Emerging Role of miR-106b-25/miR-17-92 Clusters in the Control of Transforming Growth Factor β Signaling

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Abstract

Inactivation of the transforming growth factor β (TGFβ) tumor suppressor pathway is a main step in the development of a variety of human tumors. The miR-106b-25 and miR-17-92 clusters are emerging as key modulators of TGFβ signaling in gastrointestinal and other tumors, interfering with cell cycle arrest and apoptosis when overexpressed in cancer cells. Genetic ablation of these microRNAs (miRNAs) reveals their physiologic role in the control of liver and central nervous system apoptosis, supporting the notion that miRNA-based homeostatic mechanisms can be usurped by cancer cells to resist TGFβ tumor suppression. [Cancer Res 2008;68(20):8191–4]

Escaping the Transforming Growth Factor β Tumor Suppressor Pathway

Transforming growth factor β (TGFβ) is a signaling molecule with a wide spectrum of functions that depend on the specific biological context. This cytokine activates the serine/threonine kinase TGFβ type-2 receptors (TGFBRII) that, in turn, recruit type-1 receptors (TGFBRI) to form active heterotetrameric complexes. Signal transduction is mediated by receptor-activated SMADs that are phosphorylated by TGFBR, associate with common mediator SMADs, and translocate to the nucleus where they interact with DNA and transcription factors to control the expression of target genes.

Although TGFβ promotes growth and development during early embryogenesis and in some adult mesenchymal cells, most mature tissues respond to TGFβ by undergoing cell cycle arrest and/or apoptosis (1). This differential response to TGFβ is dictated by mostly unknown molecular switches that divert TGFβ signaling toward activation of either prosurvival or proapoptotic genes.

In the gastrointestinal tract, spatial gradients of TGFβ and other cytokines instruct epithelial cells to transiently proliferate, differentiate, and eventually die as they migrate away from the stem cell niche, coordinated by a complex system of cross-talk between the epithelial and the stromal compartments (2). This mechanism ensures the proper turnover of gastrointestinal epithelial cells, preventing the propagation and accumulation of acquired genetic defects.

Because transformed cells must escape TGFβ-induced cell cycle arrest and apoptosis to proliferate, impairment of TGFβ signaling is a condicio sine qua non for the development of gastrointestinal tumors. As a matter of fact, inactivation of the TGFβ pathway is a hallmark of gastrointestinal malignancies and several escape routes have been described, from loss of the TGFBR1 and/or TGFBRII receptors to inactivating mutations of signal transduction SMAD molecules up to dysfunctional alterations of TGFβ downstream effectors, such as the cell cycle inhibitor CDKN1A (p21) and the proapoptotic gene BCL2L11 (BIM; ref. 3). However, only a fraction of gastrointestinal sporadic tumors exhibit inactivating mutations in early tumorigenesis, suggesting that other mechanisms must play a role in the inactivation of this pathway (1).

Furthermore, because inactivating mutations are more frequent downstream of SMAD proteins, a major portion of the TGFβ pathway remains intact in many tumors. In such cases, tumor cells may use other noncytostatic aspects of TGFβ signaling, reminiscent of early development, to acquire a more malignant phenotype. For example, TGFβ can stimulate the epithelial-mesenchymal transition in gastrointestinal tumor cells and promote metastasis (1).

MicroRNAs (miRNAs) have recently emerged as key regulatory molecules playing a role in a variety of cellular process, including differentiation, self-renewal, proliferation, apoptosis, stress response, and metabolism (4–6). miRNA genes are transcribed as noncoding transcripts, processed through a series of sequential steps involving the enzymes Drosha and Dicer, and finally incorporated into the RNA-induced silencing complex to mediate target miRNA repression of translation and/or degradation. It is thought that more than 30% of human genes are posttranscriptionally regulated by miRNAs (7).

