Breast Cancer RESEARCH

EDITORIAL

miR-200c at the nexus of epithelial–mesenchymal transition, resistance to apoptosis, and the breast cancer stem cell phenotype

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Abstract

Decreased expression of miRNAs of the miR-200 family has been implicated in the growth and metastasis of breast cancer cells. Of this family, miR-200c has garnered particular attention as a consequence of its ability to target ZEB1 and ZEB2, mediators of epithelial–mesenchymal transition. An article in the previous issue of *Breast Cancer Research* identifies additional targets of miR-200c that link increased cancer cell invasiveness, resistance to apoptosis, and induction of breast cancer stem cell characteristics.

miRNAs are a family of small noncoding RNA molecules that downmodulate gene expression through post-transcriptional mechanisms, and individual miRNAs have been found to play critical roles in mammary gland development and breast cancer progression. In the previous issue of *Breast Cancer Research*, Howe and colleagues find that miR-200c plays a broader role in suppression of breast cancer development than had been previously suspected [1]. Members of the miR-200 family had previously been investigated for their ability to inhibit epithelial–mesenchymal transition (EMT), a developmental process in which epithelial cells acquire the migratory, invasive, and apoptosis-resistant properties of mesenchymal cells [2-4]. To date, the most investigated targets of miR-200 family members have been the transcription factors ZEB1 and ZEB2, regulators of EMT that maintain the mesenchymal phenotype by downmodulating expression of E-cadherin as well as other mediators of epithelial cell polarity and function [5]. Previous studies from the Richer laboratory, however, have shown that reintroduction of miR-200c into Hey ovarian cancer cells led to decreased migration and invasion even though E-cadherin was not re-expressed [6], suggesting that miR-200c could also affect invasion through processes independent of the ZEB1/2–E-cadherin axis.

To identify ZEB1-independent mechanisms by which miR-200c could inhibit cell motility, Howe and colleagues used a panel of breast and endometrial cancer cell lines previously identified as miR-200c deficient [1]. MDA-MB-231 and BT549 breast cancer cells and Hec50 and AN3CA endometrial cancer cells express very low levels of miR-200c, high levels of ZEB1, and little E-cadherin; expression of miR-200c in these cells is sufficient to inhibit ZEB1 and increase expression of E-cadherin [1,7]. Analysis of microarray profiles of Hec50 endometrial cells in which miR-200c had been re-expressed revealed several additional genes that were potential targets of miR-200c and which had been previously implicated in cell motility, including the extracellular matrix protein fibronectin 1 and the actin-organizing protein moesin. Luciferase reporter assays were used to show that miR-200c directly targeted the 3' UTR of these genes. While expression of miR-200c in these cells led to significantly decreased cell motility and re-expression of E-cadherin, further addition of plasmids encoding either fibronectin 1 or moesin that could not be targeted by miR-200c restored cellular migratory ability without affecting E-cadherin expression levels – effectively demonstrating that miR-200c can affect cell motility through both ZEB1/E-cadherin-dependent and ZEB1/E-cadherin-independent pathways.

Another important mesenchymal characteristic that can be acquired through activation of the EMT program in tumor cells is increased ability to tolerate conditions that should trigger apoptotic cell death. Researchers from the Richer laboratory had previously shown that re-expression of miR-200c in breast, endometrial, and ovarian cancer cells led to increased susceptibility to apoptosis induced by microtubule-targeting chemotherapeutic agents [7]. In the present study, Howe and colleagues identify the neurotrophic receptor tyrosine

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kinase 2 (which encodes the protein TrkB) as a specific target of miR-200c that confers resistance to anoikis [1]. Anoikis is the cell death program activated in anchorage-dependent cells upon separation from the extracellular matrix, suppression of which is believed to be a necessary step in development of breast ductal carcinoma *in situ* [8]. TrkB was shown to act as a mediator of anoikis resistance in both BT549 and Hec50 cells, and restoration of miR-200c caused decreased expression of TrkB protein concomitant with increased death of cells cultured on nonadhesive substrata; resistance to anoikis in the miR-200c-expressing cells was specifically regained by expression of a TrkB construct that could not be targeted by miR-200c. These experiments identify a novel and unexpected potential function for miR-200c in blocking tumor progression. Intriguingly, recent studies with rat kidney epithelial cells have found that ZEB1 is a required downstream effector for TrkB-induced anoikis resistance [9], and other investigators have found that the miR-200c target Fas-associated phophatase 1 is involved in resistance to Fas-mediated apoptosis [10], suggesting that miR-200c can also control apoptosis through both ZEB1-dependent and ZEB1-independent processes.

Induction of the EMT program has also been linked with the breast cancer stem cell (CSC) phenotype, characterized by increased malignant potential and resistance to chemotherapeutic agents [11]. A series of recent studies have implicated miR-200 family members and targets in activation and maintenance of the CSC phenotype, as miR-200c is downregulated in breast cancer cells that express CSC markers [12] and re-expression of miR-200 family members can reverse CSC characteristics [12,13]. Furthermore, while ZEB1 and ZEB2 were previously shown to target miR-200 family members in a self-reinforcing feedback loop [3], recent studies have indicated that ZEB1 also inhibits other miRNAs involved in stem cell characteristics, including miR-203 and miR-183 [14]. It is striking that the key characteristics of the CSC phenotype – the ability to grow on nonadhesive substrata and increased cell motility [15] – are both identified in the current study as being directly regulated by miR-200c [1].

An important consideration when evaluating these pathways in cancer cells is that the function of miR-200 in controlling EMT is likely to be highly dependent upon specific characteristics of the developing tumors; indeed, the targets of miR-200c identified by Howe and colleagues are not invariably induced in every cell line with reduced levels of the miRNA [1]. Unlike developmental EMT, which proceeds as an orchestrated program of many different mediators and effectors to induce an organized outcome, the more chaotic tumor microenvironment can stimulate incomplete or transient activation of the EMT program. As activation of even a subset of EMT-associated processes may be sufficient to confer increased motility or resistance to apoptotic stimuli without complete conversion to a mesenchymal cell, it may be necessary for cellular mediators that regulate EMT to be sufficiently flexible to inhibit many different targets – a job that is perhaps particularly well suited for miRNA. Defining the full range of targets through which miR-200c functions as a tumor suppressor could provide insight into how (and why) activation of the EMT program in tumors is linked to apoptosis resistance and the CSC phenotype, a critical question yet to be fully addressed.

Abbreviations

CSC, cancer stem cell; EMT, epithelial–mesenchymal transition; miRNA, microRNA; UTR, untranslated region; ZEB, zinc-finger enhancer binding.

Competing interests

The author declares that he has no competing interests.

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