Restoration of Let-7: a possible approach for increased sensitivity to paclitaxel in ovarian cancer

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Abstract

Ovarian cancer is one of the common cancers of the female reproductive system. Paclitaxel is the first-line treatment of ovarian cancer and the second-line treatment of advanced ovarian cancer. Unfortunately, many patients cannot be treated because of drug resistance. miRNAs comprise a group of small non-coding RNAs 18-25 nucleotides in length that specifically interact with their own mRNAs. Many miRNAs that have so far been identified play a role in cancer. miRNAs regulate formation of cancer stem cells (CSCc) and drug resistance-associated epithelial-mesenchymal transition (EMT) phenotype. The let-7 miRNA is a founding member of the miRNA family and is conserved in invertebrates and vertebrates. In this review paper we have tried to describe a possible approach for increased sensitivity to paclitaxel in ovarian cancer by restoration of Let-7. In addition to suppressing tumorigenic activities and negatively regulating a number of oncogenes (Kras-Hras-HMGA2-c-myc-BF2), let-7 affects the main regulators of cell cycle, cell differentiation, and apoptosis pathway. Let-7 via RNA decomposition of the IMP-1 gene increased sensitivity to paclitaxel drug. Various compounds such as Isoflavone specifically can affect expression of Let-7. Although let-7 is a potential therapeutic target for therapy resistant ovarian cancer, further studies should be conducted to investigate clinical use of let-7 to treat or suppress ovarian cancer.

Key words: miRNA, Ovarian cancer, Drug resistance, Let-7

Introduction

Today cancer as a deadly disease causes many problems for all of the people in the world. Ovarian cancer is one of the common cancers of the female reproductive system and one of the most life-threatening cancers such that it is the cause of over 50% of deaths due to gynecological cancers. At early stages, ovarian cancer is asymptomatic or its symptoms may be so vague that they cannot be detected by physician or the patient (1). There are many drugs for prevention and treatment of cancers such as ovarian cancer. Natural products such as medicinal plants have been used as one of the main resources for production of anticancer drugs (2-7). Paclitaxel as a natural compound is the first-line treatment of ovarian cancer and the second-line treatment of advanced ovarian cancer that prevents microtubule depolymerization in the process of cell proliferation. Hence, paclitaxel inhibits cell cycle. If ovarian cancer is diagnosed at early stages, treatment consists of surgery and chemotherapy. At advanced stages, chemotherapy is started as well, but unfortunately treatment may not be successful in many cases because of drug resistance (8) such that following surgery, combination chemotherapy (paclitaxel+carboplatin) is also used with an 80% response rate.

However, in most patients, unfortunately, recurrent cancer develops and the disease becomes resistant to chemotherapy after 18 months. Currently, the cell line NCI/ADR-RES, which has become resistant to paclitaxel, and the cell line OVCAR8, as control, are used to investigate resistance to ovarian cancer drugs in vitro. In this review paper we have tried to describe a possible approach for increased sensitivity to paclitaxel in ovarian cancer by restoration of Let-7.

miRNAs

miRNAs comprise a group of small non-coding RNAs approximately 18-25 nucleotides in length that cause destruction of mRNA and inhibition of its translation. miRNA genes comprise approximately 1% of the genome of different species. Each miRNA gene has hundreds of target genes. Over 2500 miRNAs have been identified in the human genome that regulate 30% of protein-coding genes. Most of these small regulatory molecules that were first identified in 1983 are located on Chromosomal fragile regions that are predisposed to removal, addition, chromosomal replacements, and epigenetic changes in different diseases such as cancer. miRNAs target several genes simultaneously such that the number of target genes may exceed 100 (9, 10). Since 2002, disruption of miRNA regulation has been found to be associated with cancer (11, 12).