A role for miRNAs in human tumorigenesis firstly became apparent in 2002 when our laboratory identified a small genomic region involved in translocations and deletions of chromosome 13q14, frequently altered in chronic lymphocytic leukemia, which contained miR-15a and miR-16-1 genes (8). Extensive analysis of miRNA expression profiles in most human tumors subsequently revealed that several miRNAs are deregulated in human cancer (9, 10) and can be used to predict tumor origin (11). As a proof of concept that at least some miRNA alterations predispose to cancer and are not a consequence of cell transformation, the Hammond and Hannon laboratories showed that lentiviral transduction of the miR-17-92 cluster into hematopoietic cells dramatically accelerates lymphomagenesis in MYC transgenic mice (12), whereas we found that conditional overexpression of miR-155 in the B-cell lineage causes the development of pre-B-cell leukemias (13).

Nonetheless, the mechanisms and pathways targeted by cancer-related miRNAs are beginning to be uncovered. A collection of studies recently published on Cell, Cancer Cell, and Nature Immunology has shed new light on the role of miR-17-92 and its paralog miR-106b-25 in development and tumorigenesis (14–17). These clusters of miRNAs, located on different chromosomes, derive from a unique gene that underwent a series of duplications, mutations, and loss of individual miRNAs during the early evolution of vertebrates, resulting in the selection of similar but
not identical clusters (Fig. 1A). The most obvious difference is the lack of miR-18 and miR-19 homologues in the miR-106b-25 cluster. However, subtle differences between homologue miRNAs may well have functional importance: for example, miR-106b matches miR-17-5p sequence but it is three nucleotides shorter at the 3’ end, possibly affecting its intracellular localization, whereas miR-93 sequence somehow diverges from homologue miRNAs and resembles stem cell–specific miR-291-3p, miR-294, and miR-295.

Whether such differences reflect a gain of functional specificity by each cluster, which might justify their segregation into separate transcriptional units, is still unclear. Moreover, the role of a third paralog cluster, miR-106a-92, almost identical to miR-17-92 but rarely expressed in adult human tissues, remains obscure.

**New Insights into miR-106b-25/miR-17-92 Oncogenic Mechanisms**

Besides early studies from the Mendell laboratory establishing a role for miR-17-92 in controlling E2F1 expression (18) and promoting tumor angiogenesis (19), little is known about the molecular mechanisms at the basis of its oncogenicity. Moreover, the role of miR-106b-25 paralog cluster had been ignored thus far.

In the March issue of *Cancer Cell*, we describe a mechanism whereby TGFβ signaling is impaired at multiple levels by overexpression of the miR-106b-25 cluster in gastric cancer (14). A complementary study by the Jacks laboratory, published on *Cell*, addresses the role of these clusters in animal development and sheds new light on the functional relationship between miR-106b-25 and miR-17-92 clusters (15).

The miR-106b-25 cluster is composed of the highly conserved miR-106b, miR-93, and miR-25 that accumulate in different types of cancer, including gastric, prostate, and pancreatic neuroendocrine tumors, neuroblastoma, and multiple myeloma. These miRNAs are located in a 515-bp region at chromosome 7q22, in the intron 13 of the host gene *MCM7*, where they are cotranscribed in the context of *MCM7* primary transcript. Although amplification of this region in gastric cancer has been reported in several studies, *MCM7* overexpression is a bad prognostic indicator in prostate and endometrial cancer. This raises the possibility that *MCM7* oncogenic properties could be linked, at least in part, to the hosted miRNAs.

*MCM7* is induced by E2F1, a transcription factor that controls the G1-S transition activating a variety of genes involved in DNA replication, and belongs to a family of specialized proteins that...
licenses chromosomal DNA to undergo replication once, and not more than once, at each cell cycle. It has been proposed that overexpression of MCM7 may "relicense" chromosomal DNA within the same cell cycle, thus causing aneuploidy. However, because MCM7 mRNA and the intronic miR-106b-25 cluster derive from the same transcript, it is conceivable that MCM7 and miR-106b-25 cooperate in exerting their oncogenic function through different complementary mechanisms.

