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MicroRNA-29a plays a prominent role in PRIMA-1^{Met}-induced apoptosis in ovarian cancer cells

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Abstract: The structural analog of the small 2,2-bis(hydroxymethyl)-1-azabicyclo[2,2,2]octan-3-one molecule named PRIMA-1^{Met}for "p53 reactivation and induction of massive apoptosis" has been shown to inhibit cell growth and induce apoptosis in human tumor cells by restoring the tumor suppressor function of tumor protein p53. In several microRNA (miRNA) profiling studies related to ovarian cancer, different miRNAs associated with PRIMA-1^{Met} have been reported, but miRNAs related to PRIMA-1^{Met}-induced apoptosis remain unclear. This study was designed to explain the potential mechanism of PRIMA-1-induced apoptosis. According to the MTSassay and fluorescence-activated cell sorting (FACS) analysis results, PRIMA-1^{Met} induced a significant decrease in cell viability and an increase in apoptosis in both A2780 and Caov-3 cells, regardless of p53 status. PRIMA-1^{Met} upregulated miRNA-29a in both cell lines. To determine the effect of miRNA-29a on PRIMA-1^{Met}-induced apoptosis, A2780 and Caov-3 cells were transfected with miRNA-29a inhibitor. After treatment with PRIMA-1^{Met}, cell viability increased and apoptosis decreased in the transfected cells. The results of this study suggest that miRNA-29a potentially regulates PRIMA-1^{Met}-induced apoptosis in ovarian cancer cells.

Keywords: apoptosis; miRNA29a; ovarian cancer; PRIMA-1^{Met}

INTRODUCTION

The P53 tumor suppressor protein is a transcription factor that suppresses tumor growth by regulating many target genes involved in central cellular processes such as transcription, DNA repair, genomic stability, aging, cell cycle control and apoptosis [1,2]. It has also become a target for mechanism-based anticancer drug discovery because p53 mutations are found in more than 50% of human cancers [3-7]. In recent years, attempts have been made to use p53 as a target for cancer treatment, and various small molecules have been identified as capable of reversing the oncogenic properties of mutant p53 [8,9]. Among these small molecules, the most studied are PRIMA-1 and the methylated derivative PRIMA-1^{Met}[10,11]. PRIMA-1^{Met} was shown to inhibit cell proliferation and to induce apoptosis in malignant cell cultures containing different p53 mutations and in some xenograft animal tumors by restoration of the p53 tumor suppressor function [12-16].

MiRNAs are a short non-coding and highly conserved RNA class of 18-24 nucleotides. MiRNAs function in a variety of physiological and pathological processes, such as cell proliferation, cell differentiation, apoptosis and tumor growth, by regulating gene expression at the levels of transcription and translation[17]. According to recent studies, miRNAs play a prominent role in the regulation of the tumor phenotype by modulating the expression and signaling pathways of important genes involved in tumor pathogenesis, acting as oncogenes or tumor suppressor genes[18-20]. Several miRNA profiling studies related to ovarian cancer identified various miRNAs associated with chemotherapy resistance and disease progression [21-26]. In another study, miR-138 was shown to suppress invasion and metastasis by targeting SRY-related HMG-box (SOX)-4 and hypoxia-inducible factor 1-alpha (HIF-1a) genes in ovarian cancer cells [27]. It was found that members of the miR-29 family (miR-29a, miR-29b, and miR-29c) increase p53 levels and trigger apoptosis in a p53-dependent manner [28].We have recently

identified altered expression of miRNAs in response to PRIMA-1^{Met} treatment in ovarian cancer cell lines with mutant and wild-typep53; we demonstrated that miRNA-29a was among the most regulated miRNAs in response to PRIMA-1^{Met} treatment, however, it was not shown whether PRIMA-1^{Met} induces apoptosis by regulating specific miRNA expression. Therefore, in this study a potential role of miRNA-29a for PRIMA-1^{Met}-induced apoptosis has been investigated.

