miR-375 Modulates Radiosensitivity of HR-HPV-Positive Cervical Cancer Cells by Targeting UBE3A through the p53 Pathway

Background: Prediction of radioresistance of HR-HPV-positive (+) cervical cancer, especially before the course of radiotherapy, is quite beneficial to develop an optimal treatment strategy for individual patients. Unfortunately, the mechanisms responsible for radioresistance of cervical cancer are still largely unexplored. HR-HPV infection leads to a series of changes to normal biophysical process, including miRNAs expression. In this study, we explored the association between miR-375 and radioresistance in HR-HPV (+) cervical cancer.

Material/Methods: qRT-PCR analysis was performed to determine miR-375 expression in HR-HPV-positive (+) cervical cancer patients and in HPV-16-positive SiHa and HPV-18-positive HeLa cervical cancer cell lines. The influence of miR-375 on radiosensitivity and the downstream regulative network were further explored in the cell line models.

Results: The results verified a putative binding site between miR-375 and UBE3A. miR-375 overexpression could significantly reduce UBE3A expression. UBE3A knockdown led to significantly reduced cell survival under radiation treatment. miR-375 promoted radiosensitivity of HR-HPV (+) cancer through decreasing p53 degradation and thereby increasing radiation-induced apoptosis.

Conclusions: The miR-375-UBE3A axis is important in modulating radiosensitivity of HR-HPV (+) cervical cancer.

MeSH Keywords: Human Papillomavirus 16 • Human Papillomavirus 18 • Uterine Cervical Neoplasms

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/893859
Background

Cervical cancer is the third most frequent cancer in women [1]. For these patients, radiotherapy is still the most common intervention, either as a primary or an adjuvant therapy [2,3]. Although radiotherapy is used for over 60% of cervical cancers cases, local recurrence is common due to radioresistance [4,5]. Therefore, the prediction of radioresistance, especially before the course of radiotherapy, is quite beneficial to develop an optimal treatment strategy for individual patients. Unfortunately, the mechanisms responsible for radioresistance of cervical cancer are still largely unexplored.

Persistent high-risk HPV (HR-HPV) infection is involved in more than 90% of cervical cancer cases and has been identified as a causal factor in cancer development [6]. Typically, HPV16 and HPV18 cause about 70% of cervical cancer cases [7]. E6 and E7 are 2 primary oncoproteins of HR-HPV. As a pivotal viral oncogene, E6 can combine with cellular protein ubiquitin-protein ligase E3A (UBE3A), also known as E6AP, and initiate proteasomal degradation of p53 [8]. p53 degradation results in reduced p53-mediated apoptosis and p21-mediated cell cycle arrest [9]. In addition, p53 degradation is also involved in the mechanism of radioresistance in several types of tumors [10,11], including cervical cancer [12,13]. However, the upstream regulative network of p53 in radioresistance of HR-HPV-positive (+) cervical cancer is still not clear.

miRNAs are a group of small, conservative, and non-coding RNAs degrading or repressing the translation of target mRNAs through directly binding to the 3’-UTR [14]. Several miRNAs were found involved in radioresistance of cervical cancers, such as miR-630, miR-1246, miR-1290, miR-3138, miR-181, miR-375, and miR-21 [15,16]. However, the downstream regulative network of these miRNAs is not yet fully understood. In this study, we explored the association between miR-375 and radioresistance in HR-HPV (+) cervical cancer. We demonstrated that miR-375 can modulate radiosensitivity of HR-HPV (+) cervical cancer cells by directly downregulating UBE3A expression and thereby promoting p53 regulated cell apoptosis.

Material and Methods

Patient selection and human tissues

We recruited 22 patients from Cangzhou City Central Hospital who were histologically diagnosed as having IA with lymphovascular space invasion (IVSI) or IA2 cervical cancer, confirmed who were histologically diagnosed as having IA with lymphocytic stromal infiltration. We excluded those with severe concurrent medical diseases. Informed consent was obtained from each patient before collecting the specimens.

