



A novel p53-mediated miR-30a/ZEB2 regulatory network in the control of breast cancer progression

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Understanding of the complexity of the microRNA (miRNA)-mediated regulatory network is still largely unknown for many diseases, including cancer. Recent evidences on the control of miRNA-mediated circuits in complex networks have contributed to address the intricate interplay through which miRNAs achieve their regulatory role (1). This editorial provides a summarizing view of a novel p53-mediated regulatory role of miR-30a/ZEB2 circuit that has been validated in the context of triple negative breast cancers (TNBC). A canonical regulatory network includes transcription factors, miRNAs and target genes (2). The article of di Gennaro *et al.* offers an example of an experimentally p53-regulated miRNA circuit involved in TNBC. *TP53* (tumor protein p53) is the most frequently mutated gene in human cancers. It encodes a tetrameric transcription factor that in wild-type (wt; not mutant) conformation exerts tumor suppressor activities (3,4). Wt-p53 protein binds to response elements on regulatory regions of its target genes, and transcriptionally induces the production of proteins that reduce cell proliferation, trigger apoptosis and orchestrate DNA damage response (4). Besides transcribing coding genes, p53 also induces the transcription of miRNAs that participate in the p53 network contributing to the control on tumor progression (5).

p53-deficient cancers are often unstable, aggressive, and resistant to therapy, indicating a strong selective pressure for *TP53* inactivation during tumor progression (3). p53 results mutated in approximately 80% of TNBC, a subgroup of basal-like breast cancers that lack both the estrogen and

progesterone receptors and HER2. This subtype of breast cancers, that is actually poorly cured, is characterized by *TP53* alterations, which are largely disrupting mutations (loss of tumor suppressive functions) (6-9). The loss of function of wt-p53 can confer a selective advantage to TNBC progression controlling the transcription of miRNAs on a variety of processes, including epithelial-mesenchymal transition (EMT) and cell plasticity (5-10). Therefore, a deep analysis of miRNA-mediated mechanisms through which p53 inactivation contributes to the invasive behavior of BC is of paramount importance to understand the interplay of known molecules in functional regulatory circuits.

In their study di Gennaro *et al.* contributed to highlight the p53/miRNA crosstalk in TNBC progression, by demonstrating the role of a new p53/miR-30a/ZEB2 axis. Starting from an *in silico* prediction, by employing The Cancer Genome Atlas (TCGA) dataset (9), the authors focused only on miR-30a, being the only miRNA downregulated in *TP53*-mutated tumors. miR-30a belongs to the miR-30 family that consists of five highly conserved miRNAs (miR-30a-e), which lie in various chromosomes or neighboring sites (11). miR-30a exerts different functions by regulating processes involved in tumor progression. In particular, miR-30a controls EMT by targeting Snai1 and Slug expression (12-14). In this regards, in high-grade-serous ovarian cancers, which represent the majority of OC characterized by 90% of *TP53* mutations, miR-30a is associated with poor prognosis and chemoresistance (15). In ovarian cancer cells miR-30a directly targets the endothelin A receptor (ET_AR) and, consequently, inhibits the ET_AR-

