Editorial

Clinical significance of miR-15 and miR-16 in ovarian cancer

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Due to the fact that ovarian cancer is frequently late symptomatic and, therefore, detected too late, it is the most lethal malignant gynecological cancer. Approximately 70% of patients are diagnosed with advanced FIGO stages (III or IV) and have a 5-year survival rate of less than 40%, whereas patients who are diagnosed with FIGO stage I or II have a longer 5-year survival rate of 70–90% (1). Treatment failure in ovarian cancer patients caused by the emergence of chemo-resistance towards cytotoxic drugs, such as cisplatin or carboplatin, is attributable to its high mortality. In particular in advanced tumor, low response to platinum-based chemotherapy may result in poor prognosis and recurrence (2). Therefore, new therapies are urgently needed to overcome chemo-resistance in treatment of cancer. Since microRNAs (miRNAs) vary in expression during the course of disease and therapy, they could represent potential candidates for replacement or targeted therapy (3).

Investigations of the role of miRNAs have opened up possible applications in molecular diagnostics and prognostics, particularly for cancer. Screening of deregulated miRNAs that are involved in numerous gene regulatory networks and identification of their target mRNAs could deliver evidence of the impact of drugs on the diverse signal transduction pathways (3). As a component of the gene regulatory network, a single miRNA is capable of controlling the expression of hundreds of protein-coding genes and modulating a wide spectrum of biological functions, such as proliferation, differentiation, stress response, DNA repair, cell adhesion, motility, inflammation, cell survival, senescence and apoptosis—all of these processes are important program sequences during tumorigenesis (4). This family of evolutionary conserved, small non-coding RNA molecules inhibits post-transcriptionally expression of their target genes. Their specific binding to the 3'untranslated-region (3'UTR) of their target mRNAs leads to the suppression of protein expression or cleavage of their target mRNAs (5). As miRNA loci frequently map to fragile chromosomal regions harboring DNA amplifications, deletions or translocations, their expression is often deregulated in cancer, contributing to tumor progression and metastasis. The identification of onco-miRNAs and tumor suppressor-miRNAs showed that many of them are strictly expressed in tumor type- and stage-specific manner (6).

The family of miR-16, comprising representative miRNA members of miR-15a/b, miR-16, miR-103, miR-107 and miR-195, has been widely studied in different cancer types. However, inconsistence of the results referring to the diagnostic and prognostic value of these miRNAs has been reported (7-12). In their recent study, Dwivedi *et al.* (7) reported that multiple mechanisms that promote ovarian cancer progression and chemo-resistance may be inhibited by miR-15a and miR-16. They found that BMI1 (B lymphoma Mo-MLV insertion region 1), a member of the Polycomb Repressor Complex 1 (PRC1) that mediates gene repression by regulating chromatin structure and is involved in the self-renewal of stem cells (13), is a direct target of both miRNAs (7). Ectopic expression of these miRNAs reduced cell proliferation rate, decreased anchorage independent clonal growth of ovarian cancer cells and inhibited expression of the cisplatin efflux pump ATP7B, resulting in enhanced sensitization of ovarian cancer cells to cisplatin. In addition to inhibiting epithelial to mesenchymal transition (EMT), miR-15 and miR-16 decreased degradation of the extracellular matrix (ECM) by ovarian cancer cells. In this context, both miRNAs are associated with the suppression of tumor invasiveness, cell migration and tumor progression (7). Compared to previous studies (8,11,12,14,15) on the analyses of miR-15 and miR-16 in

ovarian cancer, the strength of the study by Dwivedi *et al.* (7) lies in testing the therapeutic efficacy of these miRNAs in a pre-clinical chemo-resistant orthotopic mouse model of ovarian cancer. In these mice nanoliposomal administration of a combination of miR-15a and miR-16 demonstrated a striking reduction in tumor burden. Of particular interest is that the combination therapy of miR-15a and miR-16 without cisplatin demonstrated a better therapy response in the mice compared to cisplatin alone or either a single miRNA in combination with cisplatin. The increased sensitivity to cisplatin is assumed to be caused by the cisplatin accumulation in the cells, possibly by inhibition of the ATP7B pump mediated by miR-15a and miR-16 (7). This excellent and elaborate study (7) well supports the tumor-suppressive effects of miR-15 and miR-16, and encourages their entry into future clinical trials, particularly, because apart from their characteristics, no obvious toxicity was noticed in the animals during the experiment.

