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The roles of microRNAs and epithelial-mesenchymal transition in colorectal cancer metastasis*

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Abstract Colorectal cancer (CRC) is the second most common cause of cancer death worldwide. Distant metastasis is the major cause of death in patients with CRC. During progression to metastasis in which malignant cells disseminate from the primary tumor to seeding other organs, a multistep process is involved. Cancer cells proliferate, invade microenvironment, enter into the blood circulation, then survive and colonize into distant organs. MicroRNAs (miRNAs) and epithelial-mesenchymal transition (EMT) are key regulators and mechanism in tumorigenesis and cancer metastasis. We review the roles of EMT and microRNAs, especially EMT related microRNAs in the metastatic pathway of CRC. MicroRNAs provide us a set of potential therapeutic applications and molecular target for CRC.

Key words colorectal cancer (CRC), microRNA, epithelial-mesenchymal transition (EMT), metastasis

Colorectal cancer (CRC) is the second most common cause of cancer death worldwide and its incidence is expected to increase in association with the ageing of western populations [1–2]. Distant metastasis is the major cause of death in patients with CRC [3]. Approximately 50% of patients diagnosed with CRC die from distant metastasis, in especial liver metastasis [4]. Therefore, distant metastasis leads to most of the mortalities and has an important role in the poor prognosis with a 5-year survival rate of 60% [5]. Primary CRC originates from epithelial cells that locate the colon and rectum. During progression to metastasis in which malignant cells disseminate from the primary tumor to seeding other organs, a multistep process is involved. Cancer cells proliferate, invade microenvironment, enter into the blood circulation, then survive and colonize into distant organs [5].

Epithelial-mesenchymal transition (EMT) and CRC metastasis

Epithelial-mesenchymal transition (EMT) was firstly discovered as a morphogenic program during the process of several tissues, organs and in wound healing [6]. A large amount of researches reveal that during many critical biological processes including tissue remodeling, restitution, wound repair and even embryonic development, epithelial cells lose adhesion and cytoskeletal components [7]. At the same time, they acquire the capability of adopting a phenotype more preferable to cell migration and movement, which are known as EMT [8]. EMT is a complex process, which includes dissolution of cell-to-cell junctions and loss of apicobasolateral polarity, resulting in the formation of migratory mesenchymal cells with invasive properties [9]. Recent studies show that one of the key molecular steps in the process of distant metastasis includes the processes by which cells switch between epithelial and mesenchymal phenotypes are which permits invasion and emigration in various cancers. Epithelium-derived tumor cells undergo EMT, which actively downregulate cell-cell adhesion systems, lose their polarity, and acquire a mesenchymal phenotype with reduced intercellular interactions and increased migratory capacity as well as invasive properties [10]. In addition, cancer cells that pass through an EMT acquire the self-renewing trait associated with stem cells and cancer stem cells, which allows them to leave the site of the primary tumor, invade surrounding tissues, migrate to distant organs and is associated with a poor prognosis in CRC [10]. Thereby activation of EMT at the invasive front allows tumor cells to detach, migrate, and disseminate through blood or lymphatic vessels. EMT provides a mechanism for carcinoma cells to acquire this

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more aggressive phenotype.

MicroRNAs (miRNAs) and CRC metastasis

MicroRNAs are a family of diverse, small, highly conserved noncoding RNAs that are processed from precursors with a characteristic hairpin secondary structure [11]. More and more researches have documented that microRNAs have essential roles in multiple biological processes, including cell differentiation, proliferation, angiogenesis, invasion and migration [12–15]. MicroRNAs usually exert their biological function as critical gene regulators. They regulate gene expression post-transcriptionally and suppress specific target genes in mammalian cells by repressing translation [16]. Depending on its target genes, a microRNA can function either as an oncogene or a tumor suppressive gene. MicroRNAs play important regulatory roles in basic biological processes that form the hallmarks of cancer, such as cellular differentiation, proliferation, invasion, migration, and apoptosis [16]. In recent years, a large number of studies have confirmed that microRNAs have important roles in tumorigenesis and metastasis by targeting different miRNAs [17–18]. The expression of microRNAs was deregulated in various kinds of human cancer. It has suggested that the expression of up to 30% of genes may be affected by microRNAs; thus microRNAs can potentially regulate thousands of genes. Approximately 50% of the human microRNAs are located at chromosomal breakpoints and therefore susceptible to dysregulation in human cancer [19].

Accumulating evidence indicates that dysregulated microRNAs are involved in cancer development, progression and metastasis. MicroRNAs are involved in the pathogenesis of CRC, partly by regulating the expression of oncogenes and tumor suppressors and partly by functioning as oncogenes or tumor suppressors themselves.

