



miR-100 and miR-125b regulate epithelial-mesenchymal transition and drug resistance in tumors

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MIR100HG is a miRNA-host long non-coding RNA (lncRNA) gene encoding in its third intron for pre-miR-100, pre-miR-125b-1 and pre-let-7a-2 giving rise to mature miR-100, miR-125b and let-7a after processing. lncRNAs are transcripts longer than 200 nucleotides that can be capped, spliced, and polyadenylated but are not translated into proteins. They often function as cis and trans-acting regulatory molecules that regulate gene transcription by recruiting chromatin-remodelling complex to a specific chromatin locus. Several lncRNAs loci show aberrant expression and mutations in different cancer types possibly playing an important role in tumor biology and representing interesting potential therapeutic targets (1). Among the different lncRNAs recently studied, MIR100HG has been implicated in several tumor types and has been shown to exert regulatory functions both by miRNA-dependent and independent mechanisms, thus adding to the complexity and redundancy of the miRNA (miR) control of gene expression. MIR100HG resulted overexpressed in early stage cervical cancer, where it was associated to lymph-node metastases (2), and in triple negative breast where overexpression associated with poor prognosis and to the regulation of p27-promoted cell proliferation through the formation of an RNA-DNA triplex (3). Moreover, the polymorphism rs1816158 in intron 1 of MIR100HG was associated with poor survival in oral cancer, and the expression of miR-100, one of the three MIR100HG-embedded miRs, resulted significantly associated with survival in head and neck squamous cell carcinoma (4). Although the studies are still at the beginning, important regulatory functions of lnc MIR100HG in tumors are

emerging, while the studies on the MIR100HG encoded miRs, namely miR-100, miR-125b and let-7a, have already provided evidence of their critical regulatory functions in cancer.

miR-100 and miR-125b regulate epithelial-mesenchymal transition of tumor cells

In a recent study aimed at identifying miRs implicated in pancreatic ductal adenocarcinoma (PDAC), Ottaviani and coworkers demonstrate that TGF- β promotes epithelial-to-mesenchymal transition (EMT) in cellular models of PDAC by upregulating the transcription of MIR100HG and of the expression of two of its encoded miRs, miR-125b and miR-100, through the induction of SMAD2/3 transcription factors (5). The authors show that TGF- β also induced the expression of LIN28B gene that inhibits at the post-transcriptional level the maturation of let-7a, the third miR encoded by MIR100HG previously described as an oncosuppressor miR in PDAC, thus marking a further step forward in the characterization of the regulatory mechanisms underlying MIR100HG expression.

In this study, both miR-100 and miR-125b induced EMT in human and mouse PDAC cells, with miR-125b showing a stronger effect compared to miR-100, thus resulting the most important TGF- β effector miR encoded by lncRNA MIR100HG. TGF- β is one of the major inducers of EMT, a fundamental event in tumor dissemination that is characterized in cells of epithelial origin by the decreased expression of the epithelial marker E-cadherin and the acquisition of different mesenchymal markers including

vimentin, N-cadherin, SNAIL, SLUG, TWIST and ZEB1 transcription factors. In previous studies, TGF- β was reported to regulate miR-125b expression both positively, in melanoma cells and negatively, in hepatocellular carcinoma (6,7). Of note, in hepatocellular carcinoma, the link between TGF- β and miR-125b expression is complex and different according to the type and EMT state of hepatocellular carcinoma cell line model used in the study (7).

The association between miR-100 and miR-125b and EMT has been reported in several tumor types, although in some cases with opposing effects. Considering the studies in the most common cancer types (i.e., colorectal, breast, lung, and liver carcinomas), a high expression of both miR-100 and miR-125b was shown in colon cancer cells with mesenchymal-like phenotype (8,9), and miR-125b overexpression was reported to promote EMT and to associate to a mesenchymal subtype in breast tumors (10), while in other studies, including hepatocellular, breast and lung carcinomas, it was negatively associated with EMT (7,11-15). The opposing role of miR-125b in EMT may depend on the different expression levels of its target genes, and on the entire regulation of the molecular complex controlling the pathway, which can be tissue and tumor specific, thus determining a different impact of the miR in the signaling pathways critical for EMT processes. In breast cancer, the levels of miR targets can be affected also by specific breast cancer biopathologic features, including the expression of receptors for estrogen and progesterone (10).