MATERIALS AND METHODS

Cell culture and reagents

The human ovarian cancer cell line Caov-3 (mutant p53) was obtained from the American Type Culture Collection (ATCC, USA). A2780 cell line (wild-type p53) was purchased from the Sigma (Sigma, USA). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) with 10% fetal bovine serum and 1% penicillin/streptomycin in a humidified incubator (37°C, 5% CO_2). PRIMA-1^{Met} (purchased from Santa Cruz, USA)was diluted in Ultra Pure water (Sigma, USA) and was used for cellular treatments.

Cell viability analysis

Cell viability in PRIMA-1^{Met} treated and untreated (control) cells was assessed using a Cell Titer 96 aqueous nonradioactive cell proliferation assay (Promega, USA). The cells were seeded at 1×10^4 cells/well in 200 µL complete medium in a 96-well plate and allowed to attach for 24 h [29]. Subsequently, A2780 (p53-wild type) and Caov-3 (p53-mutant) cells were treated with PRIMA-1^{Met} at doses of 20 µM and 40 µM, respectively, for 48 h [30]. At the end of the incubation time, 20 µL of MTS[3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium]/PMS (phenazine methosulfate) solution was added to each well for 4 h. The absorbance at 490 nm was measured in a microplate reader (Thermo Labsystems Multiskan Spectrum, Thermo Lab Systems, USA), using wells without cells as background. The sample readings were calculated by subtracting the average of background absorbances. All experiments were performed at least four times.

Apoptosis assay

A2780 and Caov-3 cells were treated with PRIMA-1^{Met} at doses of 20 µM and 40 µM, respectively, for 48 h. Apoptotic cells were determined using the Annexin V-FITC apoptosis kit (BD Biosciences, USA). All procedures were performed according to the manufacturer's protocol. Briefly, $1x10^6$ cells were rinsed with a binding buffer and were suspended in 200 µL of the same buffer. Then 5 µL of Annexin V and 5 µL of propidium iodide were added to the suspension. The cells were incubated for 15min at room temperature in the dark and then assayed by FACS.

Quantitative real-time PCR

Total RNA, including miRNAs, was isolated from the cells using the miRNA Easy kit (Qiagen, Germany) according to the manufacturer instructions, and was then quantified with a spectrophotometer (Thermo Fisher Scientific, Inc., USA) at wavelengths of 230, 260, and 280 nm. For miRNA analysis, samples of isolated RNA were reverse-transcribed into cDNA using anmiScript II RT kit (Qiagen, Germany). Briefly, the RT master mix included the following: 5 µL template RNA (250 ng), 4 µL 5xmiScriptHiSpec buffer, 2 µL 10x nucleic mix, 7 µL RNase free water and 2 µL miScript reverse transcriptase mix up to a total volume of 20 µL. The reaction mixture was centrifuged, placed on ice and then incubated at 37°C for 60 min and 95°C for 5 min. cDNA samples were transferred to a -20°C freezer. The expression of miRNA-29a was examined in the two cell lines after exposure to PRIMA-1^{Met} using the miScript SYBR Green PCR kit (Qiagen, Germany) according to the manufacturer's protocol. Briefly, the PCR amplification was conducted in a 25-µL reaction using a 2X Quanti Tect SYBR Green PCR master mix (12.5 µL), 10xmiScript Universal primer (2.5µL), 10xmiScript miRNA-29a-spesific primer (2.5µL), RNase free water (5.5 µL) and 10 ng template cDNA (2 µL). Real-time PCR was performed in a 96-well plate using a Step One Plus™ Real-Time PCR System (Thermo Fisher Scientific, Inc., USA) at 95°C for 15 min, followed by 40 cycles at 94°C for 15 s, 55°C for 30 s and 70°C for 30 s. The expression of miRNA-29a was calculated relative to small nucleolar RNA (SNORD)72, and fold-changes were calculated by the $2^{-\Delta\Delta Ct}$ method [31]. Each reaction was repeated three times.