miRNA biogenesis

miRNA in the nucleus transcribes the gene and produces primary miRNA (pri-miRNA). Then, Drosha creates a precursor called pre-miRNA under RNaseIII (endonuclease), and pre-miRNA is transferred to cytoplasm by exportin-5. This molecule is cleaved by an enzyme called Dicer and produces a double-stranded sequence 20-22 nucleotides in length. One of the strands is degraded and the miRNA's, another strand, is loaded into RNA-induced silencing complex (RISC). This active complex targets the mRNA of interest and binds to the end of 3`-UTR mRNA, and exerts inhibitory effect. miRNA induces its effect in regulating gene expression through inhibiting the protein translation and decomposing the target mRNA (13).

miRNA and Cancer

Many miRNAs that have so far been identified play a role in cancer. Comparing tumor tissues with healthy tissues has indicated that miRNAs are located at fragile sites of the human genome and are likely to face gene deletion or duplication at chromosomal rearrangement. Besides that, it is possible that epigenetic mechanisms lead to inappropriate expression of miRNA genes and cause abnormal expression of miRNAs in tumor tissues leading to numerous changes in regulation of the target miRNA expression. Many miRNAs play no part in development of cancer. In contrast, certain miRNAs play an oncogenic role in cancer phenotype, and dysregulation of these miRNAs has been reported in a wide spectrum of cancers (14). Oncogenic miRNAs include miR-10b, miR-155, miR-21, and miR-17-92 (15), and out of repressive miRNAs, miR-26a, miR-335, and members of the families let-7 and miR-34 can be mentioned. Different miRNAs affect different stages of cancer. For example, miR-10b regulates metastasis and is highly expressed in advanced malignancies. Inhibition of miR-10b can prevent metastasis of cancer cells but has no effect on already developed metastases. Expression of miR-335 can prevent metastasis but cannot prevent proliferation of tumor cells and has no effect on cell apoptosis rate. However, some miRNAs can prevent proliferation of tumor cells and metastasis (16). miRNA expression has been reported to change (increase or decrease) in different human cancers (17, 18) .

miRNA and chemotherapy resistance

Recently, some studies have found miRNA and chemotherapy resistance to be associated (19, 20). In recent years, considerable advancements have been made to figure out drug resistance mechanism in ovarian cancer consisting of drug efflux, changes in DNA repair pathway, apoptosis suppression, and epithelial-mesenchymal transition and cancer stem cells. However, more effective therapeutic purposes are still needed to improve overall survival rate and therapeutic strategies for ovarian cancer patients. miRNAs play a critical role in cell processes such as cellular differentiation, proliferation, and apoptosis. The recent discovery of miRNAs in cancer has offered new paths for research on basic mechanisms of response to chemotherapy. Besides that, several studies have demonstrated that certain miRNAs such as let-7 and miR-34a can affect response to chemotherapy in different types of tumors including ovarian cancer. Use of miRNAs to overcome resistance to treatment is being studied. This study investigated the role of let-7 in overcoming resistance to treatment through several molecular mechanisms and with emphasis on potential therapeutic uses.