Because MCM7 mRNA and preliminary miR-106b-25 are cotranscribed, E2F1 regulates the expression of both MCM7 and miR-106b-25. In fact, E2F1 increases the expression of MCM7 and miR-106b-25 precursors with identical kinetics, whereas E2F1 silencing by RNA interference (RNAi) parallels miR-106b-25 precursor down-regulation. Although the possibility of a miR-106b-25-independent promotor cannot be excluded, there are not known E2F binding sites up to ~5,000 bp from the miR-106b-25 locus, whereas MCM7 mRNA and miR-106b-25 precursor levels perfectly correlate in primary gastric tumors and normal mucosa. Therefore, at least in the case of E2F1-dependent regulation, MCM7 drives miR-106b-25 cluster expression.

It is very reasonable that other MCM7 transcriptional regulators, such as MYC, MYCN, and AIB-1, can also activate this cluster in specific contexts. For example, we observed MYC-dependent regulation of miR-106b-25, whereas MYCN, an oncogene frequently amplified in neuroblastoma, may cause the reported elevation of miR-106b-25 in these tumors. However, only E2F1 showed a clear correlation in its expression with miR-106b-25 in primary gastric tumors.

In addition, tumors with elevated miR-106b-25 precursors display variable expression of each mature miRNA, predicting a further level of posttranscriptional regulation. Thus, multiple alterations occur during tumorigenesis, other than loss of transcriptional control, resulting in the accumulation of one, two, or all the three mature miRNAs in gastric tumors.

The picture becomes even more complex as mature miR-106b and miR-93 directly regulate E2F1 expression interacting with highly conserved binding sites on its 3'-untranslated region. The miR-17-92 cluster also regulates E2F1 expression through the same sites (19). This mechanism establishes a negative feedback loop between miR-106b-25/miR-17-92 and E2F1 that may work as a sensor to control E2F1 protein levels, providing cancer cells a way to escape E2F1-induced apoptosis. In fact, as with most oncogenes, overexpression of E2F1 above a critical threshold is perceived as an apoptotic signal.

The functional implications of miR-106b-25 overexpression in gastric cancer are tightly linked with the TGFβ tumor suppressor pathway. In fact, these miRNAs silence two main downstream effectors of TGFβ signaling: the cell cycle inhibitor CDKN1A (p21) and the proapoptotic gene BCL2L11 (BIM). Whereas miR-106b and miR-93 suppress p21 expression, which is required for TGFβ-induced cell cycle arrest, miR-25 silences BIM expression, which is essential for TGFβ-dependent apoptosis. Similarly, miR-17-5p and miR-20a repress p21 expression, whereas miR-92 inhibits BIM expression, suggesting that miR-106b-25 and miR-17-92 cooperate in inactivating the TGFβ pathway: gene dosage is critically important in this context as loss of even a single SMAD4 or BIM allele results in haploinsufficiency phenotypes leading to cancer (1).

On the other hand, the physiologic role of these miRNAs emerges exclusively in the context of active TGFβ signaling in fact, their silencing does not visibly alter the proliferation and the survival of gastric cancer cells in the absence of TGFβ, still supporting the notion that these miRNA clusters mainly exert their function within the TGFβ pathway.

Nonetheless, miR-106b-25/miR-17-92 targets occupy critical nodes at the intersection between the TGFβ pathway and the MYC network. Whereas the TGFβ pathway suppresses proliferation by down-regulating MYC and inducing p21 and other cyclin-dependent kinase inhibitors, MYC impairs the TGFβ pathway mainly inhibiting p21 transcription. A fine balance between these pathways is crucial to maintain the proper control of cell proliferation and apoptosis. However, in normal cells, MYC is programmed to activate BIM and trigger apoptosis as a safeguard mechanism should TGFβ control fail (20). Therefore, overexpression of miR-106b-25/miR-17-92 may not only impair TGFβ signaling but also allow undisturbed accumulation of MYC in a context where BIM cannot be translated, providing a further mechanism of evasion from apoptosis. Because MYC and E2F1 can transactivate each other and the miR-106b-25/miR-17-92 clusters, this may trigger a positive feedback loop that would support MYC, E2F1, and miR-106b-25/miR-17-92 elevated expression while inactivating the TGFβ pathway (Fig. 1B).

