IN THE SPOTLIGHT

MicroRNAs Play a Big Role in Regulating Ovarian Cancer–Associated Fibroblasts and the Tumor Microenvironment

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Summary: Mitra and colleagues analyzed microRNA expression profiles of fibroblasts isolated from ovarian cancer patients, searching for dysregulated microRNAs in the stromal compartment of human cancer. They found that decreased *miR-31* and *miR-214* and increased *miR-155* expression can reprogram normal fibroblasts into tumor-promoting cancer-associated fibroblasts. They identified CCL5, a protumorigenic chemokine that is highly expressed in tumors, as a key target of *miR-214*, thus showing that microRNA perturbation in the stromal microenvironment can affect tumor growth. *Cancer Discov; 2(12); 1078–80. ©2012 AACR.*

Commentary on Mitra et al., p. 1100 (10).

The tumor microenvironment, which includes cells such as macrophages, T cells, endothelial cells, and fibroblasts, as well as extracellular matrix components, plays an important role during tumor evolution and metastasis (1, 2). Although these stromal cells are not themselves transformed, they are often induced by tumor cells to contribute to tumorigenesis and are believed to adapt and continuously coevolve with tumor epithelial cells to foster transformation, angiogenesis, growth, and invasion (3, 4). These desmoplastic changes to the tumor stroma are observed in many cancer types, including breast, prostate, skin, pancreatic, and ovarian cancers. As a result, these nonmalignant tumor components have emerged as attractive therapeutic targets.

Fibroblasts are one of the principal constituents of the tissue microenvironment. In nonmalignant tissues, fibroblasts are capable of adapting to tissue injury, and during wound healing, change their phenotype to become reactive. The reactive fibroblast, also known as a myofibroblast, shares properties with both fibroblasts and smooth muscle cells. In addition to wound healing, reactive fibroblasts are also found in malignant cancers and referred to as cancer-associated fibroblasts (CAF), which differ from normal fibroblasts by their unusually high expression of smooth muscle actin and their protumorigenic properties (1, 5, 6). They secrete a repertoire of proinflammatory molecules, growth factors, and proteinases, including interleukins (e.g., IL-6), chemokines [e.g., C-X-C motif ligand 12/stromal cell–derived factor (CXCL12/SDF-1α)], vascular endothelial and platelet-derived growth factors (e.g., VEGF and PDGF), matrix metalloproteinases, and extracellular matrix components (e.g., tenascin C, fibronectin, and collagen type I; ref. 7). These factors recruit other cell types to the primary tumor, and even to future sites of metastatic colonization (8), and actively remodel the surrounding tumor microenvironment to facilitate growth, invasion, and metastasis. Indeed, perturbation of fibroblast homeostasis by *Pten* deletion in the mammary gland accelerates the initiation, progression, and malignant transformation of mammary epithelial tumors (9). This phenomenon is associated with extensive extracellular matrix remodeling, immune infiltration, and angiogenesis, thus illustrating the powerful effects CAFs exert on the tumor microenvironment, which in turn affect tumor development.

The origin of CAFs and the mechanisms that regulate their development remain largely unknown. One model posits that CAFs are generated locally by inducing normal fibroblasts to take on CAF properties, a process that is mediated by soluble paracrine signals secreted by the tumor (6). One striking finding is that the CAF phenotype is stable and sustainable over multiple passages *in vitro* (5). Although the signals that govern the transition are not fully understood, recent evidence suggests that TGF-β and IL-1β play important roles (6, 7). Other work has suggested that the epithelial–mesenchymal transition can convert malignant epithelial cells into CAFs under certain conditions (7), although it remains controversial whether the genomes of CAFs are identical to those of the malignant cells. Another possibility is that CAFs originate from bone marrow–derived cells or mesenchymal stem cells, which differentiate *in situ* into CAFs. Support for each of these hypotheses can be found in the literature. However, several outstanding questions remain: What drives CAFs to acquire tumor-promoting functions? What genetic networks are activated in normal fibroblasts that convert them into CAFs? How is the CAF phenotype sustained over time, and is the phenotype reversible?

In this issue of *Cancer Discovery*, Mitra and colleagues (10) investigated the role of microRNAs (miRNA) in regulating the CAF phenotype in human ovarian cancer. miRNAs are small noncoding RNA molecules that negatively regulate gene expression at the posttranscriptional level and have recently been implicated in a number of developmental processes, including cell differentiation and reprogramming.

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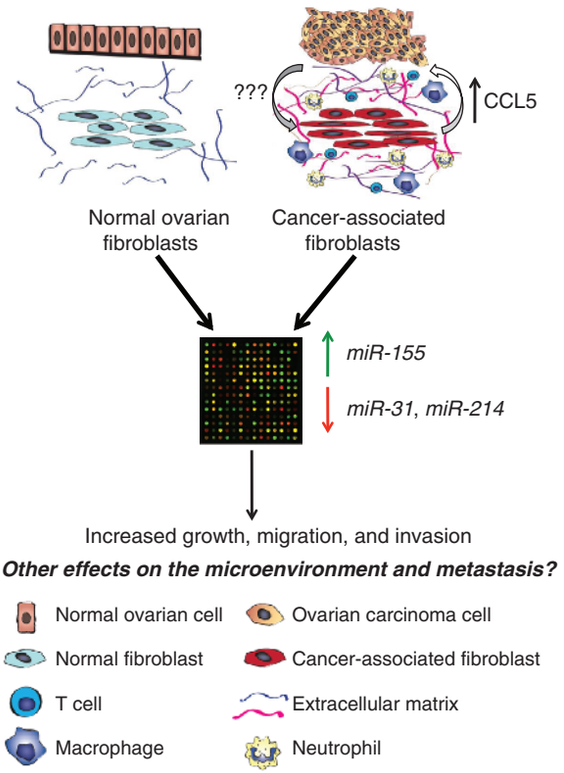