Such investigations and the involvement of specific miRNAs in the regulation of protein expression in different steps and stages of cancer program, along with the possibility to manipulate their expression, have provoked interest in the development of miRNA-based strategies or personalized and targeted therapies to treat cancer as well as to predict and monitor chemotherapy response. To date, miR-34 has become the first cancer-targeted miRNA drug (MRX34, http://www.clinicaltrials.gov; Identifier: NCT01829971), entering phase I clinical trials in patients with advanced hepatocellular carcinoma (16). The multicenter trial on miR-34 has shown that MRX34 has a manageable safety profile in patients with hepatocellular carcinoma, as well as other solid tumors with or without liver metastasis and hematological malignancies. miR-34a is able to sensitize a variety of metastatic cancer types to agents, such as fluorouracil, doxorubicin, adriamycin, cisplatin and sorafenib. However, miR-34a is also reported to be associated with metabolic function, development of diabetes and cardiac dysfunction (17,18). These findings suggest that, while beneficial in the cancerous setting, miR-34a could have serious side effects which could disrupt normal function. In this regard, before novel therapeutics based on modulators of such miRNAs can be translated into the clinic, it should be kept in mind that miRNAs have biological functions and that a single miRNA can target numerous mRNAs. Consequently, they prevent the translation of many different proteins that are involved in numerous cancer-relevant signal transduction pathways. To avoid adverse effects, the actual role of miRNAs and the identification of their key targets relevant to development and progression of diverse (benign or malignant) diseases need to be intensely defined in preclinical models.

In respect of miR-15 and miR-16, this dual behavior may also apply to them. Since their first discovery in chronic lymphocytic leukemia where they are commonly deleted, miR-15a and miR-16 have been reported to act as tumor suppressors by inhibiting a number of oncogenic mRNA targets and various aspects of cancer progression *in vitro* and *in vivo* (19). For example, their tumor suppressive characteristics have been ascribed to the reduction of cell proliferation, migration, and angiogenic capacity of endothelial cells (7,20). However, some studies have also implicated them in oncogenic pathways in different cancer types (21,22). For example, miR-16 is positively associated with the cell division cycle 25 homolog C (Cdc25C), which is a critical gene involved in cell division that dephosphorylates cyclin B-bound Cdc2 (CDK1) and triggers entry into mitosis. MiR-16 may also have a role in suppressing p53-induced growth arrest (21,22). These findings indicate that these miRNAs could also possibly accelerate tumor progression. Thus, the role of miR-16, as well as miR-15, might be more complex in cancer.

Numerous studies quantified the expression levels of miR-15 and miR-16 in tumor tissues or plasma/serum samples from ovarian cancer patients with different clinical parameters. Whereas downregulated in ovarian cancer cell lines and primary ovarian tissues (7,8), they were also shown to be overexpressed in all 3 ovarian tumor histologic subtypes relative to normal primary ovarian surface epithelium (9) and associated with both poor survival and recurrence (11). MiR-16 was also elevated in osteoclast differentiation and correlated with bone metastasis burden (10). My laboratory showed that the serum levels of circulating miR-16 were neither up- nor downregulated in ovarian cancer patients compared with healthy women (12). Moreover, miR-16 has also been frequently used as a reference miRNA for normalization of miRNA data, because it was shown to be highly expressed and relatively invariant across various samples derived from patients and healthy controls (23). The heterogeneity of the studies could be explained by the different stability of these miR-16 family members extracted from different sources, such as tumor tissue, plasma or serum. Other factors, including different laboratory conditions, techniques and normalization methods, can also have an impact on the data. Thus, the lack of a standardized approach may result in these conflicting results and that miRNAs have not been successfully implemented in the clinical setting. In addition, certain environmental factors, such as bioactive dietary agents (e.g., folate, curcumin,

polyunsaturated fatty acids) are also thought to influence the progression and severity of cancer by modulating tissue levels of miR-16 (24). Nevertheless, these studies suggest the prominent role of miR-15 and miR-16 to act as tumor suppressor genes or oncogenes.