Inhibition of miRNA-29a

To test the efficacy of miRNA-29a on PRIMA-1^{Met}-induced apoptosis in ovarian cancer cells A2780 and Caov-3,cells (4×10^4) were transfected with either 10 nM of mirVana inhibitor of miRNA-29a (Invitrogen, USA) or 10 nM of the negative control, mirVana miRNA inhibitor (Invitrogen, USA) by the lipofectamine RNAi MAX transfection method (Invitrogen, USA) according to the manufacturer's protocol. After 24 h incubation, the medium containing lipofectamine RNAi MAX reagent was removed and normal DMEM was added. The transfected cells were treated with PRIMA-1^{Met} and incubated for additional 48 h, and cell viability and apoptosis were evaluated in the transfected cells.

Statistical analysis

All values are presented as the mean±standard deviation. The Student's t-test was used for comparison of the two groups. Analyses were performed with Graph Pad In Stat v.10.0 software (GraphPad Software, Inc., USA). $P<0.001$ was considered statistically significant. Sigma Plot v.10.0 were used for illustration.

RESULTS

PRIMA-1^{Met} causes a significant decrease in cell viability and an increase in apoptosis in ovarian cancer cells

To examine the effect of PRIMA-1^{Met} on apoptotic cell death in ovarian cancer cells, the A2780 (p53-wild type) and Caov-3 (p53-mutant) cell lines were treated with 20 μM and 40 μM PRIMA-1^{Met}, respectively, and cell viability was determined using the MTS assay after 48 h of treatment. It was determined thatPRIMA-1^{Met} was significantly cytotoxic for both cell lines, independently of p53 status ($p<0.001$). After the treatment with PRIMA-1^{Met}, the Caov-3 and A2780 cells had 44.75% and 42.75% viable cells, respectively (Fig.1A).These results were confirmed by FACS analysis. In Caov-3 cells, 13% of the cells in the control group and 64.6% of the cells in the PRIMA-1^{Met}-treated group were apoptotic, whereas in A2780 cells, the apoptotic cell percentages in the control and PRIMA-1^{Met}-treated group were respectively 10.8 and 57.8% (Fig. 1B).

PRIMA-1^{Met} induces miRNA-29a expression in ovarian cancer cells

Previously we observed by miRNA PCR array that miRNA-29a was among the most expressed miRNAs afterPRIMA-1^{Met}exposure in both cell lines (A2780 and Caov-3).To further confirm the miRNA array data, miRNA expression was examined in the two cell lines after treatment to PRIMA-1^{Met}. For this, the cell lines were treated with PRIMA-1^{Met}at 20 μM and 40 μM doses (A2780 and Caov-3, respectively), and miRNA-29a expression at 8, 24 and 48 h was evaluated using miScript microRNA PCR analysis. As shown in Fig. 2, PRIMA-1^{Met}raised miRNA-29a expression

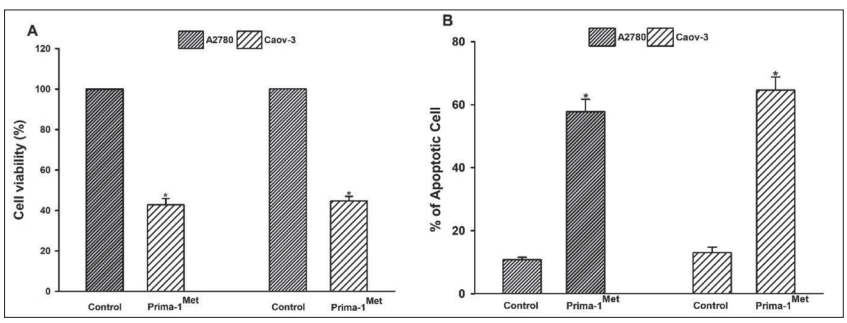

Fig. 1. Analysis of cell viability and apoptosis after PRIMA-1^{Met} treatment. A2780 (20μM) and Caov-3 (40 μM) cells were treated with PRIMA-1^{Met}, and the MTS assay was performed to determine cell viability 48h post-treatment (**A**). Cells treated with PRIMA-1^{Met} for 48 h were stained with the Annexin V-FITC apoptosis kit and analyzed by FACS (**B**). Each experiment was repeated four times. The results are presented as the mean±SD. * indicates $P<0.001$.