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miR-375 mimics and the negative control, UBE3A siRNA, and the corresponding negative controls were purchased from Ribo Life Science (China). HeLa and SiHa cells were transfected with 75 nM miR-375 mimics or 75 nM UBE3A siRNA, respectively. cDNAs were synthesized using the PrimeScript RT reagent Kit (TaKaRa). miR-375 expression was quantified by using TaqMan MicroRNA assays (Life Technologies). The 2−ΔΔ Ct method was used to calculate relative miR-375 expression, with RNU6B as a control gene. UBE3A mRNA expression was measured using qRT-PCR with SYBR Green PCR Master Mix (Life Technologies) and UBE3A-specific primers: F, 5’- CCTGGGGAAATGTCATCCA-3’; R, 5’- TTTTCAGCTGTTGTGGAGG-3’. GAPDH served as the internal control.

Cell transfection

miR-375 mimics and the negative control, UBE3A siRNA, and the corresponding negative controls were purchased from Ribo Life Science (China). HeLa and SiHa cells were transfected with 75 nM miR-375 mimics or 75 nM UBE3A siRNA, respectively, by using lipofectamine 2000 (Invitrogen). Human UBE3A lentiviral expression vector (Lenti-UBE3A) without 3’-UTR was purchased from GENECHEM. To generate sufficient lentiviral particles for transfection, Lenti-UBE3A and the corresponding packaging mix were cotransfected to HEK-293T cells. Viral supernatant was collected for further experiments at 48 h after transfection. To over-express UBE3A in SiHa and HeLa cells, the cells were treated with the viral supernatants with 8 µg/ml Polybrene (Sigma-Aldrich).
MiR-375 expression is negatively related to radiosensitivity in HPV (+) cervical cancer

Based on serum and tumor tissue samples from the cervical cancer patients and healthy controls, qRT-PCR results showed that miR-375 expression was significantly lower in the cancer patients than in the controls (Figure 1A, 1C). Its expression was even lower in radioresistant patients than in radiosensitive patients (Figure 1B, 1D). HPV-16-positive SiHa, which had lower response to radiation than HPV-18-positive HeLa cells (Figure 1E), also had lower expression of miR-375 (Figure 1F). These results suggest that miR-375 might be involved in the radiosensitivity of HPV (+) cervical cancer.

MiR-375 modulates radiation-induced apoptosis of HPV (+) cervical cancer cells

To explore the influence of miR-375 expression on radiation-induced apoptosis, SiHa and HeLa cells were transfected for miR-375 overexpression (Figure 2A, 2C). miR-375 overexpression induced apoptosis, SiHa and HeLa cells were transfected for 10–13 days and then the cells were fixed with 10% paraformaldehyde and stained with 1% crystal violet in 70% ethanol. Then, the plates were further incubated in a cell incubator for 10–13 days and then the cells were fixed with 10% paraformaldehyde and stained with 1% crystal violet in 70% ethanol. Colony formation and apoptosis analysis

Western blot analysis of UBE3A expression

Total protein from cells was extracted by using RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 1% NP-40, and 0.1% SDS). Total protein concentration was measured by using BCA protein assay (Pierce, Thermo Scientific) and then separated on 10% SDS PAGE gel and transferred onto nitrocellulose membranes for a conventional Western blot analysis. Antibodies used were Anti-UBE3A (1:2000, ab10488, Abcam), anti-p53 (1:1000, ab131442, Abcam), anti-p21 (1:2000, ab7960, Abcam), anti-survivin (1:1000, ab24479, Abcam), anti-Bax (1:1000, ab7977, Abcam), and anti-active caspase 3 (1:1000, ab2302, Abcam). GAPDH served as loading control and was detected by using anti-GAPDH (1:2500, ab9485, Abcam). Anti-Rabbit IgG (HRP) (1:10000, ab191866, Abcam) was used as a second antibody. Protein signals were detected using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific).