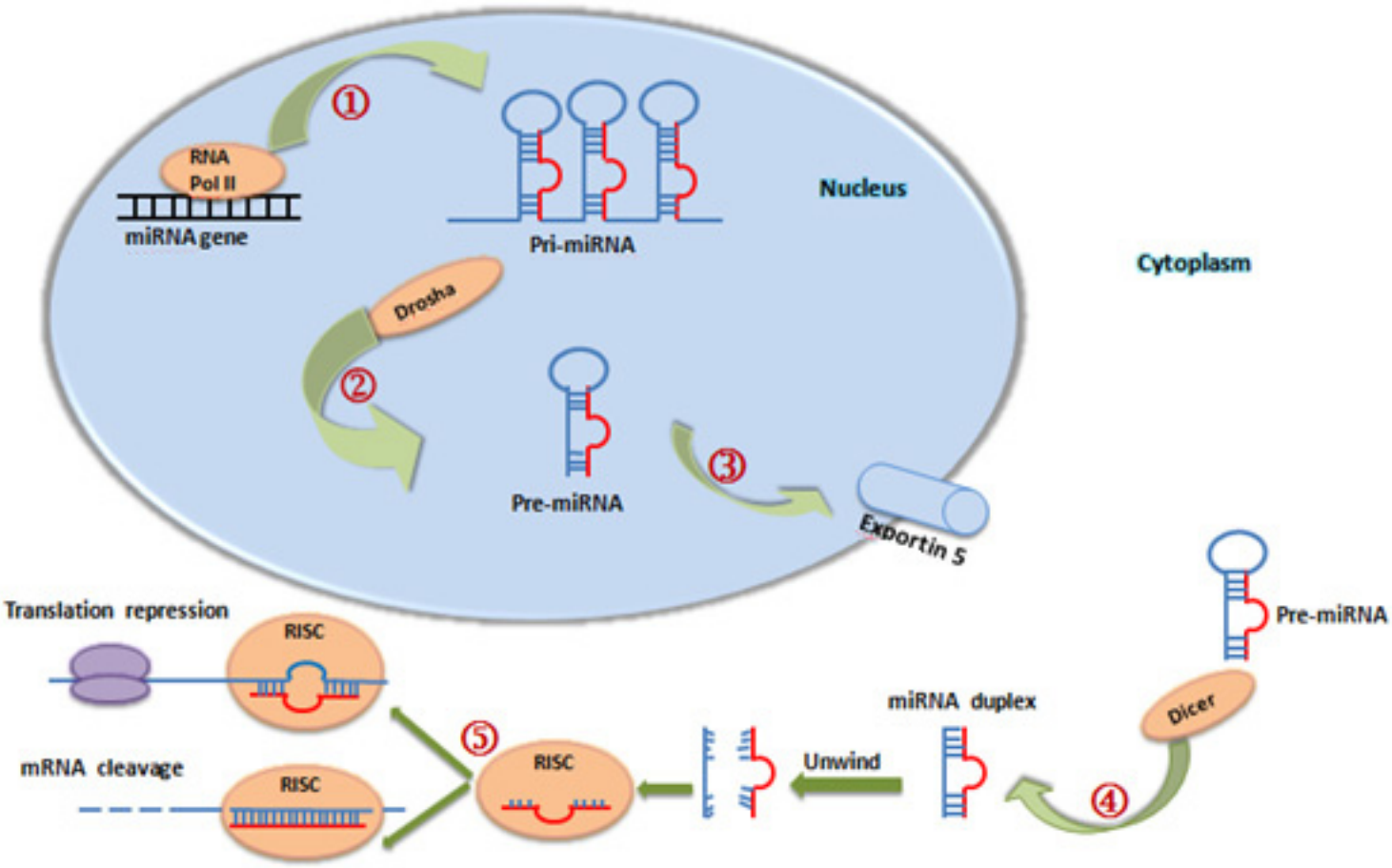

Figure 1: Biogenesis of miRNA: miRNA biogenesis is a multistep process. First, miRNA genes are transcribed by RNA polymerase II in the nucleus. The resulting primary transcript is cleaved by Drosha and DGCR8 to produce pre-miRNA. After exportin-5- and RanGTP-mediated transport to the cytoplasm, the pre-miRNA undergoes its final processing step, which consists of Dicer-dependent cleavage just below the stem loop to produce a duplex molecule. The duplex is then separated and usually one strand is selected as the mature miRNA and directed to target-specific mRNAs

miRNA Let-7

Let-7 is from a 13-member family localized on nine different chromosomes. An association between let-7 and drug resistance has been demonstrated. Recent studies have demonstrated that let-7 specifically affects 3-Urt-BCL-XL in hepatocellular cell line and let-7 high expression makes the cells susceptible to sorafenib (21). Since let-7 expression has been reported to decrease in many cancers, the changes in the expression of this miRNA are likely to be associated with chemotherapy resistance, but the data are scant in this field. Igf2 mRNA binding protein1 (IMP-1) is a drug resistance-associated protein and it has recently been demonstrated that IMP-1 level is associated with let-7 level. In fact, let-7 negatively regulates IMP-1 which in turn exerts protective effect on multi-drug resistance (MDR-1). Measuring let-7 in different cell lines indicated that the members of this family were co-regulated and co-expressed. Let-7 expression has been demonstrated to decrease before and after treatment with chemotherapy drugs, which is associated with increased production of IMP-1 and MDR-1.