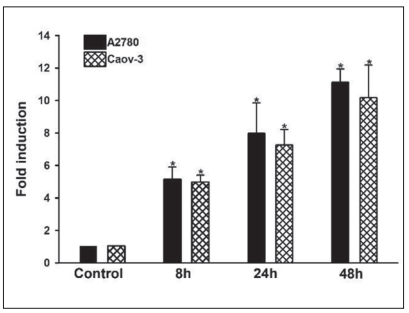

Fig. 2. PRIMA-1^{Met} upregulates expression of miRNA29a in ovarian cancer cells. RT-qPCR analysis was used to evaluate the expression of miRNA29a. The cells were treated with PRIMA-1^{Met} for 8, 24 and 48 h. RT-qPCR was carried out in a 96-well plate using a Step One Real Time-PCR instrument. Fold induction was calculated by the $2^{-\Delta\Delta Ct}$ method. Each reaction was repeated three times. The results are presented as the mean±SD. * indicates $P<0.001$.

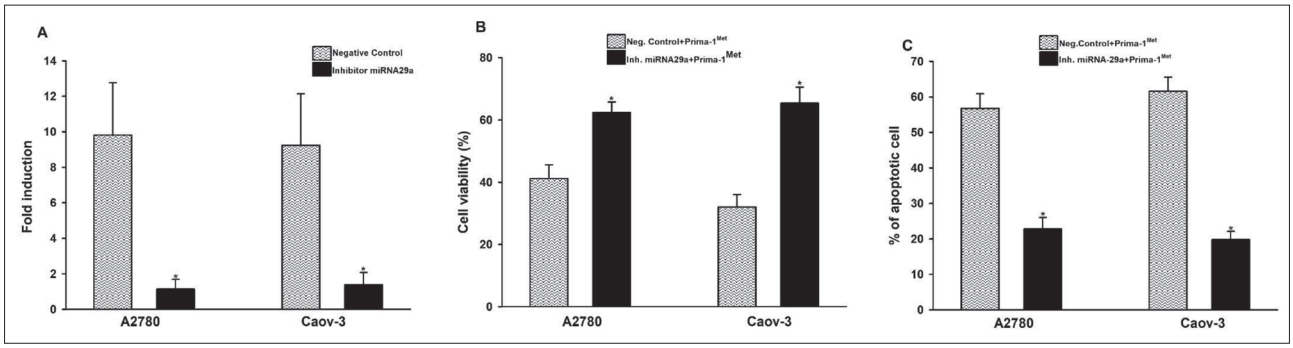

Fig. 3. Inhibition of miRNA-29a reduces PRIMA-1^{Met}-induced apoptosis. A2780 and Caov-3 cells were transfected with the mirVana miRNA inhibitor to inhibit the expression of miRNA-29a. Transfected cells were treated with PRIMA-1^{Met} for 24 h.RT-qPCR was used to confirm the specific knockdown of miRNA-29a in the transfected cells (**A**). After treatment with PRIMA-1^{Met}, cell viability was determined by MTS (**B**), and apoptosis was evaluated by FACS analysis of the transfected cells (**C**). All values were obtained from three independent analyses. The results are presented as the mean±SD, * indicates $P<0.001$.

after 48 h treatment 11.12- and 10.16-fold in A2780 and Caov-3 cells, respectively, when compared with untreated controls (p<0.001).