For example, the miR-135 family affects the Wnt signalling pathway by downregulating the tumor suppressor gene adenomatous polyposis coli (APC), regardless of mutation status a region that also contains the TP53 tumor suppressor gene. To date, abnormal expression of several microRNAs, such as miR-21 [20–21], miR-124 [22], miR-625 [23], miR-339-5p8 [24], miR-29b [25], miR-133a [26], miR-497 [27], miR-106a [28], and miR-335 [29], miR-221 [30] and miR-27b [31], has been identified in CRC and may contribute to the development and progression of CRC.

Mudduluru *et al* found that inhibition of miR-21 suppressed HT-29 cell proliferation, tumour growth, invasion and *in vivo* metastasis [20]. Furthermore, miR-21 was involved in Pdcd4, a tumor-suppressor's role in CRC transformation and invasion and metastasis [21]. Other studies indicated that miR-625 was significantly downregulated in CRC tissues and cell lines. Its decreased expression was positively associated with advanced lymph node metastasis, liver metastasis and poor overall survival for CRC patients [23]. These results suggested that miR-625 served as an efficient clinical biomarker and a therapeutic tool for the inhibition of metastasis in CRC. Recently, the role of miR-29b in CRC development was investigated. Data showed that decreased expression of miR-29b usually occurred in CRC cell lines and tissue samples. miR-29b was illustrated a suppressor has a critical role in CRC progression. miR-29b suppressed CRC cell proliferation and migration. In addition, miR-29b mediated the inhibition of EMT and the inactivation of MAPK and PI3K/AKT signal transduction pathway. Dysfunction of microRNA is associated with CRC tumorigenesis and progression. Furthermore, aberrant expression of specific microRNA may be used as potential prognostic and predictive markers in CRC.

EMT related microRNAs in CRC metastasis

During EMT, cancer cells promote their malignant phenotype and stem cell characteristics. EMT facilitates CRC metastasis. Recent researches reveal that EMT related microRNAs are important regulators in the progression of CRC from a primary process to metastatic disease.

Increased expression of miR-141 and miR-200c after knockdown of ZEB1, which is triggered by transforming growth factor beta (BCLXL) [32], an EMT activator, in CRC cells induced an increased E-cadherin expression and cell-cell adhesion [33]. This transition to epithelial phenotype was companied by reduced CRC cell migration and invasion. These results suggested that during metastasis, CRC cells lose their epithelial features and simultaneously gain the mesenchymal characteristics required for the EMT. Interestingly, further study showed that increased expression of miR-200c also translationally inhibits the expression of ZEB1 and induces mesenchymal-epithelial transition (MET) in cells that had previously undergone EMT [34]. This feed-forward loop of ZEB1 and the miR-200 family is important in invading cancer cells and might explain the strong phenotypic heterogeneity often seen within individual tumors and metastasis.

Yang *et al* found increased miR-182 expression in metastatic CRC cells [35]. Overexpression of miR-182 enhanced CRC cell proliferation, invasion, and migration *in vitro* and *in vivo*. MiR-182 targets SATB2 and key cellular molecules in EMT. Overexpression of miR-182 results in increased expression of Snail and mesenchymal maker Vimentin and enhanced CRC tumorigenesis and metastasis.

MiR-124 also plays a pivotal role in CRC metastasis [36]. MiR-124 was significantly down-regulated both in CRC-derived cell lines and clinical CRC tumors. By blocking

the expression of PRRX1, another EMT inducer, miR-124 also increases the radiosensitivity of CRC cells.

Another study concluded that the overexpression of miR-181a decreased expression of the epithelial markers E-cadherin and β-catenin, and enhanced expression of the mesenchymal markers vimentin [37]. These findings suggest that miR-181a plays a critical role in regulating epithelial-mesenchymal cell transition and, ultimately, promotes the invasive and/or metastatic potential of CRC by its direct target on WIF-1 gene. Higher expression of miR-181a in CRC specimens is associated with liver metastasis and poor survival, and in particular, the higher expression of miR-181a is associated with metachronous liver metastasis.

Furthermore, via suppression of SMAD4 expression, miR-20a was found to be involved in invasion and EMT programs, with its aberrant expression having been observed in a variety of malignant tumors [38]. The data revealed that miR-20a was an independent prognostic factor in CRC.

Conclusion

MicroRNAs are important regulators of gene expression, and current data suggest an important role for microRNAs in the process of EMT in CRC metastasis. MicroRNAs provide us a set of potential therapeutic applications and molecular target for CRC.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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