

Clinical and molecular aspects of miR-200 family in gastric cancer

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Gastric cancer, like all tumors, is a complex disease in which many predisposing and triggering factors, both environmental and genetic, are summed and combined until the development of the malignancy. Among the genetic causes and modifiers of the clinical course of gastric cancer, microRNAs (miRNAs) as wide-spectrum post-transcriptional regulators, play a central role. Their special characteristics, i.e., their tissue-, and even cell-type-, specificity, their stability in different biological fluids, and their deregulation during tumorigenesis, make to miRNAs the focus of a huge amount of studies searching for their application as potential biomarkers and therapeutic targets in cancer.

Tang and co-workers (1) published last year an interesting paper in which they analyze the expression of two miRNAs from miR-200 family, miR-200b and miR-200c, as putative prognostic biomarkers in gastric cancer. In addition, the authors provide novel data and insights into the function of these miRNAs and their contribution to the gastric cancer development and progression. These data include the establishment of DNMT3A and DNMT3B as direct targets for miR-200b and miR-200c. Moreover, the authors demonstrate that DNMT1 is also indirectly regulated by miR-200b and miR-200c through the control of expression of the transcriptional factor SP1. DNMT3A, DNMT3B and DNMT1 are DNA-methyltransferases which catalyze the methylation of CpG islands contributing thus to the epigenetic silencing of gene expression. The importance of these findings lies in the central role that the malfunction of epigenetic regulation plays in cancer, and specifically in gastric cancer, in which there is a generalized hypermethylation along the entire genome. Therefore, we can hypothesize that the downregulation of miR-200b and miR-200c may be part of the molecular mechanism involved in the aberrant hypermethylation found in the

majority of gastric cancers. So, when miR-200b and miR-200c are overexpressed in gastric cancer cells, as determined by the authors, it is generated a decrease in the global DNA methylation: miR-200b and miR-200c directly repress the expression of DNMT3A and DNMT3B and indirectly inhibit the expression of DNMT1 repressing the activator, SP1. As consequence of this global decrease in DNA methylation, it is triggered the restoration of expression of many genes, including those involved in the control of tumor progression (tumor suppressors), such as E-cadherin, p16 and RASSF1A. This has important implications for tumor behavior, both to clinical and biological levels. First, the overexpression of miR-200b and miR-200c reduces the proliferative and invasive capability of gastric cancer cells in vitro. And second, in the majority of gastric cancer tissues analyzed by the authors, miR-200b and miR-200c are underexpressed regarding paired non-tumor adjacent samples. These interesting findings add a novel piece to the functional puzzle already depicted in literature about the role of miR-200 miRNA family in cancer. The miR-200 miRNA family is well known to be a key determinant of epithelial phenotype of cancer cells through the regulation of E-cadherin transcriptional repressors, ZEB1 and ZEB2 (2-4). Additionally, ZEB1 is able to reciprocally repress the expression of miR-200 miRNA family members, miR-200c and miR-141 (5) thus constituting a double negative feedback regulatory loop. This highly unstable system depends on small changes in the levels of miR-200 miRNA family members and ZEB transcriptional factors to induce switches between the epithelial and mesenchymal cell states. This epithelial-to-mesenchymal plasticity (EMP; term that embraces the phenomena of epithelial-to-mesenchymal transition, EMT, and the mesenchymal-to-epithelial transition, MET)