Inhibition of miRNA-29a reduces PRIMA-1^{Met}-induced apoptosis

As mentioned, PRIMA-1^{Met} increases miRNA-29a expression in A2780 and Caov-3 cells. To investigate whether miRNA-29a regulates PRIMA-1^{Met}-induced apoptosis, A2780 and Caov-3 cells were transfected with mirVana, the mir-29a inhibitor and negative control. A significant reduction in miRNA-29a expression in cells transfected with mirVana mir-29a inhibitor was indicative of successful transfection (Fig.3A). After 24 h of PRIMA-1^{Met} treatment, cell viability in transfected cells was measured as previously described. As shown in Fig.3B, after treatment with PRIMA-1 ^{Met}, cell viability increased in cells transfected with the miRNA-29a inhibitor when compared to cells transfected with the negative control in both cell lines (p<0.001).This result showed that miRNA-29a plays an important role in sensitizing ovarian cancer cells to PRIMA-1^{Met}. To examine in more detail whether miRNA-29a regulates PRIMA-1^{Met}-induced apoptosis, cells transfected with miRNA-29a inhibitor were treated with PRIMA-1^{Met} for 24 h, and apoptosis was evaluated by FACS analysis (Fig.3C). The apoptosis results were also consistent with viability. Compared to cells transfected with inhibitor miRNA and cells transfected with its negative control, cell viability increased from 42% to 62.4% in A2780 cells and from 32% to 65.4% in Caov-3 cells. As expected, inhibition of miRNA-29a reduced PRIMA-1-induced apoptosis, namely; the proportion of apoptotic cells in cells transfected with inhibitor miRNA-29a decreased to 22.8% and 19.8%, in A2780 and Caov-3 cells, respectively. These results, when evaluated together, showed that miRNA-29a could play a prominent role in the regulation of PRIMA-1^{Met}-induced apoptosis in ovarian cancer cells.

DISCUSSION

Recently, different strategies have been developed to use P53 as a target in cancer treatment. Identification of various small molecules that can reverse the oncogenic properties of mutant p53 is among these strategies[9,32]. PRIMA-1^{Met} is a methylated derivative of one of these low molecular weight compounds, PRIMA-1[33]. Studies have shown that PRIMA-1^{Met} is more effective in cancer cells expressing mutant p53 than wild-type p53 or null cells [16,34], while several studies have shown that PRIMA-1^{Met} exhibits cytotoxic effects independent of the p53 mutation status[35-38]. It was reported that PRIMA-1^{Met} has sufficient activity to suppress growth in epithelial ovarian cancer cells, regardless of the mutation status of p53 [30].PRIMA-1 was shown to trigger apoptosis by altering the expression of p53 downstream genes such as p53 upregulated modulator of apoptosis (PUMA), phorbol-12-myristate-13-acetate-induced protein 1 (NOXA) and apoptosis regulator BAX, also known as bcl-2-like protein 4 in the mutant p53 bladder cancer cell line [39]. PRIMA-1 induces apoptosis by promoting the activation of

proapoptotic signaling pathways and inhibiting JNK cell viability in mutant p53 breast cancer cells [40].In this study, PRIMA-1^{Met} was shown to cause a significant reduction in cell viability and increased apoptosis in ovarian cancer cells carrying both wild-type p53 and mutant p53. There is no precise information about the mechanism of PRIMA-1^{Met}-induced apoptosis, especially in ovarian cancer. The fact that PRIMA-1^{Met} is effective in both mutated and unmutated ovarian cancer cells can only be explained by p53-independent mechanisms of action [41].

