymal-like cells. This essential step is characterized by increased motility and invasiveness, allowing the cancer cell to become metastatic. In addition to its prominent role in cancer metastasis, EMT is also a process that occurs during normal growth and development. However, occurrence of EMT outside of normal development is a sign of malignancy. Loss of miR-200b expression

has been associated with carcinogenesis and EMT, along with a marked increase in the transcriptional repressor proteins that regulate extracellular matrix recycling, namely ZEB1 and ZEB2. Increased ZEB1 and ZEB2 expression can lead to binding of E-boxes in the E-cadherin gene promoter, causing transcriptional inhibition. Down-regulation of E-cadherin expression leads to the loss of cellular adhesion. As a result, tumor cell motility and ability to metastasize is increased. Some researchers have also reported a relationship between miR-200 family and chemoresistance, showing that restoration of miR-200c levels led to increased sensitivity to antitumor drugs [5]. These studies show that the miR-200 family is not only important in cancer metastasis but also for the efficacy of chemotherapeutics. Metastasis is generally characterized into six sequential steps: 1) growth of the primary tumor and invasion of nearby tissue, 2) disassociation and migration away from the primary tumor, 3) intravasation into circulatory system, 4) ability to survive transportation through vascular system, 5) attachment to the vascular wall and successful extravasation, and 6) colonization of the new organ and growth of the secondary tumor [6]. In this review, we will examine the role of miR-200b in gastric cancer cell transformation, migration, and invasion.

## Transformation

Neoplastic cellular transformation in gastric cancer is thought to be the result of the accumulation of DNA damage resulting from a variety of factors such as *Helicobactor pylori* (H. pylori) infection, smoking, obesity, and genetic susceptibility [7]. Studies have shown that miR-200b plays an important role in preventing malignant cell transformation. In a study investigated by Tang et al., miR-200b was found to be strongly down-regulated in gastric cancer specimens. It was reported that miR-200b directly targeted DNA methyltransferases, which is responsible for the enzymatic addition of a methyl group at the C5 position of cytosine. This down-regulation of miR-200b results in a decrease in global DNA methylation, leading to dysregulation of gene expression and malignancy. Additionally, it was shown that ectopic expression of miR-200b in gastric cancer cells impaired proliferation and invasion through direct targeting of DNMT3A and DNMT3B and decreasing DNMT1 expression indirectly. These data suggest miR-200b plays an important role in DNA hypomethylation of gastric cancer [8]. The biological effects of overexpressing miR-200b have also been investigated, with the results showing that miR-200b overexpression leads to significantly attenuated cell proliferation [8]. In a study by Yang et al., miR-200b was revealed to be down-regulated in malignant nasopharyngeal carcinoma (NPC) cells when quantified using qRT-PCR [9]. It was also found in the same study that restoration of miR-200b was capable of inhibiting the growth of NPC cells, indicating that miR-200b may be cancer-suppressive. Others have also reported the effect of miR-200b on maintaining epithelial morphology. Kurashige et al. was able to show that up-regulation of miR-200b in gastric cancer cells caused cell morphology to change from fibroblast- to epithelial-like, an indication that expression of miR-200b is correlated with the ability to reverse EMT [10]. The results of these studies demonstrate the importance of miR-200b in inhibition of cell transformation and EMT.

## Migration

After a tumor cell transforms into a malignant tumor cell, it needs to gain the ability to migrate before metastasis can occur. Paterson et al. recently showed that altered expression of miR-200b at the leading edges of colorectal adenocarcinomas has significant effects on metastasis and leads to a poorer survival [11]. The study confirms that loss of miR-200b expression is associated with EMT, increased ZEB1, and degradation of the basement membrane, all of which contribute to the migratory ability of tumor cells. Paterson's *in vitro* study used SW480 colon cancer cells to demonstrate that leading edge epithelial cells transformed to the more malignant and elongated mesenchymal phenotype in cells with down-regulated miR-200b expression [11]. These results show that loss of miR-200b expression is critical for the migratory characteristics of colon cancer cells.