In melanoma, an EMT-like phenotype switching is associated to the acquisition of migratory and invasive cell functions. In the majority of melanomas, EMT transcription factors are induced by genetic mutations activating MAPK, PI3K/AKT/mTOR, Wnt/ β -catenin and Src signaling pathways. For this reason, the genetic makeup of melanoma cells is probably a key factor in regulating tumor aggressiveness. The role of miR-125b in the acquisition of an EMT-like phenotype appears controversial. In fact, Rambow and coworkers showed that miR-125b is upregulated in melanoma cells characterized by a high invasive and migratory potential (6). In these cells, miR-125b upregulation is induced by TCF4, a transcription factor involved in EMT, and increases melanoma cell migration and invasive potential by reducing the expression of NEDD9, a focal adhesion protein regulating cell attachment. On the contrary, the study of Zhang reported that miR-125b expression suppressed cell invasion and tumor metastasis by downregulating ITGA9, a gene coding for an integrin receptor binding to different extracellular

matrix proteins (16). The effects of miR-125b and miR-100 in different tumor types are summarized in *Table 1*.

miR-100 and miR-125b expression levels in tumor cells influence drug sensitivity

The acquisition of a mesenchymal phenotype by PDAC cells was shown associated to the acquisition of stem cell markers and properties, including tumor initiating capacity and resistance to cytotoxic drugs (5), a feature with paramount clinical importance. In fact, the association between miR-125b and miR-100 expression levels and resistance to therapeutic treatments has been extensively studied. In BRAF-mutated metastatic melanoma, we reported that miR-100 and miR-125b are up-regulated in cell lines and tumor specimens resistant to BRAF inhibitor treatment. In particular, our studies demonstrated that miR-100 and miR-125b overexpression promoted the survival of resistant cells by negatively regulating apoptotic genes. In resistant melanoma cells, the augmented expression of miR-100 and miR-125b was not dependent on TGF- β production but rather to the increased production of CCL2 chemokine that sustained cell growth and resistance to apoptosis by a CCL2/HIF1/miR loop (18).

In other published studies, miR-100 and miR-125b were found to promote resistance to cetuximab in colorectal cancer (17); high miR-125b expression levels were shown to increase chemoresistance in colon cancer, while, on the contrary, miR-125b downregulation was associated to resistance to oxaliplatin and 5-fluorouracil in hepatocellular carcinoma, and to paclitaxel in lung and breast cancer cells (11,14,15,22). Several other studies reported contrasting data about the association between miR-125b and drug resistance in breast cancer, indicating that the effect of miR-125b is tumor and drug-dependent (10,19). In line with its definition as oncosuppressor miR, low expression levels of miR-100 were related to chemoresistance in breast and lung cancer (20,21,23,24).

The molecular mechanisms regulating miR-100 and miR-125b effects in tumor cells

To identify miR-100 and miR-125b direct targets, Ottaviani and collaborators set up a novel method based on sequencing of 3'UTR transcripts loaded onto AGO2 protein enriched for sequence complementarity to miR-100 and miR-125b seed regions; the obtained gene lists were then selected for overlap with those of genes down-regulated in

Table 1 Role of miR-100 and miR-125b in EMT and drug resistance

miR	Tumor	EMT	Target genes A	Drug resistance	Target genes B	References
miR-100 and miR-125b	PDAC	Promote	-	↑ (gemcitabine)	-	(5)
miR-100 and miR-125b	CRC	Promote	APC	↑ (cetuximab, 5-fluorouracil)	DKK1, DKK3, ZNRF3, RNF43, APC2	(8,9,17)
miR-100 and miR-125b	MM	-	-	↑ (vemurafenib)	BCL2	(18)
miR-125b	MM	Promote	NEDD9	-	-	(6)
miR-125b	MM	Inhibit	ITGA9	-	-	(16)
miR-125b	BC	Promote	-	↑ (5-fluorouracil)	-	(10)
miR-125b	BC	Inhibit	SEMA4C	↓ (paclitaxel, doxorubicin)	SEMA4C, HAX-1	(14,19)
miR-125b	TNBC	Inhibit	MAP2K7	-	-	(13)
miR-100	BC	-	-	↓ (paclitaxel, cisplatin)	mTOR, HAX-1	(20,21)
miR-125b	HCC	Inhibit	EVA1A, CDK16, SMAD2	↓ (oxaliplatin, 5-fluorouracil)	HK II	(7,11,12,22)
miR-125b	NSCLC	Inhibit	SEMA4C	↓ (paclitaxel)	SEMA4C	(15)
miR-100	NSCLC	-	-	↓ (cisplatin, docetaxel)	PLK1	(23,24)