MiRNAs act in a variety of physiological and biological processes, such as cell proliferation, differentiation, and hematopoiesis, by regulating the expression of multiple target genes [42]. Recently, dysregulation of miRNAs has been associated with cancer initiation and progression. The role of miRNAs in apoptosis is not fully understood, but there is evidence that miRNAs are important in this process [43]. For example, when the role of miR-34 family members in regulating PRIMA-1 induced apoptosis was investigated [44], it was reported that PRIMA-1 upregulated miR-34a in mutant p53 human lung cancer cells, which suggests that PRIMA-1 upregulates miR-34a to induce apoptosis in lung cancer cells. Investigation of the role of miRNA-29a in response to PRIMA-1^{Met} in multiple myeloma cells revealed that miRNA-29a plays an important role in sensitizing the cells to PRIMA-1^{Met} [42]. Comparison of miRNA expression levels among PRIMA-1-treated and untreated groups in a lung tumor model in transgenic mice expressing human mutant p53showed that 9 miRNAs changed at least 6-fold after PRIMA-1 treatment compared to the control group; these are miR-194, miR-1937a, 1937b, miR-34c, miR-192, miR-1949, miR-2135, miR-3472, miR-712 and miR-1931. However, thus far, it has not been determined which miRNAs regulate PRIMA-1^{Met}-induced apoptosis in ovarian cancer cells. In the present study, we showed that miRNA-29a expression was increased by PRIMA-1^{Met} treatment in A2780 and Caov-3 ovarian cancer cells carrying wild-type p53 and mutant p53, respectively. These results indicate that miRNA-29a is associated with PRIMA-1^{Met}-induced apoptosis. Thus, cells transfected with the mirVana miRNA-29a inhibitor were treated with PRIMA-1^{Met}, and cell viability and apoptosis were analyzed. The reduction of apoptosis in these transfected cells provides important evidence that miRNA-29a plays a prominent role in PRIMA-1^{Met}-induced apoptosis. The increase in cell viability in these transfected cells supports the importance of miRNA-29a in PRIMA-1^{Met}-induced apoptosis.

CONCLUSION

The results of this study show that the mechanisms of action of PRIMA-1^{Met} differ in part from those previously described. In the present report, the antitumor activity of PRIMA-1^{Met} was shown to be independent of p53 mutation status in ovarian cancer cells and that apoptosis was induced by increasing miRNA-29a. Accordingly, PRIMA-1^{Met} should be examined as a potential anti-cancer agent in *in vivo* and clinical trials. Further studies are needed to examine the role of miRNA-29a in the mechanism of PRIMA-1^{Met}-induced apoptosis in more detail, including the apoptotic genes that it regulates.

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REFERENCES

1. Harris CC. Structure and function of the p53 tumor suppressor gene: Clues for rational cancer therapeuetic strategies. J Natl Cancer Inst. 1996;88(20):1442-55.
2. Sullivan KD, Galbraith MD, Andrysik Z, Espinosa JM. Mechanisms of transcriptional regulation by p53. Cell Death Differ. 2018;25:133-43.
3. Teneriello MG, Ebina M, Linnoila R, Henry M, Nash JD, Park RC, Birrer MJ. p53 and Ki-ras gene mutations in epithelial ovarian neoplasms. Cancer Res. 1993;53:3103-08.
4. Mazare R, Pujol P, Maudelonde T, Jeanteur P, Theillet C. p53 mutations in ovarian cancer: a late event? Oncogene. 1991;6:1685-90.
5. Eccles DM, Brett L, Lessells A, Gruber L, Lane D, Steel CM, Leonard RC. Overexpression of the p53 protein and allele loss at 17p13 in ovarian carcinoma. Br J Cancer. 1992;65:40-4.
6. Kohler MF, Kerns BJ, Humphrey PA, Marks JR, Bast RC, Berchuck A. Mutation and overexpression of p53 in earlystage epithelial ovarian cancer. Obstet Gynecol. 1993;81:643-50.
7. Wang Z and Sun Y. Targeting p53 for novel anticancer therapy. Transl Oncol. 2010;3(1):1-12.
8. Hoe KK, Verma CS, Lane DP. Drugging the p53 pathway: understanding the route to clinical efficacy. Nat Rev Drug Discov. 2014;13:217-36.

