IN THE SPOTLIGHT

miR-23a, a Critical Regulator of "migR"ation and Metastasis in Colorectal Cancer

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Summary: Jahid and colleagues have shown that *miR-23a* promotes the transition from indolent to invasive colorectal cancer through inhibition of the MTSS1 tumor suppressor. This study reveals a novel role of *miR-23a* in the acceleration of colorectal cancer progression. *Cancer Discov; 2(6); 489-91. ©2012 AACR.*

Commentary on Jahid et al., p. 540 (6).

Human colorectal cancer is a highly aggressive malignant disease, which remains the fourth most common human cancer and the second leading cause of cancer-related mortality in the United States. It is estimated that approximately 103,170 new colorectal cancer cases will be diagnosed and 51,690 deaths will occur in 2012 (1). Despite recent therapeutic advances, patients are often diagnosed with colorectal cancers at late stages that have already metastasized either as micro- or macro-metastatic disease. Because metastasis is the leading cause of treatment failure and tumor recurrence, there is an urgent need for achieving earlier diagnosis and developing innovative treatment strategies for improving the overall treatment outcome of this deadly disease (1).

Although the molecular mechanisms for colorectal cancer metastasis are not fully elucidated, accumulating evidence suggests that microRNA (miRNA) could play a critical role. miRNAs bind to the 3′ untranslated region (UTR) of target mRNAs, subsequently leading to target mRNA translational repression or degradation (2). Intriguingly, certain miRNAs with deregulated expression display antitumor activities in human malignances, whereas others exhibit oncogenic activities. Furthermore, unlike the "one mRNA, one transcribed protein" theme, a single miRNA could regulate multiple mRNA targets, and one given gene could also be governed by several different miRNAs (2).

Recent studies have shown that several miRNAs (miR), including *miR-23a* and *miR-27a,* are involved in the development and progression of human cancers, although details of the underlying molecular mechanisms remain unclear and may be context-dependent. Numerous studies have shown that expression of *miR-23a* is suppressed by c-Myc in prostate cancer and lymphoma cells and is downregulated by the oncogenic promyelocytic leukemia protein–retinoic acid receptor-α (PML–RARA) fusion protein in acute promyelocytic leukemia (3). In contrast, other reports have documented upregulation of *miR-23a* in a variety of human cancers including bladder cancer, gastric cancer, glioblastoma, hepatocellular carcinoma, breast cancer, and pancreatic cancer (3). *miR-27a* has been shown to exert an oncogenic function in human tumorigenesis. For example, in breast cancer, *miR-27a* exhibits oncogenic activity through downregulating zinc finger ZBTB10 protein, a putative specificity protein (SP) repressor, leading to overexpression of SP and SP-dependent prosurvival and proangiogenic genes including survivin, VEGF, and VEGF receptor 1 (VEGFR1; ref. 4). Moreover, downregulation of *miR-27a* decreases the percentage of breast cancer cells in the S-phase. Consistent with this finding, downregulation of *miR-27a* inhibited the proliferation of gastric cancer cells which was, in part, mediated through inhibiting cyclin D1 and upregulating p21 expression (5). Interestingly, *miR-27a* was also found to be involved in drug resistance through regulation of the expression of the drug transporter MDR1 (P-glycoprotein), a protein implicated in paclitaxel resistance in a variety of human cancers. However, the roles of *miR-23a* and *miR-27a* in the development and progression of colorectal cancers have not been mechanistically investigated.

In this issue of *Cancer Discovery*, Jahid and colleagues (6) revealed the novel roles of *miR-23a* and *miR-27a* in colorectal cancer progression. To determine whether these two miRNAs are deregulated in human colorectal cancers, they measured their expression in colorectal cancer clinical tissue samples at different malignant stages. They found that *miR-27a* displayed higher expression in all tumor tissues, whereas *miR-23a* expression was more restricted to invasive colorectal cancers. Consistent with this, the authors also showed that both *miR-23a* and *miR-27a* expression levels are increased in the mouse model with intestinal invasive adenocarcinomas (6). Because colon cancer stem cells (CCSC) have been characterized to play a critical role in the invasion and metastasis processes, this group conducted locked nucleotide analogue (LNA) microarray–based miRNA profiling of CCSCs. As expected, both *miR-23a* and *miR-27a* were highly expressed in CCSCs as well as in the invasive non-CCSC colorectal cancer cell lines. Taken together, *miR-23a* and *miR-27a* are highly expressed in colorectal cancer cell lines and mouse intestinal tumor as well as in human colorectal cancer tumor tissue specimens.

To further determine the molecular mechanisms by which *miR-27a* exerts its oncogenic functions in colorectal cancers, the authors used several molecular approaches to reveal FBXW7 as a direct target of *miR-27a* (6). FBXW7 is a well-studied E3

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ubiquitin ligase that is reported to target various oncogenic proteins for ubiquitination, including cyclin E, Notch, Mcl-1, c-Myc, and c-Jun (7), and is considered a tumor suppressor largely due to its negative regulation of the stability of these oncogenic proteins. Indeed, it has been shown that FBXW7 is frequently inactivated by mutation, deletion, or promoter hypermethylation in multiple neoplasms including colon cancer (7). In addition, FBXW7 mutation was found in 11% of colorectal cancer. Lerner and colleagues (8) found that *miR-27a* suppresses FBXW7 expression during specific cell-cycle phases and promotes the degradation of proteins regulating G_1 to S-phase transition, such as cyclin E. They also showed that overexpression of *miR-27a* induces DNA replication stress and causes cyclin E dysregulation, in part, through inhibition of the FBXW7 expression (8). Moreover, Wang and colleagues (9) also independently discovered that FBXW7 is a potential *miR-27a* target and that the regulation of FBXW7 substrates such as cyclin E, c-Jun, and Notch1 might account for the observed elevation of cell growth arising from specific inhibition of FBXW7 mediated by overexpressing *miR-27a*. Consistent with previous reports, Jahid and colleagues confirmed that the E3 ubiquitin ligase FBXW7 is a direct *miR-27a* target in colorectal cancers and showed that depletion of endogenous *miR-27a* leads to the downregulation of oncogenic FBXW7 substrates such as Myc, Jun, and Notch (6). Taken together, *miR-27a* was shown to function as an oncogene, which is, in part, mediated through regulating the expression of the FBXW7 tumor suppressor in colorectal cancers.