Molecular mechanisms of chemotherapy resistance in cancer

Chemotherapy resistance develops molecularly via two pathways consisting of de novo or internal pathway through CSCs, and external or acquired pathways including genetic and epigenetic changes. However, the precise mechanisms of chemotherapy resistance generally have not yet been identified. In de novo pathway, limited drug absorption, increased efflux, and activated detoxification and in the second pathway, epigenetic changes such as DNA methylation-histone modification and mRNA regulation play part in drug resistance. For example, in colorectal cancer, the transcription factors AP2E and DKK4 undergo methylation changes that cause them to become resistant to fluorouracil. In ovarian cancer, the gene MLH1-TAP73 is hypermethylated and predisposed to acquiring resistance to d-azacitidine-hydralazine. miRNA deregulation has been demonstrated to be associated with cancer drug resistance. For example, in breast cancer, increased expression of miR-21 leads to trastuzumab resistance. In case of resistance to cisplatin in ovarian cancer, the expression rates of miR 376 and miR-214 increase and therefore it is necessary to study miRNAs so

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Potential mechanisms of let-7 action on ovarian cancer

Let-7 regulates CSCs and EMT formation which is associated with drug resistance (23, 24). Let-7 exerts regulatory effects on p53 (25). Let-7 negatively regulates MDR and indeed exerts effect on MDR1 indirectly through IMP1. Let-7 causes decomposition of mRNA related to IMP-1 which is both a target of let-7 and inhibits endolithic activity of MDR1 (26). In experimental studies, silencing the gene EZH2 has been demonstrated to cause decrease in cell proliferation, M-G2 arrest, and cell drug susceptibility. Increased expression of let-7 causes the expression of the gene EZH2 to decrease but it has not yet been discovered how this occurs. Let-7a, let-7b, and let-7c exert inhibitory effects on EZH2. Let-7 can also exert inhibitory or down-regulatory effect on CCND1. CCND1 is a member of the family of cyclins that affects cell cycle and its expression in tumors increases in cisplatin resistance (26). Studies have demonstrated that the expression of the common miRNAs that exist in most paclitaxel-resistant cell lines is associated with ovarian cancer. These miRNAs include miR: pre218-let-7e-130a-130b-pre204-0c-335-106-pre106, and let-7 (27). MS-PCR results have indicated that in chemotherapy drug-resistant cell lines, let-7-related CPG hypermethylation occurs in DNA in most cancers including ovarian cancer, and since one of the mechanisms of disrupted expression (deregulation) and decreased expression of let-7 is hypermethylation, then hypermethylation is likely to occur in ovarian cancer as well.