FTFCAATATGAAATCCCTTATTATTATT-3'; R, 5'-aaacGATTTCAGCTACATATGAAATCCCTTATTATTATT-3'; MUT: F, 5'-aaacGATTTCAGCTACATATCCCTTATTATTATTATTATTATTATT-3'; R, 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miR-375 can directly modulate apoptosis of HR-HPV (+) cervical cancer cells induced by radiation.

**MiR-375 directly targets UBE3A and regulates its expression in HR-HPV (+) cervical cancer cells**

Since miR-375 has a regulative role over radioresistance, we further explored its downstream targets and regulative network in HR-HPV (+) cervical cancer cells. A recent study demonstrated that UBE3A, also known as E6AP, is a direct target of miR-375. In this study, we further verified this target in both SiHa and HeLa cells. miR-375 mimics could inhibit luciferase activity of pmirGLO-UBE3A-WT vector, but had no inhibiting effect over pmirGLO-UBE3A-MUT vector (Figure 3A, 3B). In both of the cell lines, miR-375 overexpression, similar to si-UBE3A expression, effectively interfered with UBE3A expression at both mRNA and protein levels (Figure 3D, 3E). These results suggest that miR-375 can directly target UBE3A and regulate its expression in both HeLa and SiHa cells.
miR-375 promotes radiosensitivity through p53 pathway

We then further studied the downstream regulation network of miR-375. In both HeLa and SiHa cells, UBE3A knockdown significantly lowered cell viability under radiation treatment (Figure 4A, 4B). Overexpression of UBE3A without the 3'UTR region rescued miR-375-induced lowered radiosensitivity in both HeLa and SiHa cells, and also lowered p53 expression (Figure 4C, 4D). The downstream regulation of miR-375 was further explored in HeLa cells. miR-375 overexpression resulted in significantly increased p53 and p21 expression (Figure 4E), both of which are involved in p53-dependent cell cycle G1 phase arrest [18]. miR-375 overexpression also led to significantly higher expression of the apoptotic markers Bax and active caspase 3 under radiation treatment (Figure 4E). In contrast, the expression of survivin, a negative regulation of apoptosis, was remarkably inhibited by miR-375 overexpression (Figure 4E). These results suggest that miR-375 modulates radiation-induced apoptosis of cervical cancer cells at least partially through the p53 pathway.

Discussion

HR-HPV infection can alter a series of biophysical process in cervical tissues. In fact, the infection can also affect the expression of multiple miRNAs, thereby affecting the downstream regulations. miR-375 is a miRNA that is significantly downregulated in HR-HPV infection [19,20]. However, how this expression change affects the downstream regulation is still not clearly understood. According to previous studies, miR-375 is involved in a wide range of regulation in different cancers. In gastric cancer, miR-375 is downregulated and can inhibit migration and invasion of cancer cells by targeting Janus kinase 2 (JAK2) [21]. It can also target the p53 gene and regulate cellular response to ionizing radiation and etoposide in gastric cancer cells [22]. In breast cancer, miR-375 directly targets SHOX2 and thereby modulates epithelial-to-mesenchymal transition (EMT) [23]. In cervical cancer, miR-375 is also involved in cancer development processes. For example, in squamous cervical cancer, miR-375 can inhibit cell migration and invasion via targeting transcription factor SP1 [24]. It can
also mediate acquired chemo-resistance in cervical cancer by facilitating EMT [25]. Therefore, it is highly possible that miR-375 has a wide regulative network and that its regulation might be different in different cancers. In the current study, we observed that miR-375 was related to radioresistance of HR-HPV (+) cancer. A lower level of miR-375 expression resulted in a higher level of radioresistance. In contrast, miR-375 overexpression in HR-HPV-positive cancer cells significantly promoted radiosensitivity. Due to the significant association between miR-375 and radioresistance, we decided to explore how it affects radioresistance in HR-HPV (+) cervical cancer. A recent study reported that miR-375 has multiple targets in HPV-associated cancers, including HPV type 16 and 18 transcripts, E6AP and CIP2A [26]. The HPV type 16 and 18 transcripts consist of E6 and E7 protein, the most oncogenic proteins. This study also demonstrated that replenishment of miR-375 in HPV (+) cervical cancer cells significantly reduced the levels of HPV transcripts [26]. In fact, the complex of E6 and UBE3A is quite important for the oncogenic properties of HR-HPVs. It initiates proteasomal degradation of p53 [8] and results in reduced p53-mediated apoptosis and p21-mediated cell cycle arrest. Therefore, we hypothesized that the downregulation of miR-375 might regulate radiosensitivity through the p53 pathway. In both SiHa and HeLa cells, we verified the putative binding site between miR-375 and UBE3A 3'UTR. miR-375 overexpression significantly reduced UBE3A expression at both mRNA and protein level. Each bar represents the mean ± S.D. of 3 experiments. * P<0.05; ** P<0.01, *** P<0.001.