9. Duffy MJ, Synnott NC, McGowan PM, Crown J, McGowan PM, Crown J, O'Connor D, Gallagher WM. p53 as a target for the treatment of cancer. Cancer Treat Rev. 2014;40(10):1153-60.
10. Bykov VJ, Issaeva N, Shilov A, Hultcrantz M, Pugacheva E, Chumakov P, Bergman J, Wiman KG, Selinova G. Restoration of the tumor suppressor function to mutant p53 by a low molecular-weight compound. Nat Med. 2002;8:282-8.
11. Selinova G, Wiman KG. Reactivation of mutant p53: molecular mechanisms and therapeutic potential. Oncogene. 2007;26(15):2243-54.
12. Bykov VJ, Zache N, Stridh H, Westman J, Bergman J, Selivanova G, Wiman KG. PRIMA-1MET synergizes with cisplatin to induce tumor cell apoptosis. Oncogene. 2005;24:3484-91.
13. Shi H, Lambert JM, Hautefeuille A, Bykov VJ, Wiman KG, Hainaut P, Caron de Fromentel C. In vitro and in vivo cytotoxic effects of PRIMA-1 on hepatocellular carcinoma cells expressing mutant p53ser249. Carcinogenesis. 2008;29:1428-34.
14. Zache N, Lambert JM, Wiman KG, Bykov VJ. PRIMA-1MET inhibits growth of mouse tumors carrying mutant p53. Cell Oncol. 2008;30:411-8.
15. Liang Y, Besch-Williford C, Hyder SM. PRIMA-1 inhibits growth of breast cancer cells by re-activating mutant p53 protein. Int J Oncol. 2009;35:1015-23.
16. Zandi R, Selivanova G, Christensen CL, Gerds TA, Willumsen BM, Poulsen HS. PRIMA-1Met/APR-246 induces apoptosis and tumor growth delay in small cell lung cancer expressing mutant p53. Clin Cancer Res. 2011;17:2830-41.
17. Oliveto S, Mancino M, Manfrini N, Biffo S. Role of microRNAs in translation regulation and cancer. World J Biol Chem. 2017;8(1):45-56.
18. Zhang B, Pan X, Cobb GP, Anderson TA. MicroRNAs as oncogenes and tumor suppressors. Dev Biol. 2007;302:1-12.
19. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet. 2007;39:673-7.
20. Yang D, Sun Y, Hu L, Zheng H, Ji P, Zhao Y, Reynolds S, Cheng H, Rupaimoole R, Cogdell D, Nykter M, Broaddus R, Rodriguez-Aguayo C, Lopez-Berestein G, Liu J, Shmulevich I, Sood AK, Chen K, Zhang W. Intregrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. Cancer Cell. 2013;23:186-99.
21. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H, Calin GA, Menard S, Croce CM. MicroRNA signatures in human ovarian cancer. Cancer Res. 2007;67:8699-707.
22. Yang N, Kaur S, Volinia S, Greshock J, Lassus H, Hasegawa K, Liang S, Leminen A, Deng S, Smith L, Johnstone CN, Chen XM, Liu CG, Huang Q, Katsaros D, Calin GA, Weber BL, Bützow R, Croce CM, Coukos G, Zhang L. MicroRNA microarray identifies Let-7i as a novel biomarker anti-therapeutic target in human epithelial ovarian cancer. Cancer Res. 2008;68:10307-14.
23. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih IM, Zhang Y, Wood W, Becker KG, Morin PJ. MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One. 2008;3(6):e2436.
24. Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, Kim JW, Kim S. MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res. 2008;14:2690-95.
25. Dahiya N and Morin PJ. MicroRNAs in ovarian carcinomas. Endocr Relat Cancer. 2010;17:77-89.
26. Mateescu B, Batista L, Cardon M, Gruosso T, de Feraudy Yi Mariani O, Nicolas A, Meyniel JP, Cottu P, Sastre-Garau X, Mechta-Grigoriou F. miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response. Nat Med. 2011;17:1627-35.
27. Yeh YM, Chuang CM, Chao KC, Wang LH. MicroRNA-138 suppresses ovarian cancer cell invasion and metastasis by targeting SOX4 and HIF-1a. Int J Cancer. 2013;133:867-78.
28. Park SY, Lee JH, Ha M, Nam JW, Kim VN. miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42. Nat Struct Mol Biol. 2009;16(1):23-9.
29. Aydemir EA, Oz ES, Gokturk RS, Ozkan G, Fiskin K. Glycyrrhizaflavescens subsp. antalyensis exerts antiproliferative effects on melanoma cells via altering TNF-α and IFN-α levels. Food Chem Toxicol. 2011;49(4):820-8.
30. Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods. 2001;25:402-8.
31. Parrales A and Iwakuma T. Targeting oncogenic mutant p53 for cancer therapy. Front Oncol. 2015;5:288.
32. Bykov VJ, Issaeva N, Selivanova G, Wiman KG. Mutant p53-dependent growth suppression distinguishes PRIMA-1 from known anticancer drugs: a statistical analysis of information in the National Cancer Institute database. Carcinogenesis. 2002;23:2011-8.
33. Izetti P, Hautefeuille A, Abujamra AL, de Farias CB, Giacomazzi J, Alemar B, Lenz G, Roesler R, Schwartsmann G, Osvaldt AB, Hainaut P, Ashton-Prolla P. PRIMA-1, a mutant p53 reactivator, induces apoptosis and enhances chemotherapeutic cytotoxicity in pancreatic cancer cell lines. Invest New Drugs. 2014;32:783-94.
34. Russo D, Ottaggio L, Foggetti G, Masini M, Masiello P, Fronza G, Menichini P. PRIMA-1 induces autophagy in cancer cells carrying mutant or wild type p53. BiochimBiophysActa. 2013;1833:1904-13.
35. Aryee DN, Niedan S, Ban J, Schwentner R, Muehlbacher K, Kauer M, Kofler R, Kovar H. Variability in functional p53 reactivation by PRIMA-1(Met)/APR-246 in Ewing sarcoma. Br J Cancer. 2013;109:2696-704.
36. Tessoulin B, Descamps G, Moreau P, Maïga S, Lodé L, Godon C, Marionneau-Lambot S, Oullier T, Le Gouill S, Amiot M, Pellat-Deceunynck C. PRIMA-1Met induces myeloma cell death independent of p53 by impairing the GSH/ROS balance. Blood. 2014;124:1626-36.
37. Li XL, Zhou J, Chan ZL, Chooi JYi Chen ZR, Chang WJ. PRIMA-1met (APR-246) inhibits growth of colorectal cancer cells with different p53 status through distinct mechanisms. Oncotarget. 2015;6(34):36689-99.
38. Yoshikawa N, Kajiyama H, Nakamura K, Utsumi F, Niimi K, Mitsui H, Sekiya R, Suzuki S, Shibata K, Callen D, Kikkawa F. PRIMA-1MET induces apoptosis through accumulation of intracellular reactive oxygen species irrespective of p53 status and chemo-sensitivity in epithelial ovarian cancer cells. Oncol Rep. 2016;35:2543-52.