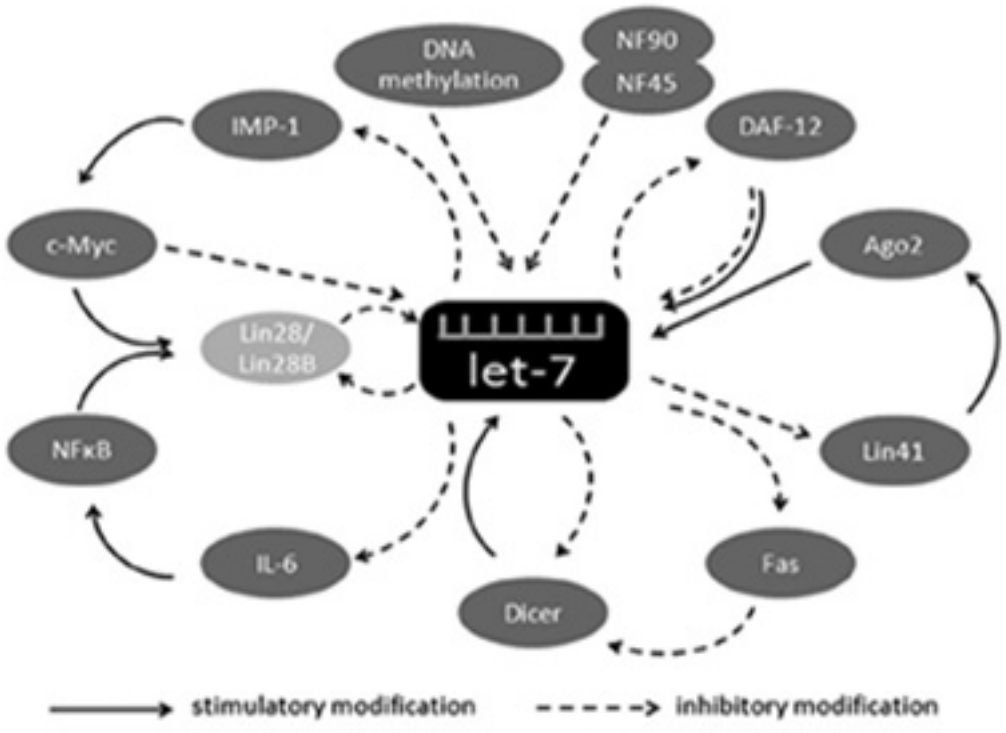

Figure 2: illustrates the mechanisms and factors that affect let-7. IMP-1 plays a role in drug resistance and inhibits its let-7. DNA methylation exerts inhibitory effect on let-7 (28).

Flavonoid's effect on let-7 expression

Recently, nature-based compounds such as isoflavone and DIM have been demonstrated to affect the expression of miRNAs including let-7 and can induce its expression; therefore, flavonoid's effect on paclitaxel transporters can be investigated in a resistant cell line, termed NCI/ADR-RES. Flavonoids affects miR-21 expression and increases its production, and causes increase in production of the molecules PTEN-PDCD4-RECK that progress the cell mainly toward apoptosis. Although no study has yet reported clinical use of these compounds, clinical trials at different phases are being conducted (25). Synthetic and/or nature-based compounds derived from plant flavonoids mainly target malignant cells. In neuroblastoma, the flavonoid and retinoid compound, called cyclincyc, can exert effect on miRNA with oncogenic role and miRNA with tumor-suppressing role (23). Since synthetic let-7 has limited use and is easily degraded, use of flavonoids to increase let-7 expression seems appropriate.

Conclusion

Introduction of miRNAs and their role represents a new level of controlling gene expression. Studies have demonstrated that disrupted regulation of miRNAs can be an important stage of progression in most cancers. Dysregulation of miRNAs can be due to genetic mutations or regulation at transcription level which are important mechanisms of increased expression of the target genes causing tumorigenesis. miRNA-based treatments are based on two bases; use of mimics miRNAs that is mainly conducted by miRNA replacement therapy and causes the expression levels of tumor-suppressing miRNAs (undergoing decrease in expression) to reach normal levels. The second approach is use of their antagonists which are mainly used to inhibit function of oncogenic miRNAs. A drug called AS1411 is from a group of compounds called G-rich aptamer. This drug acts via blocking production of oncogenic miRNAs in the cell whose expression levels increase in cancer. AS1411 inhibits a protein called nucleolin that plays an important role in miRNA maturation (29). In studies miRNA microarray, decreased expression of miR30C-miR130a-let7 was demonstrated to cause paclitaxel resistance and cisplatin. Moreover, let-7 inhibits certain oncogenic proteins such as Kras-Hras-HMGA2-c-myc-NF2. Studies have demonstrated that the removal rate of DNA in ovarian cancer is over 44% for Let-7a-3 and let-7b (30). In the near future, use of nanoparticle technology, particularly in cancer drug resistance, will facilitate use of let-7 and other miRNAs in treatment. Let-7 nanoparticle is being used In vitro (31).