Figure 3. MiR-375 directly targets UBE3A and regulates its expression in HR-HPV (+) cervical cancer cells. (A) The predicted pairing between miR-375 and 3’-UTR of UBE3A. Designed UBE3A-mutant (MUT) sequence without miR-375 binding sites is shown (B, C). HeLa (B) and SiHa (C) cells were co-transfected with either 75nM miR-375 mimics or NC oligos and 200 ng dual-luciferase reporter plasmids carrying either WT or MUT 3’-UTR of UBE3A. The relative firefly luciferase activity was measured at 24 h after transfection and was normalized with Renilla luciferase activity. miR-375 mimics decreased luciferase activity of WT reporter but not MUT reporter. (D, E) HeLa and SiHa cells were transfected with 75 nM miR-375 mimics or 75 nM UBE3A siRNA, respectively. miR-375 and UBE3A siRNA significantly inhibited UBE3A expression at both mRNA and protein level.
apoptosis [27], significantly decreased with miR-375 overexpression. These results suggest that miR-375 promotes radiosensitivity of HR-HPV (+) cancer through decreasing p53 degradation and thereby increasing radiation-induced apoptosis. Although p53 degradation has already been found in the mechanism of radioresistance in several types of tumors [10,11], our study is the first to demonstrate its upstream regulation in HR-HPV (+) cervical cancer cells.

Conclusions

Based on the evidence obtained above, it is evident that the miR-375-UBE3A axis is important in modulating radiosensitivity of HR-HPV (+) cervical cancer. The modulation occurs at least partly through inhibiting p53 degradation and promoting p53-mediated apoptosis under radiation treatment.

References:

1. Siegel R, Ma J, Zou Z, Jemal A: Cancer statistics, 2014. Cancer J Clin, 2014; 64(1): 9–29
2. Rogers L, Siu SS, Luesley D et al: Radiotherapy and chemoradiation after surgery for early cervical cancer. Cochrane Database Syst Rev, 2012; 5: CD007583
3. Waggoner SE: Cervical cancer. Lancet, 2003; 361(9376): 2217–25
4. Powell ME: Modern radiotherapy and cervical cancer. Int J Gynecol Cancer, 2010; 20(11 Suppl.2): S49–51
5. Frega A, Sopracordevole F, Scirpa P et al: The re-infection rate of high-risk HPV and the recurrence rate of vulvar intraepithelial neoplasia (VIN) usual type after surgical treatment. Med Sci Monit, 2011; 17(9): CR532–35
6. Dunne EF, Park IU: HPV and HPV-associated diseases. Infect Dis Clin North Am, 2013; 27(4): 765–78
7. Durst M, Giessmann L, Ikenberg H, zur Hausen H: A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci USA, 1983; 80(12): 3812–15
8. Scheffner M, Hulbregtse JM, Vierstra RD, Howley PM: The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell, 1993; 75(3): 495–505
9. Buyru N, Altinisik I, Isin M, Dalay N: p53 codon 72 polymorphism and HPV status in lung cancer. Med Sci Monit, 2008; 14(9): CR493–97
10. Concin N, Zeillinger C, Stimpfel M et al: p53-dependent radioresistance in ovarian carcinoma cell lines. Cancer Lett, 2000; 150(2): 191–9