Figure 1. Summary of the experimental strategy and key findings of Mitra and colleagues (10). The authors isolated CAFs and adjacent normal fibroblasts from ovarian cancer patients and looked for differentially expressed miRNAs using microarrays. They found increased expression of *miR-155* and decreased expression of *miR-214* and *miR-31* in CAFs. Perturbation of these miRNAs was sufficient to generate induced CAFs from normal fibroblasts. One key target of *miR-214* was *CCL5*, which provided tumor-promoting signals back to the carcinoma cells. The tumor-derived signals that regulate expression of these miRNAs remain to be determined, as well as effects on other components of the tumor microenvironment. CCL5, C-C motif ligand 5.

Although many studies have focused on miRNAs expressed by tumor cells, few studies have analyzed miRNAs expressed by CAFs. Mitra and colleagues (10) sought to determine differences in the miRNA expression profiles of normal fibroblasts and CAFs (Fig. 1). Accordingly, they isolated primary CAFs and adjacent normal fibroblasts from ovarian cancer patients, generated induced human CAFs by coculturing normal fibroblasts (isolated from human patients undergoing benign gynecologic surgery) with tumor cells, and conducted miRNA array studies. Interestingly, several differentially expressed miRNAs emerged as interesting candidates to control the CAF phenotype.

To show that these candidates played an important role in CAF induction, the authors used a combination of *in vitro* and *in vivo* assays to study the effects of *miR-31*, *miR-214*, and *miR-155*. They showed that transient perturbation of these 3 miRNAs was sufficient to convert normal ovarian fibroblasts into induced CAFs that promoted ovarian tumor growth and increased tumor cell invasiveness and migration. Conversely, they showed the reverse was also true, and that CAFs could be retro-converted into more normal fibroblasts by overexpressing the downregulated miRNAs (*miR-31* and *miR-214*) and knocking down the upregulated miRNA (*miR-155*).

Using a screen for chemokines and cytokines, Mitra and colleagues (10) identified a target of *miR-214* to be *CCL5* and showed that *miR-214* inversely regulated *CCL5*. Importantly, the authors showed that downregulation of *miR-214* increases CCL5 production, leading to increased tumor growth. An anti-CCL5 antibody blocked the effect of CAFs on tumor growth and cell migration. This work illustrates that miRNA dysregulation in CAFs, not epithelial tumor cells, is capable of affecting tumorigenesis and highlights the importance of miRNAs in controlling the tumor microenvironment.

One of the most notable aspects of the study is that the authors used primary ovarian fibroblasts from omental metastases of human patients to conduct their microarray study. The authors compared these fibroblasts with normal adjacent fibroblasts from the same patient, as well as ovarian fibroblasts from patients with benign gynecologic disease. In addition, they created induced CAFs by coculturing the normal primary human fibroblasts with ovarian cancer cells. This is a key strength of the study, as it immediately validates the clinical relevance of the findings and uses complementary strategies to isolate CAFs. In addition, the study sheds light on how the CAF phenotype is maintained by reversible epigenetic modifications, namely, miRNA regulation. This gives us insight into how CAFs sustain their tumor-promoting properties, which may be a therapeutically targetable pathway. Finally, the study emphasizes the complexities of the cross-talk between the tumor and stromal compartments, adding miRNAs to the conversation.

However, several missing links remain that will certainly be important to address in future studies. For example, what upstream tumor-derived signals promote and inhibit expression of these 3 miRNAs? In their experiments, the authors show that ovarian cancer cells are capable of inducing normal ovarian fibroblasts to become CAFs. Yet the nature of this signal is not explored, and it will be important to determine whether the signal is soluble or if it depends on cell–cell interactions. Although it is known that molecules such as TGF-β and IL-1β regulate CAF induction, it is unclear whether these 3 miRNAs are downstream of these signaling pathways.

In addition, what other sources of these signals might exist *in vivo*? It is an intriguing possibility that cells other than the carcinoma might also secrete factors that trigger fibroblasts to downregulate *miR-31* and *miR-214* and upregulate *miR-155*. This may establish multiple feed-forward loops to drive tumor progression and metastasis. It will be important to determine the upstream signals within the tumor microenvironment that regulate the expression of *miR-31, miR-214*, and *miR-155*.

Furthermore, although Mitra and colleagues (10) nicely show that inhibition of CCL5 using a blocking antibody abrogates the tumor-promoting properties of miR-CAFs on ovarian cancer growth, it is not yet clear by what mechanism this effect occurs. In other words, how does CCL5 regulate ovarian cancer progression and metastasis? Other chemokines such as CXCL12/SDF-1α have been shown to increase

tumor growth as well as to recruit endothelial progenitor cells to stimulate tumor angiogenesis (5). Whether CCL5 promotes tumor progression through a non–cell-autonomous mechanism by modification of the microenvironment in ovarian cancer remains to be determined.

Finally, the authors identified 3 miRNAs that are important in ovarian CAFs, yet have only elucidated one target of one miRNA. How do *miR-31* and *miR-155* function in CAF induction, and what downstream chemokines, cytokines, or growth factors are involved? Do the targets of *miR-31* and *miR-155* interact with other cells in the microenvironment? *miR-31* was recently shown to be antimetastatic in mammary epithelial cancer cells by modulating integrin α5, radixin, and RhoA (11). Whether these targets are operative in CAFs remains to be determined. These questions, and others that will stem from this exciting work, will be important to answer in the near future and may provide important considerations and new strategies for therapeutic intervention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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