Moreover, the fluctuating changes in miRNA expression may be caused by their interference with mRNA targets, as well as their indirect regulation of the expression of other genes and even miRNAs. To emphasize this argumentation, a study investigating the transient overexpression of miR-7 in ovarian cancer cells reported a change in the expression of hundreds of genes in diverse signaling pathways, but one-fifth of the regulated genes were only predicted to be direct targets of miR-7. Consequently, the majority of the observed changes in gene expression may be an indirect effect by miRNAs (25). This may also apply to miR-15 and miR-16, and it is assumed that they are mingled in numerous signaling pathways and regulate numerous cell physiologic processes.

Prior to starting a replacement therapy with miR-15 and miR-16, their multiple behaviors should be considered. Thus, the potential up-regulation of these miRNAs in the temporal and local context needs to be taken into contextual evaluation, where their delivery may be beneficial in ovarian cancer patients. A major effort to start a replacement therapy is the development of miRNA delivery systems that can be used in a clinical setting. In particular, miRNA therapeutics should specifically be delivered to tumor cells, to avoid liver clearance. Currently, many liposomal preparations of RNA-based agents primarily accumulate in the liver. Bypassing this organ will be challenging to deliver miRNAs to their target sites. Restoring miRNA function may be accomplished by administration of different types of miRNA molecules able to mimic native miRNAs, such as synthetic double-stranded or precursor RNA oligonucleotides or viral vectors encoded for miRNA mimics. A main advantage of viral vectors in the context of a miRNA-based therapy is due to the possibility of injecting them directly in the tumor mass. For example, miR-34a has been successfully delivered in mouse models using a number of methods, including various liposomal complexes, hyaluronic acid chitosan nanoparticles, atelocollagen and a class of 7C1 nanoparticles (16).

In conclusion, Dwivedi *et al.* (7) demonstrated that miR-15 and miR-16 are involved in multiple mechanisms to inhibit ovarian cancer progression and chemo-resistance, such as EMT, drug transport and clonal growth. In particular, their *in vivo* data show that miR-15 and miR-16 could be highly valuable as therapeutics in anti-ovarian cancer therapy. Furthermore, their investigations may contribute to explain the molecular pathway leading to enhanced sensitization of ovarian cancer cells to cisplatin and to reduction in tumor burden. Further studies on the multiple functions of these miRNAs and their widespread impact on gene expression programs in ovarian cancer are warranted to improve our understanding of miRNA biology before they can be claimed to be relevant therapeutic agents of anti-cancer therapy. In addition, large-scale studies on the expression levels of these miRNAs during different tumor phases and stages of ovarian cancer are needed. Since their action is often nonspecific, careful investigations of target miRNAs must be performed to ensure that miRNA-based therapeutics can modulate the endogenous target of interest and accordingly to minimize off-target effects. Nevertheless, miRNA-based therapies have the advantage to treat diseases at a network level rather than targeting a single gene. Consequently, a miR-15/miR-16-based replacement therapy in combination with cisplatin that concurrently regulates multiple components of various pathways could reduce the acquirement of resistances, because such resistances usually develop by inhibitors that only target a single molecule or pathway. Although the direct and indirect regulatory effects of miRNAs are interactive and complex, they may be of potential future use for miRNA-based strategies to treat various cancer types and other diseases.