Taken together, regarding the potential role of let-7 in paclitaxel resistance in ovarian cancer and that let-7 can suppress the expression of the genes involved in this cancer, let-7 has attracted attention as a potential therapeutic target in therapy resistant ovarian cancer. In addition to suppressing tumorigenic activities and negatively regulating a number of oncogenes (Kras-Hras-HMGA2-c-myc-BF2), let-7 affects the main regulators of cell cycle, cell differentiation, and apoptosis pathway. Therefore, let-7 can be used to inhibit the expression of these genes and therefore therapy resistant cancer. Regarding the findings of recent studies, we can use increase in expression of let-7 using medicinal plants, or mimic production of it as a synthetic and transporting it into the cell to enhance treatment and control the growth of ovarian cancer, and mimic let-7 most probably can be used as an adjuvant drug in the treatment protocol for patients with paclitaxel resistance. However, this issue requires further investigation. Increase in Let-7 expression is expected to serve as an effective treatment for therapy resistant cancer. Although acceptable advancements have been made to figure out regulation of let-7 synthesis and role in signalling pathways, its regulation in cancer and normal cells, and mechanism of cell proliferation control and cell survival need further investigation. Further studies are needed to use let-7 in clinical settings to treat or suppress therapy resistant cancer.

References

1. Cho KR, Shih Ie M. Ovarian cancer. Annual review of pathology. 2009;4:287-313.
2. Asadi-Samani M, Kooti W, Aslani E, Shirzad H. A systematic review of Iran's medicinal plants with anticancer effects. Journal of evidence-based complementary & alternative medicine. 2016;21(2):143-53.
3. Kooti W, Servatyari K, Behzadifar M, Asadi-Samani M, Sadeghi F, Nouri B, et al. Effective Medicinal Plant in Cancer Treatment, Part 2. Journal of evidence-based complementary & alternative medicine. 2017:2156587217696927.
4. Gholamian-Dehkordi N, Luther T, Asadi-Samani M, Mahmoudian-Sani MR. An overview on natural antioxidants for oxidative stress reduction in cancers; a systematic review. Immunopathologia Persa. 2017;3(2):e12.
5. Afkhami-Ardakani M, Hassanzadeh S, Shahrooz R, Asadi-Samani M, Latifi M, Luther T. Phytotherapy and phytopharmacology for reduction of cyclophosphamide-induced toxicity in the male urinary system. Journal of Renal Injury Prevention 2017;6(3):164-70.
6. Rahimifard M, Sadeghi F, Asadi-Samani M, Nejati-Koshki K. Effect of quercetin on secretion and gene expression of leptin in breast cancer. Journal of Traditional Chinese Medicine. 2017;37(3):321-5.
7. Chaleshtori JS, Soreshjani EH, Reisi F, TabaTabaiefar MA, Asadi-Samani M, Navid Z, et al. Damage intensity of carvacrol on prostatic cancer cells lineDu145 and molecular dynamic simulation of it effect on apoptotic factors. International Journal of PharmTech Research. 2016;9(6):261-73.
8. Kim A, Ueda Y, Naka T, Enomoto T. Therapeutic strategies in epithelial ovarian cancer. Journal of experimental & clinical cancer research 2012;31:14.
9. Heneghan HM, Miller N, Kerin MJ. MiRNAs as biomarkers and therapeutic targets in cancer. Current opinion in pharmacology. 2010;10(5):543-50.
10. Slabý O, Svoboda M, Fabian P, Svoboda M, Garajová I, Šachlová M, et al. Association of miR-21, miR-31, miR-143, miR-145 and let-7a-1 levels with histopathologic