As for the physiologic role of *miR-27a*, knockdown by LNA or short hairpin RNA led to inhibited CCSC growth and suppressed clonogenicity *in vitro*. Conversely, *miR-27a* overexpression promoted cell growth and increased clonogenicity in colorectal cancers. Furthermore, *miR-27a* knockdown significantly retarded *in vivo* tumor growth in a mouse xenograft model.

Interestingly, *miR-23a* knockdown had modest and minimal effect on cell proliferation and clonogenicity, respectively. Consistent with this notion, *miR-23a* knockdown did not cause a reduction in tumor volume *in vivo* either. Taken together, these results suggest that *miR-27a*, but not *miR-23a*, promotes cell proliferation in colorectal cancers both *in vivo* and *in vitro*. Having excluded a role for *miR-23a* in cell proliferation, the authors continued to further dissect a role for *miR-23a* in CCSC cell migration and invasion (6). To this end, one recent study has shown that *miR-23a* promotes colon cancer cell growth, invasion, and metastasis through inhibiting metastasis suppressor (*MTSS*) gene expression (10). Furthermore, the upregulation of *miR-23a* expression was associated with an advanced clinical stage and the depth of invasion, as well as lymph node metastasis, indicating that *miR-23a* could be a biomarker for the prognosis of colorectal cancers. In line with this finding, Jahid and colleagues (6) found that *miR-23a* knockdown inhibited cell motility, cell migration, and invasion. Moreover, they showed that suppression of migration and invasion by *miR-23a* knockdown is primarily due to upregulation of its target MTSS1 and downstream inhibition of Src signaling pathway (6), indicating that *miR-23a* could play an important role in cell migration and invasion, leading to the development of invasive colorectal cancers.

To further determine the roles of *miR-23a* and *miR-27a* in metastasis, Jahid and colleagues (6) conducted an *in vivo* assay of metastasis by injection of cells into the tail vein of immunodeficient mice. They showed that both *miR-23a* and *miR-27a* knockdown led to fewer lung tumors. Moreover, knockdown of *miR-23a* or *miR-27a* increased overall survival of mice. Together, they obtained experimental evidence to support the notion that *miR-23a* primarily increases cell motility, whereas *miR-27a* mainly promotes cell proliferation (Fig. 1).

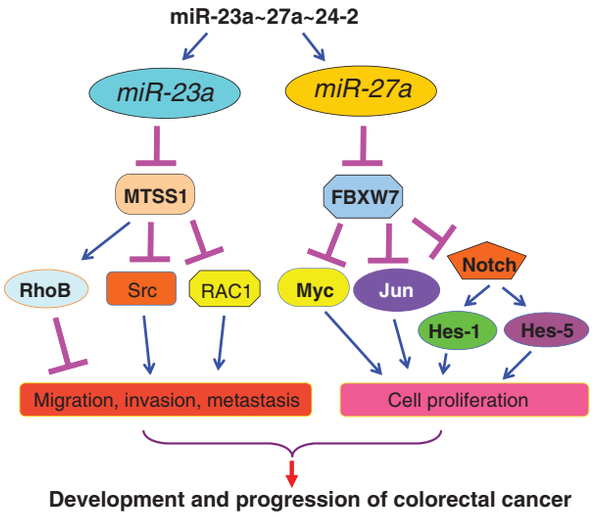

Figure 1. The critical roles of *miR-23a* and *miR-27a* in colorectal cancer progression. *miR-23a* primarily increases cell motility through downregulation of its target MTSS1 and upregulation of the Src signaling pathway, whereas *miR-27a* promotes cell proliferation, in part, via inhibition of FBXW7, leading to the upregulation of FBXW7 ubiquitin substrates including Myc, Jun, and Notch.

These interesting studies shed light on the roles of *miR-23a* and *miR-27a* in colorectal cancer progression; however, several questions still need to be addressed in follow-up studies. For example, why was *miR-23a* expression lower in the late stage of patients with colorectal cancers? Why is there high expression of *miR-27a* target genes even though *miR-27a* levels do not decrease in colorectal cancers? Why did expression of cyclin E, a FBXW7 target, not change after *miR-27a* knockdown? Are additional miRNAs involved in colorectal cancer progression? Could *miR-27a* and *miR-23a* be biomarkers of prognosis for colorectal cancers, or are they only restricted to invasive colorectal cancers? Without a doubt, further studies will be ignited and are warranted to determine the physiologic functions of *miR-23a* and *miR-27a* in the development and progression of colorectal cancers.

Although further investigation is required to fully answer the questions raised above, the results reported by Jahid and colleagues (6) open a new avenue to target *miR-23a* and *miR-27a* for clinical benefits. Intriguingly, one recent study has shown that natural compound derivatives could decrease the expression of *miR-27a* in colorectal cancer cells (11) and inhibit cell growth and induction of apoptosis, suggesting that *miR-27a* could be a useful therapeutic target for colorectal cancers. We anticipate that establishing oncogenic roles of *miR-23a* and *miR-27a* in colorectal cancer progression will thus open new research directions for clinical management of this deadly disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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