The role of BIM in mediating miRNA-dependent apoptosis is supported by recent work by the Rajewsky and Jacks laboratories showing extensive apoptosis in the pre-B-cell compartment in B-cell–specific Dicer knockout (16) and constitutive miR-17-92 knockout mice (15) and expansion of the lymphocytic compartment in B-cell–specific miR-17-92 transgenic mice (17). In these models, the observed phenotypes are associated with alterations in apoptosis and BIM expression. Most importantly, the apoptotic phenotype observed in Dicer knockout mice can be partially rescued by generation of B-cell–specific Dicer knockout mice on a BIM-null genetic background.

Inactivation of the TGFβ pathway by miRNA interference in gastric tumors can be ascribed to p21 and BIM suppression because specific silencing of these genes by RNAi abrogates the response to TGFβ and recapitulates the effects of miR-106b-25. Similarly, p21 silencing recapitulates the cell cycle effect of the miR-106b family in breast cancer (21). This is quite surprising as these miRNAs have hundreds of predicted targets. A recent study from the Weinberg laboratory also showed that miR-10b enhances cell motility and invasiveness of breast cancer cells, although this phenotype is completely reverted on constitutive expression of the miR-10b target HOXD10 (22). Although these observations do not exclude biological contexts where parallel regulation of multiple targets by a single miRNA is necessary to exert a specific function, they implicate that "primary miRNA targets" become critical in specific contexts.

It is possible that miR-106b-25 and miR-17-92 play a role in dictating the pacing of TGFβ physiologic response in the gastrointestinal tract, repressing p21/BIM expression in proliferating cells and allowing sequential expression of p21 and BIM as cells mature and undergo TGFβ-induced cell cycle arrest and apoptosis. That miR-106b-25 and miR-17-92 physiologically control apoptosis is supported by the work of Ventura and colleagues (15) showing that miR-17-92/miR-106b-25 double knockout mice exhibit a much more severe phenotype compared with miR-17-92 single knockout mice, characterized by prenatal lethality and extensive apoptosis in the liver, spinal cord, and lateral ganglionic eminences.

\[ F. \text{ Petrocca, unpublished data.} \]
In cancer disease, uncontrolled expression of miR-106b-25 and/or miR-17-92 would hijack this regulatory mechanism, providing a route of escape from TGFβ-dependent cell cycle arrest and apoptosis to transformed cells.

Establishing the Diagnosis of miRNA-Dependent Tumors

Taken together, these studies suggest that physiologic miRNA-based regulatory mechanisms can be usurped by cancer cells and diverted to favor their growth and provide evasion from apoptosis. As the functional characterization of cancer-related miRNAs proceeds, more and more similar mechanisms are emerging whereby aberrant miRNA expression can silence the expression of tumor suppressor genes or release oncogene expression. Unraveling the complexity of these mechanisms will provide the rationale to identify, and eventually treat, human tumors driven by miRNA alterations.

The clinical potential of these findings resides in the nature itself of miRNAs, whose function can be mimicked by small interfering RNA (siRNA)-like molecules or inhibited by small antisense oligonucleotides. Therefore, the progress in siRNA delivery achieved in nonhuman primates and the experience gained from ongoing clinical trials with siRNA-based formulations may be directly transferred to manipulate miRNA expression in a clinical setting. Discovering that, in well-defined gastric tumors, miRNAs interfere with TGFβ tumor suppression will certainly make miR-106b-25/miR-17-92 antisense oligonucleotides interesting candidates for future translational studies and personalized therapies.

However, the therapeutic potential of these and other cancer-related miRNAs needs to be thoroughly weighed against their physiologic role in normal tissues. Because miR-106b-25 and miR-17-92 seem to be expressed at low levels in normal, terminally differentiated cells while they tend to accumulate in less differentiated, proliferating cancer cells, they may represent ideal targets for cancer therapy. On the other hand, the same miRNAs may be critical in promoting the proliferation of normal, transit-amplifying progenitor cells in the context of tissue repair or renewal, transiently relieving the TGFβ antiproliferative pressure in the earliest steps of cellular differentiation. Current knowledge supports the notion that these miRNAs are essential at least in developing organisms (15, 16). Hence, it will be of primary importance to define to which extent these miRNAs are dispensable in adult tissues and, eventually, realizing the promise of nanotechnology to develop cancer-specific RNAi delivery agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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