39. Piantino c, Reis ST, Viana NI, Silva IA, Morais DR, Antunes AA, Dip N, Srougi M, Leite KR. PRIMA-1 induces apoptosis in bladder cancer cell lines by activating p53. Clinics (Sao Paulo). 2013;68(3):297-303.
40. Wang T, Lee K, Rehman A, Daoud SS. PRIMA-1 induces apoptosis by inhibiting JNK signaling but promoting the activation of Bax. Biochem Biophys Res Commun. 2007;352(1):203-12.
41. Lambert JM, Moshfegh A, Hainaut P, Wiman KG, Bykov VJ. Mutant p53 reactivation by PRIMA-1MET induces multiple signaling pathways converging on apoptosis. Oncogene. 2010;29(9):1329-38.
42. Saha MN, Abdi J, Yang Y, Chang H. miRNA-29a as a tumor suppressor mediates PRIMA-1Met-induced anti-myeloma activity by targeting c-Myc. Oncotarget. 2016;7(6):7149-60.
43. Othman N and Nagoor NH. The Role of microRNAs in the Regulation of Apoptosis in Lung Cancer and Its Application in Cancer Treatment. Biomed Res Int. 2014;2014(Special issue):1-19.
44. Duan W, Gao L, Wu X, Wang L, Nana-Sinkam SP, Otterson GA, Villalona-Calero MA. MicroRNA-34a is an important component of PRIMA-1-induced apoptotic network in human cancer cells. Int J Cancer. 2010;127(2):313-20.