11. Yang CF, Peng LX, Huang TJ et al: Cancer stem-like cell characteristics induced by EB virus-encoded LMP1 contribute to radioresistance in nasopharyngeal carcinoma by suppressing the p53-mediated apoptosis pathway. Cancer Lett, 2014; 344(2): 260–71

12. Lindel K, Rieken S, Daffinger S et al: The transcriptional regulator gene E2 of the Human Papillomavirus (HPV) 16 influences the radiosensitivity of cervical keratinocytes. Radiat Oncol, 2012; 7: 187

13. Beskow C, Skikuniene J, Holgersson A et al: Radioresistant cervical cancer shows upregulation of the NHEJ proteins DNA-PKcs, Ku70 and Ku86. Br J Cancer, 2009; 101(5): 816–21

14. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004; 116(2): 281–97

15. Zhang B, Chen J, Ren Z et al: A specific miRNA signature promotes radioresistance of human cervical cancer cells. Cancer Cell Int, 2013; 13(1): 118

16. Ke G, Liang L, Yang JM et al: miR-181A confers resistance of cervical cancer to radiation therapy through targeting the pro-apoptotic PRKCD gene. Oncogene, 2013; 32(25): 3019–27

17. Koh WI, Greer BE, Abu-Rustum NR et al: Cervical cancer. J Natl Compr Canc Netw, 2013; 11(3): 320–43

18. Gartel AL, Radhakrishnan SK: Lost in transcription: p21 repression, mechanisms, and consequences. Cancer Res, 2005; 65(10): 3983–85

19. Li Y, Wang F, Xu J et al: Progressive miRNA expression profiles in cervical carcinogenesis and identification of HPV-related target genes for miR-29. J Pathol, 2011; 224(4): 484–95

20. Lajer CB, Garnaes E, Friis-Hansen L et al: The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer. Br J Cancer, 2012; 106(9): 1526–34

21. Xu Y, Jin J, Liu Y et al: Snail-regulated miR-375 inhibits migration and invasion of gastric cancer cells by targeting JAK2. PLoS One, 2014; 9(7): e99516

22. Liu Y, Xing R, Zhang X et al: miR-375 targets the p53 gene to regulate cellular response to ionizing radiation and etoposide in gastric cancer cells. DNA Repair, 2013; 12(9): 741–50

23. Hong S, Noh H, Teng Y et al: SHOX2 is a direct miR-375 target and a novel epithelial-to-mesenchymal transition inducer in breast cancer cells. Neoplasia, 2014; 16(4): 279–90 e271–75

24. Wang F, Li Y, Zhou J et al: miR-375 is down-regulated in squamous cervical cancer and inhibits cell migration and invasion via targeting transcription factor SP1. Am J Pathol, 2011; 179(5): 2580–88

25. Shen Y, Zhou J, Li Y et al: miR-375 mediated acquired chemo-resistance in cervical cancer by facilitating EMT. PLoS One, 2014; 9(10): e109299

26. Jung HM, Phillips BL, Chan EK: miR-375 activates p21 and suppresses telomerase activity by coordinate regulating HPV E6/E7, E6AP, CIP2A, and 14-3-3zeta. Mol Cancer, 2014; 13: 80

27. Sah NK, Khan Z, Khan GI, Bisen PS: Structural, functional and therapeutic biology of survivin. Cancer Lett, 2006; 244(2): 164–71