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References

1. Jayson GC, Kohn EC, Kitchener HC, et al. Ovarian cancer. Lancet 2014;384:1376-88.
2. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 2014;740:364-78.
3. Schwarzenbach H. The potential of circulating nucleic acids as components of companion diagnostics for predicting and monitoring chemotherapy response. Expert Rev Mol Diagn 2015;15:267-75.
4. Moreno-Moya JM, Vilella F, Simón C. MicroRNA: key gene expression regulators. Fertil Steril 2014;101:1516-23.
5. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215-33.
6. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999-3004.
7. Dwivedi SK, Mustafi SB, Mangala LS, et al. Therapeutic evaluation of microRNA-15a and microRNA-16 in ovarian cancer. Oncotarget 2016;7:15093-104.
8. Bhattacharya R, Nicoloso M, Arvizo R, et al. MiR-15a and MiR-16 control Bmi-1 expression in ovarian cancer. Cancer Res 2009;69:9090-5.
9. Wyman SK, Parkin RK, Mitchell PS, et al. Repertoire of microRNAs in epithelial ovarian cancer as determined by next generation sequencing of small RNA cDNA libraries. PLoS One 2009;4:e5311.
10. Ell B, Mercatali L, Ibrahim T, et al. Tumor-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone metastasis. Cancer Cell 2013;24:542-56.
11. Delfino KR, Rodriguez-Zas SL. Transcription factor-microRNA-target gene networks associated with ovarian cancer survival and recurrence. PLoS One 2013;8:e58608.
12. Meng X, Joosse SA, Müller V, et al. Diagnostic and prognostic potential of serum miR-7, miR-16, miR-25, miR-93, miR-182, miR-376a and miR-429 in ovarian cancer patients. Br J Cancer 2015;113:1358-66.
13. Bhattacharya R, Mustafi SB, Street M, et al. Bmi-1: At the crossroads of physiological and pathological biology. Genes Dis 2015;2:225-39.
14. Park SM, Gaur AB, Lengyel E, et al. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 2008;22:894-907.
15. Nam EJ, Yoon H, Kim SW, et al. MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res 2008;14:2690-5.
16. Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov 2013;12:847-65.
17. Chakraborty C, George Priya Doss C, Bandyopadhyay S. miRNAs in insulin resistance and diabetes-associated pancreatic cancer: the 'minute and miracle' molecule moving as a monitor in the 'genomic galaxy'. Curr Drug Targets 2013;14:1110-7.
18. Bernardo BC, Gao XM, Winbanks CE, et al. Therapeutic inhibition of the miR-34 family attenuates pathological cardiac remodeling and improves heart function. Proc Natl Acad Sci U S A 2012;109:17615-20.
19. Calin GA, Croce CM. Genomics of chronic lymphocytic leukemia microRNAs as new players with clinical significance. Semin Oncol 2006;33:167-73.
20. Braza-Boïls A, Salloum-Asfar S, Marí-Alexandre J, et al. Peritoneal fluid modifies the microRNA expression profile in endometrial and endometriotic cells from women with endometriosis. Hum Reprod 2015;30:2292-302.
21. Miles GD, Seiler M, Rodriguez L, et al. Identifying microRNA/mRNA dysregulations in ovarian cancer. BMC Res Notes 2012;5:164.
22. Lavecchia A, Di Giovanni C, Novellino E. Inhibitors of Cdc25 phosphatases as anticancer agents: a patent review. Expert Opin Ther Pat 2010;20:405-25.
23. Schwarzenbach H, da Silva AM, Calin G, et al. Data Normalization Strategies for MicroRNA Quantification. Clin Chem 2015;61:1333-42.
24. Shah MS, Davidson LA, Chapkin RS. Mechanistic insights into the role of microRNAs in cancer: influence of nutrient crosstalk. Front Genet 2012;3:305.
25. Shahab SW, Matyunina LV, Hill CG, et al. The effects of MicroRNA transfections on global patterns of gene expression in ovarian cancer cells are functionally coordinated. BMC Med Genomics 2012;5:33.

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