Target genes A are relative to EMT studies; Target genes B are relative to drug resistance studies. APC, EVA1A, CDK16, SMAD2, MAP2K7, SEMA4C, NEDD9, HAX-1, HK II and ITGA9 are direct targets of miR-125b; mTOR, HAX-1 and PLK1 are direct targets of miR-100; DKK1, DKK3, ZNRF3, RNF43 and APC2 are direct targets of miR-100 and/or miR-125b. BCL2 is a predicted target of miR-100 and miR-125b. PDAC, pancreatic adenocarcinoma; BC, breast carcinoma; TNBC, triple-negative breast cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; MM, metastatic melanoma.

cells overexpressing the miRs. These analyses revealed a significant overlap between the transcripts repressed by the two miRs, indicating that miR-100 and miR-125b regulate common pathways in PDAC cells, including p53, cell cycle checkpoints, and apoptosis (5). Interestingly, genes encoding for regulators of apoptosis and cell cycle are validated targets of miR-125b also in hepatocellular carcinoma, where miR overexpression inhibits EMT (11,12). miR-125b may act as an oncosuppressor miR also in breast cancer and melanoma where it downregulates genes promoting tumor cell growth and survival, such as the MAP kinase MAP2K7 and signaling adhesion receptors like integrin ITGA9 (13,16). Both miR-125b and miR-100 regulate EMT also by repressing the transcriptional modulators of EMT genes SEMA4C and SMAD2; moreover, miR-125b influences EMT by suppressing APC, which is a regulator of Wnt/ β -catenin pathway that activates the transcription of EMT-promoting genes (7-9,14,15).

Overall, these data indicate that both miR-100 and miR-125b target genes involved in cell growth and survival and EMT-related genes. The available experimental

information indicates that miR-125b regulates common pathways in different tumor types, and that its effect on EMT is strictly dependent upon cellular context and the expression of target mRNAs. In line with the results of Ottaviani and coworkers, also in other tumor types a direct link between miR-100 and miR-125b expression levels, EMT and drug resistance has been reported, as summarized in *Table 1*. In particular, the genes targeted by these miRs and associated to drug resistance are involved in pathways associated to EMT and regulating cell proliferation and apoptosis. For this reason, the modulation of these miRs can be considered as a possible therapeutic strategy to improve drug sensitivity in tumor cells.

miR-100 and miR-125b expression levels in tumors and disease prognosis

Analysis of PDAC tissues revealed that both miR-100 and miR-125b are upregulated in tumor samples compared to normal pancreatic tissue samples; the high expression levels of these miRs in tumor cells and not in stromal cells resulted associated to reduced progression-free survival and overall

survival. These results were obtained by the quantification of the staining signal localized in tumor cells obtained by *in situ* hybridization (ISH) for the miR in tissue sections (5).

High expression of both miR-100 and miR-125b has been associated to poor survival also in patients with colon adenocarcinoma (8). In addition, high levels of miR-125b were significantly associated with poor survival in metastatic melanoma, in agreement with its association with invasive and migratory cell states both *in vitro* and *in vivo* (6). Data from our lab showed that both miR125b and miR100 are expressed in melanoma metastatic specimens, where their expression levels resulted highly inter-correlated and correlated to myeloid markers. Moreover, by ISH both miRs showed localization not only in tumor cells but also in the immune infiltrate of myeloid origin (25). In this context, it should be mentioned that miR-125b has acknowledged roles in the regulation of immune cells, and in particular in regulating monocyte differentiation, while for miR-100 a role in the immune system has not been disclosed.

Conclusions

In conclusion, all these experimental data clearly indicate that lncRNA MIR100HG-derived miR-100 and miR-125b are key regulators of EMT and of drug resistance in several tumor types, and for this reason they can be considered for their prognostic potential. Even if these miRs regulate common pathways across different cell types, the published studies report contrasting data about their regulatory functions in tumors, suggesting that their effects are strictly connected with both the cellular context and the drugs studied. Nonetheless, a deeper characterization of the mechanisms involved in their regulative functions will contribute to the identification of potential new therapeutic targets for cancer treatment.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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