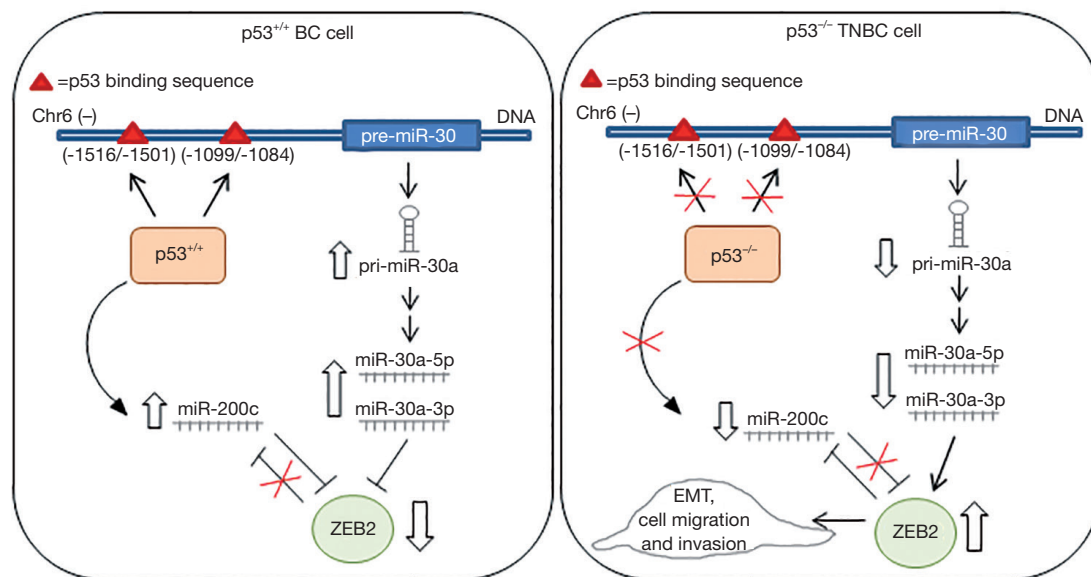


Figure 1 Proposed model for the p53/miR-30a/ZEB2 network in TNBC progression. In wt p53 (+/+) breast cancer cells (*left*), p53 upregulates miR-30a-3p/-5p expression by promoting their transcription. These miRNAs, in turn, bind the 3'UTR on ZEB2 mRNA, reduce ZEB2 expression and suppress the pro-invasive functions. In addition, p53 downregulates ZEB2 through the increase of miR-200c, a tumor-suppressor miRNA that participates with ZEB2 in a double negative feedback loop. In TNBC cells (*right*), mutated p53 (-/-) is unable to bind the miR-30a promoter. Consequently, miR-30a-3p/-5p levels are downregulated, ZEB2 increases and induces tumor progression. In parallel, as a consequence of the p53 lost and of the ZEB2 increase, miR-200c is downregulated, thus integrating the p53/miR-30a/ZEB2 circuit in which miR-30a appears as an emerging hub node, characterized by important properties that control aggressive features of breast cancers. BC, breast cancer; TNBC, triple negative breast cancer; EMT, epithelial-to-mesenchymal transition.

mediated increase of mesenchymal markers (Snail and vimentin) and E-cadherin downregulation. These events lead to reduced survival, cell migration, invasion, plasticity and platinum-response. These findings provide evidence of a hitherto unappreciated miR-30a function as a tumor suppressor in chemoresistant OC cells and its contribution to poor patient outcome. Accordingly, by downregulating vimentin, miR-30a inhibits breast cancer invasiveness and metastasis and reduced miR-30a levels are associated with a poor clinical outcome (16). In line with these evidences, di Gennaro *et al.* demonstrated that low miR-30a levels were associated with a poor clinical outcome in TNBC patients, thus suggesting a prognostic relevance of miR-30a in this BC subtype. Of note, a downregulation of both miR-30a strands (miR-30-3p/-5p) was observed in *TP53*-mutated BC, particularly TNBC, strongly supporting the existence of an *in vivo* p53/miR-30a interplay. Notably, re-expression of p53 in p53-deficient BC cell lines up-regulated both miR-30a strand expressions, acting on their primary transcript (pri-miR-30a). Accordingly with previous

findings in melanoma cells (17), di Gennaro *et al.* reported that wt-p53 protein induced miR-30a transcription in TNBC by specifically binding its promoter in two binding regions (*Figure 1*). Among the direct targets of miR-30a the authors identified ZEB2, an E-box-binding transcription factor that is implicated in the regulation of E-cadherin expression (18). Of clinical relevance, ZEB2 is up-regulated in TNBC and basal-like BCs in which it associates with an aggressive tumor phenotype (19). With the identification of miR-30a as a p53-downstream effector in TNBC, the authors contributed to put in evidence the mechanistic link between p53 and ZEB2 in the control of tumor progression. Importantly, the authors also demonstrated the existence of a complex regulatory network that integrated miR-200c. This miRNA belongs to the miR-200 family that includes five members (miR-200a/b/c, miR-141 and miR-429) (20). Of interest, p53 inhibits EMT also by modulating the miR-200 expression and in *TP53*-mutated BC cells miR-200c is downregulated, supporting the notion that inactivation of p53 promotes EMT and thereby tumor progression

and poor drug response (10,21,22). Moreover, miR-200 family and ZEB transcription factors reciprocally inhibit their expression through a negative feedback loop (23). In this work, the authors demonstrated that re-expression of miR-30a in TNBC cells led to ZEB2 downregulation and to the consequent miR-200c upregulation. Importantly, they found a positive association between miR-30a and miR-200c in TNBC patients, thus supporting the interplay of miR-200c with p53/miR-30a axis. Regarding the biological implications of this novel identified network in TNBC biology, they found that miR-30a/ZEB2 network controlled cell migration and invasiveness.

Collectively, di Gennaro *et al.* through the identification of the p53/miR-30a/ZEB2 circuit, integrated with miR-200c, contribute to reveal the molecular mechanisms by which loss of p53 induces EMT and aggressive features of BC disclosing how miR-30a achieves its regulatory role acting as a central node in this circuit (*Figure 1*). Summing up, this study highlights the role of p53 in regulating the functional properties of miRNA-mediated circuits, characterizing its pathological significance in TNBC context. Further studies are needed to validate the involvement of this network in the control of BC progression as well as its prognostic potential. To this end, it will be useful to analyze a larger cohort of BC patients with full clinical annotation available. To validate the prognostic relevance of this network, it could be important to assess the presence of the reported miRNA profile in the plasma along with the status of *TP53* gene in BC patients at diagnosis and relapse. The analysis of circulating miRNA could also allow evaluating potential changes in the network miRNA/gene at diagnosis and relapse in BC patients. Most importantly, this analysis could be predictive of the impact of first-line treatment in BC. Genome wide approaches (e.g., Nanostring analysis) will be extremely important to quantify the expression and the alterations of this network in BC patients. Last, but not least, to fully understand the mechanisms underlying the function of the miRNA-regulatory network, the development of suitable mathematical models (24,25) might improve the interpretation of the complexity of these regulatory networks and thereby provide precious information elucidating the composition and function of these circuits in cancer progression.

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Footnote

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