In another study by Yang et al., malignant NPC cells were found to be expressing significantly less miR-200b compared to non-tumor nasopharyngeal cells. Using a wound-healing assay, Yang et al. demonstrated that overexpression of miR-200b caused decreased cell migration

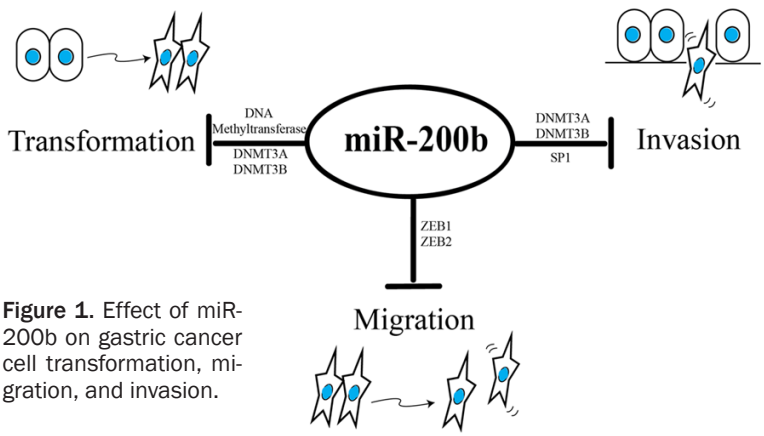


**Figure 1.** Effect of miR-200b on gastric cancer cell transformation, migration, and invasion.

in C666-1 and 5-8F cell lines compared to control. They were also able to show that miR-200b knockdown cell lines had increased migration compared to the negative control [9]. These findings are consistent with a study by Kurashige et al., who showed that overexpression of miR-200b in gastric cancer cells had the same effect of inhibiting cellular migration in a wound healing assay [10]. Kurashige used NUGC3 and AGS cell lines to show that when treated with pre-miR-200b, both were found to have distinctively less migratory abilities compared to negative control cells. The same study also revealed that miR-200b controls gastric cancer metastasis by specifically regulating ZEB2 expression. The results of these studies indicate that loss of miR-200b expression is common in migrating cancer cells.

## Invasion

Recent studies have suggested that miR-200b also plays an important role in cell invasion. Tang et al. demonstrated that ectopic expression of miR-200b in MGC-803 and AGS cells markedly attenuated cell invasion by targeting DNMT3A, DNMT3B, and SP1, when compared to gastric cancer control cells [8]. Using frozen tissue samples of gastric cancer patients, it was shown that metastasis and invasion into the lymph nodes were higher in the low miR-200b expressing group than the high expressing group. Another study by Paterson et al. showed that increased invasion of leading edge cells occur as a result of EMT and a down-regulation of miR-200b [11]. Using ISH and qPCR, Paterson was able to demonstrate that expression of miR-200b declines toward the invasive front of colorectal adenocarcinomas, leading to degradation of the border basement membrane. This finding is important because loss of basement membrane in colorectal cancer is correlated with invasive metastasis and poor survival [12]. Kurashige et al. similarly showed that cell invasion was significantly inhibited in AGS cells with upregulated miR-200b expression, which may suggest the anti-invasive properties of miR-200b. This line of evidence was also supported by Yang et al., who used the Matrigel transwell invasion assay to demonstrate that overexpression of miR-200b markedly reduced the invasive ability of C666-1 and 5-8F NPC cell lines. Across all these studies, it is evident that miR-200b has suppressive abilities on metastatic invasion in gastric cancer and numerous other types of cancer.