features of colorectal cancer. European Journal of Cancer. 2007; 5(4): 78-79.
11. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences. 2002;99(24):15524-9.
12. Mahmoudian-sani M-R, Mehri-Ghahfarrokhi A, Asadi-Samani M, Mobini G-R. Serum miRNAs as Biomarkers for the Diagnosis and Prognosis of Thyroid Cancer: A Comprehensive Review of the Literature. European Thyroid Journal. 2017:In Press.
13. Bhatt K, Mi Q-S, Dong Z. microRNAs in kidneys: biogenesis, regulation, and pathophysiological roles. American Journal of Physiology-Renal Physiology. 2011;300(3):F602-F10.
14. Orellana EA, Kasinski AL. MicroRNAs in cancer: a historical perspective on the path from discovery to therapy. Cancers. 2015;7(3):1388-405.
15. Medina PP, Nolde M, Slack FJ. OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. Nature. 2010;467(7311):86-90.
16. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature. 2000;403(6772):901-6.
17. Xie Q, Chen X, Lu F, Zhang T, Hao M, Wang Y, et al. Aberrant expression of microRNA 155 may accelerate cell proliferation by targeting sex-determining region Y box 6 in hepatocellular carcinoma. Cancer. 2012;118(9):2431-42.
18. Zhang Y, Wei W, Cheng N, Wang K, Li B, Jiang X, et al. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. Hepatology (Baltimore, Md). 2012;56(5):1631-40.
19. Wang F, Liu M, Li X, Tang H. MiR-214 reduces cell survival and enhances cisplatin-induced cytotoxicity via down-regulation of Bcl2l2 in cervical cancer cells. FEBS letters. 2013;587(5):488-95.
20. Chan JK, Blansit K, Kiet T, Sherman A, Wong G, Earle C, et al. The inhibition of miR-21 promotes apoptosis and chemosensitivity in ovarian cancer. Gynecologic oncology. 2014;132(3):739-44.
21. Ray SK. Emerging roles of microRNAs in malignant neuroblastoma. Clinical & Experimental Pharmacology. 2013;2013.
22. Cai J, Yang C, Yang Q, Ding H, Jia J, Guo J, et al. Deregulation of let-7e in epithelial ovarian cancer promotes the development of resistance to cisplatin. Oncogenesis. 2013;2(10):e75.
23. Peter ME. Regulating cancer stem cells the miR way. Cell stem cell. 2010;6(1):4-6.
24. Zheng T, Wang J, Chen X, Liu L. Role of microRNA in anticancer drug resistance. International journal of cancer. 2010;126(1):2-10.
25. Sarkar FH, Li Y, Wang Z, Kong D, Ali S. Implication of microRNAs in drug resistance for designing novel cancer therapy. Drug Resistance Updates. 2010;13(3):57-66.
26. Xie Z, Cao L, Zhang J. miR-21 modulates paclitaxel sensitivity and hypoxia-inducible factor-1α expression in human ovarian cancer cells. Oncology letters. 2013;6(3):795-800.
27. Sorrentino A, Liu C-G, Addario A, Peschle C, Scambia G, Ferlini C. Role of microRNAs in drug-resistant ovarian cancer cells. Gynecologic oncology. 2008;111(3):478-86.
28. Wang X, Cao L, Wang Y, Wang X, Liu N, You Y. Regulation of let-7 and its target oncogenes (Review). Oncology letters. 2012;3(5):955-60.
29. Kutanzi KR, Yurchenko OV, Beland FA, Vasyl F C, Pogribny IP. MicroRNA-mediated drug resistance in breast cancer. Clinical epigenetics. 2011;2(2):171.
30. Wang Y, Hu X, Greshock J, Shen L, Yang X, Shao Z, et al. Genomic DNA copy-number alterations of the let-7 family in human cancers. PloS one. 2012;7(9):e44399.
31. Gadducci A, Sergiampietri C, Lanfredini N, Guiggi I. Micro-RNAs and ovarian cancer: the state of art and perspectives of clinical research. Gynecological Endocrinology. 2014;30(4):266-71.