is key to understand many of invasive and proliferative characteristics of tumors. The system is so sensitive to micro-environmental cues that any molecular disturbance affecting the delicate balance between its members is able to drive changes in cell phenotype. In this sense, it is paradigmatic the effect that TGF-beta signaling displays on this system for the establishment and maintenance of mesenchymal phenotype (6). TGF-beta signaling not only is able to activate the sustained ZEB expression with the consequent miR-200 repression, but also can reinforce and stabilize this inhibition by hypermethylation of the miR-200 loci upon prolonged exposure to the signal. This last data is in accordance with the fact that, as demonstrated by Tang and co-workers (1), miR-200b and miR-200c are regulators of several DNMTs, and with the hypomethylation seen in liver metastasis from colorectal cancer overexpressing miR-200c and miR-141 (7). Furthermore, since TGF-beta cytokines are proven targets for miR-200 family members, the inhibition of miR-200 expression enhances the TGF-beta production and contributes to the maintenance of an autocrine/paracrine signaling and its effects, i.e., the stabilization of mesenchymal state. However, given the structure of the miR-200/ZEB/TGF-beta axis, the interruption in the inductive signal (in this case, TGF-beta) causes re-expression of miRNAs from miR-200 family and reversion to an epithelial phenotype. Thus, different cancer cells in different moments may display different phenotypic states depending on the signal affecting cells in such moments. And, in fact, inside of tumors and metastases, many molecular cues coming from microenvironment (stromal cells, immune cells, endothelial cells, etc.) interact with cancer cells to modulate their appearance and behavior. This, in combination with the elevated genetic heterogeneity existing among cells within tumors and metastases, makes that such cells can display a highly variable phenotype through the expression of different genes and miRNAs. And more importantly, cells with distinct phenotypes are not randomly distributed within tumors but they occupy a specific location in function of their capabilities, like a living organism that evolves and wants its own survival. In this way, it has been demonstrated in colorectal cancer that cells in the invasive front of tumor undergo complete EMT, associated with loss of miR-200 family members and E-cadherin expression, and increase in ZEB1 levels (8). This contrasts with the elevated expression of miR-200 in the tumor core (8) where tumor cells display epithelial characteristics necessary for their intense proliferation (7). The mesenchymal phenotype of

cells at the invasive front of tumor enables them to destroy and migrate through the basement epithelial membrane reaching other local and/or distant tissues. However, once tumor cells reach a secondary location, they become epithelial again by using the mechanism of MET, which allows their expansion and colonization. This phenotypic reversion has been seen in colorectal tumors where distant liver and regional lymph node metastasis and proximal vascular tumor deposits show elevated levels of different miRNAs from miR-200 family (7,8). In line with this, it has been demonstrated in vitro that the overexpression of miR-200 is a distinctive feature of breast cancer cell lines with elevated metastatic potential (9,10). This phenotypic duality may explain the discordance found in the expression of miR-200 and other miRNAs between primary lesions and metastases in different studies, perhaps due to the analysis of primary tumors with different preponderant pathological and phenotypic characteristics (9,11). Also, while in primary gastric tumors, low levels of miR-200b and miR-200c have been found regarding healthy tissue (1), in patients' blood, high levels of miR-200c were found regarding healthy controls (12), indicating poor prognostic. Beyond their role regulating the TGF-beta/ZEB/E-cadherin axis, the miRNAs from miR-200 family play other functions, many of them yet undiscovered, in consonance with the nature of miRNAs as multi-target regulatory tools. And these functions, when deregulated, have important implications both in the proliferation and dissemination of tumors. For example, studies in vitro demonstrated that the expression of Sec23a, a protein involved in the secretory pathway, is controlled by miR-200 (9), and that the upregulation of miR-200 is able to elicit deep changes in the secretome of cancer cells with potential effects on the metastatic process (9,13). Also, it has been recently demonstrated the role of miR-200 inhibiting angiogenesis (14), which might explain the attraction of blood vessels toward the invasive front of tumors where miR-200 is downregulated.

In the clinical setting, Tang and co-workers (1) demonstrated that the downregulation of miR-200b and miR-200c in primary gastric tissues is able to predict shorter survival. The prognostic significance of miR-200a and miR-200b downregulation in primary gastric tumors was also confirmed in a recent article in which these miRNAs were found to form part of a mesenchymal miRNA signature associated with poor outcome (15). In addition to their role in prognostic assessment, miRNAs from miR-200 family may also be related to resistance to systemic therapy in gastric cancer. Thus, in a recent paper, it has been shown that three

miRNAs from miR-200 family (miR-141, miR-200a and miR-200b) are only expressed in 10-hydroxycamptothecin-sensitive gastric cancer cell lines (16). The fact that gastric cancer cells expressing miR-200 may be more sensitive to chemotherapy could have a direct impact on the measure of outcomes: since only those patients over-expressing miR-200 are more sensitive to treatment, only these patients show better survival. And more importantly, this opens the door to the use of miRNAs from miR-200 family as hypothetical predictive biomarkers. The findings of Tang and co-workers (1) also could point to a potential therapeutic solution for gastric cancer patients with downregulation of miR-200 and poor response to treatment. Given that these patients show an overall increase in DNA methylation, a treatment option to explore could be the administration of any demethylating agent in combination with standard therapy. This therapeutic approach perhaps could restore the expression of key tumor suppressors (17), including miR-200 family, thus minimizing the invasive capacity of gastric cancer cells and increasing their sensitivity to conventional chemotherapy.

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