## Conclusion

Gastric cancer continues to be the second leading cause of cancer related mortality [1]. The carcinogenesis of gastric cancer is a complex interplay between host and environmental factors that still have not yet been fully elucidated. We now understand through a large volume of work that EMT is an important characteristic that tumor cells acquire in order to become invasive. This process is heavily influenced by the loss of miR-200b, a non-coding RNA that plays a significant role in the process of EMT and metastasis. Studies on miR-200b have increased our understanding of the critical roles it plays in gastric cancer cell transformation, migration, and invasion (**Figure 1**). Interestingly, although many studies have shown the cancer suppressive effects of miR-200b, some groups have reported that other members of the miR-200 family are actually overexpressed in other types of cancer. These findings indicate that the role of miR-200s in cancer development may be cancer-type dependent. Furthermore, Dykxhoorn et al. reported that increased miR-200b expression might be necessary for colonization of the new site, due to the effect of miR-200b in increasing E-cadherin [13]. Some have speculated that EMT is an important feature of the initial steps

**Table 1.** Summary of miR-200b functional partners in gastric cancer

| Direct targets of miR-200b | Cell types | Functions of targets | References |
|---|---|---|---|
| DNA (cytosine-5)-Methyltransferase 3 Alpha (DNMT3A) | Stomach | Adds methyl groups to DNA; Responsible for CpG Island Methylation | [8] |
| DNA (cytosine-5)-Methyltransferase 3 Beta (DNMT3B) | Stomach | Adds methyl groups to DNA; Responsible for CpG Island Methylation | [8] |
| Transcription factor Sp1 (SP1) | Stomach, Pancreas | Regulation of cell survival, growth, and angiogenesis; Regulates FGFR1, IGFR1, VEGF, thymidine kinase | [8, 17] |
| Zinc Finger E-box Binding Homeobox 1 (ZEB1) | Colon, Stomach | Transcriptional Repressor of E-cadherin and miR-200 Family Members; Expression Promotes EMT | [2, 6, 10] |
| Zinc Finger E-box Binding Homeobox 2 (ZEB2) | Colon, Stomach | Transcriptional Repressor of E-cadherin and miR-200 Family Members; Expression Promotes EMT | [2, 6, 10] |

of metastasis, while a reversion (MET) is necessary for the tumor cells to complete colonization and establish attachment at the new site. Therefore, more research is needed to investigate the role of miR-200b in different metastatic cancer types and subtypes.

Our current knowledge of miR-200b allows us to hypothesize that its expression may be a useful marker for evaluating the prognosis of gastric cancer. Similarly, E-cadherin, ZEB1, and ZEB2 may all potentially be used as markers to predict the metastatic abilities of a tumor. We know that miR-200b reduces the risk of metastasis by playing an important role in inhibiting EMT, at least in gastric cancer. It has also been reported that the miR-200 family, specifically loss of miR-200c, is found to be associated with chemoresistance of cancer cells. Thus, the distinction must be made that members of the miR-200 family may have similar but not necessarily identical functions. A summary of the functional partners of miR-200b in gastric cancer can be found in **Table 1**.

Further research on miR-200b is needed to gain a better understanding of its promising potential as a therapeutic target for gastric cancer. Recent clinical trials have explored two main strategies of miRNA manipulation. These strategies are dependent on whether the expression of a specific miRNA acts as an oncogene or as a tumor suppressor [14]. Current treatment uses anti-miRNA oligonucleotides as antagonists to inhibit oncogenes [15]. However, in gastric cancer where loss of tumor suppressor miR-200b is seen, the strategy is to use viral carriers to reintroduce miR-200b expression [16]. Although many studies have shown good results using these strategies, there are still numerous hurdles to overcome. Due to the expansive nature of the function of miRNAs, further research is needed to improve the specificity of miRNA targeting. Many studies have confirmed the inhibitory role of miR-200b on the initiation of metastasis in multiple types of cancer, and further biomedical research may offer exciting, novel strategies for miRNA-based cancer treatment.

## Acknowledgements

We wish to thank Dr. Chengfeng Yang for helpful guidance and incredible mentorship.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Leyu Chiu, College of Human Medicine, Michigan State University, East Lansing, Michigan 48824, USA. Tel: 562-481-8146; E-mail: chiule@msu.edu

## References

[1] Ang TL and Fock KM. Clinical epidemiology of gastric cancer. Singapore Med J 2014; 55: 621-628.

[2] Cong N, Du P, Zhang A, Shen F, Su J, Pu P, Wang T, Zjang J, Kang C and Zhang Q. Down-regulated microRNA-200a promotes EMT and tumor growth through the wnt/beta-catenin pathway by targeting the E-cadherin repressors ZEB1/ZEB2 in gastric adenocarcinoma. Oncol Rep 2013; 29: 1579-1587.

[3] Altuvia Y, Landgraf P, Lithwick G, Elefant N, Pfeffer S, Aravin A, Brownstein MJ, Tuschl T and Margalit H. Clustering and conservation patterns of human microRNAs. Nucleic Acids Res 2005; 33: 2697-2706.

[4] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

[5] Cochrane DR, Spoelstra NS, Howe EN, Nordeen SK and Richer JK. MicroRNA-200c mitigates invasiveness and restores sensitivity to

microtubule-targeting chemotherapeutic agents. Mol Cancer Ther 2009; 8: 1055-1066.
[6] Humphries B and Yang C. The microRNA-200 family: small molecules with novel roles in cancer development, progression and therapy. Oncotarget 2015; 6: 6472-6498.
[7] Lee YY and Derakhshan MH. Environmental and lifestyle risk factors of gastric cancer. Arch Iran Med 2013; 16: 358-365.
[8] Tang H, Deng M, Tang Y, Xie X, Guo J, Kong Y, Ye F, Su Q and Xie X. miR-200b and miR-200c as prognostic factors and mediators of gastric cancer cell progression. Clin Cancer Res 2013; 19: 5602-5612.
[9] Yang X, Ni W and Lei K. miR-200b suppresses cell growth, migration and invasion by targeting Notch1 in nasopharyngeal carcinoma. Cell Physiol Biochem 2013; 32: 1288-1298.
[10] Kurashige J, Kamohara H, Watanabe M, Hiyoshi Y, Iwatsuki M, Tanaka Y, Kinoshita K, Saito S, Baba Y and Baba H. MicroRNA-200b regulates cell proliferation, invasion, and migration by directly targeting ZEB2 in gastric carcinoma. Ann Surg Oncol 2012; 19 Suppl 3: S656-64.
[11] Paterson EL, Kazenwadel J, Bert AG, Khew-Goodall Y, Ruszkiewicz A and Goodall GJ. Down-regulation of the miRNA-200 family at the invasive front of colorectal cancers with degraded basement membrane indicates EMT is involved in cancer progression. Neoplasia 2013; 15: 180-191.
[12] Spaderna S, Schmalhofer O, Hlubek F, Berx G, Eger A, Merkel S, Jung A, Kirchner T and Brabletz T. A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal cancer. Gastroenterology 2006; 131: 830-840.
[13] Dykxhoorn DM, Wu Y, Xie H, Yu F, Lal A, Petrocca F, Martinvalet D, Song E, Lim B and Lieberman J. miR-200 enhances mouse breast cancer cell colonization to form distant metastases. PLoS One 2009; 4: e7181.
[14] Christopher AF, Kaur RP, Kaur G, Kaur A, Gupta V and Bansal P. MicroRNA therapeutics: discovering novel targets and developing specific therapy. Perspect Clin Res 2016; 7: 68-74.
[15] Garzon R, Marcucci G and Croce CM. Targeting microRNAs in cancer: rationale, strategies and challenges. Nat Rev Drug Discov 2010; 9: 775-789.
[16] Gao M, Yin H and Fei ZW. Clinical application of microRNA in gastric cancer in Eastern Asian area. World J Gastroenterol 2013; 19: 2019-2027.
[17] Zhang J, Zhu ZG, Ji J, Yuan F, Yu YY, Liu BY and Lin YZ. Transcription factor Sp1 expression in gastric cancer and its relationship to long-term prognosis. World J Gastroenterol 2005; 11: 2213-2217.