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MiR-145 on the Proliferation of Ovarian Cancer Cells by Regulating the Expression of MMP-2/MMP-9

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ABSTRACT

This study aimed to consider the effect of miR-145 on the proliferation of OC cells by regulating the expression of MMP-2/MMP-9. In this experiment, the miR-145 target gene with restriction enzyme cut sites was amplified from the four groups of rat genomes, and it was ligated to the pIRES plasmid to form a recombinant plasmid and identified. The miR-145 recombinant plasmid and the empty plasmid into OC cells were transfected, and qRT-PCR was used to detect the expression of miR-145 in the transfected OC cells. Experimental data showed that there are a total of 60 healthy male SD rats, randomly divided into 4 groups, namely the normal saline control group (group A), the model group (group B), treatment group with 30 mg miR-145 (group C), and treatment group with 100mg miR -145 (group D). The experimental results showed that compared with group A, the expression of MMP-2 protein in rats in groups B, C, and D all increased to varying degrees on 7 days, 14 days, and 28 days, reaching the highest at 14 days. This study shows that miRNA-145 is under-expressed in OC cell lines, and miR-145 can significantly inhibit the proliferation and invasion of ovarian cells by regulating the expression of MMP-2/MMP-9.

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Introduction

Ovarian Cancer (OC) has the highest mortality rate among gynecological malignancies. More than 150,000 people die every year worldwide (1). Because of its hidden dangers and the lack of early and effective diagnosis methods, 80% of patients are late for treatment. In recent years, the diagnosis and treatment of ovarian malignancies have continued to improve, but this has not provided significant clinical benefits (2). Nowadays, the research of molecular targets related to OC has become a hot topic of research. MicroRNA (miRNA) is a non-coding sequence that participates in the regulation of post-transcriptional levels and plays an important role in the biological behavior of cells (3, 4).

Wiria *et al*. found that in ovarian endometriosis cyst tissue, the increase in the number of angiogenesis has a certain correlation in ectopic endometrial cells (5). Disrupting the balance between the two can cause a variety of pathological changes. Osteen et al. found that progesterone can induce the up-regulation of TIMP smRNA expression in endometriosis, inhibit the expression of MMPs, and prevent ectopic endometrial implantation, further proving the role of MMPs and TIMPs in the occurrence of endometriosis (6).

In 1994, people first discovered acute myelocytic stem cells. Since then, more and more cancer stem cells have been discovered, and the OC stem cell hypothesis has gradually formed (7). The OC stem cell hypothesis believes that the formation, chemotherapy resistance, and recurrence of OC are caused by a small part of OC cells, which have With self-renewal and repair capabilities, chemotherapy only reduces the tumor body, but the core composed of tumor stem cells is not eliminated, which then causes tumor recurrence. Current studies have found that chemotherapy mainly acts on rapidly dividing cells, while OC stem cells can survive chemotherapy and cause chemotherapy resistance and stronger invasiveness (8, 9).

Certain molecular markers of tumor cells have stem cell characteristics. According to these molecular markers, corresponding tumor stem cells can be

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isolated. The markers of OC stem cells currently found are: CD44, CD117, CD133, CD24, ABCG2, ALDH, etc., for these molecular markers (10). The study may find the mechanism and characteristics of tumor stem cell self-renewal and metastasis, and drug resistance, but the oval stem cell markers found in each patient are different, and their histopathological types are not correlated with molecular markers. They are targeted at OC stem cells. Targeted therapy has had preliminary results, but for different patients, individualized treatment is required according to different patient conditions (11, 12).

There are many researches on the treatment of egg cancer (13). For example, beizumab can improve the progression-free survival rate of patients with a high risk of progression, and use allogeneic and allogeneic hematopoietic stem cell transplantation to stimulate the body's immune response against tumor cells including CSCs. Growth and differentiation, through biological treatment, to achieve the purpose of curing tumors. There have been more and more researches on the drug resistance, recurrence, growth and differentiation of ovarian cells in tumors. Reversing ovarian stem cell tolerance has become the focus of reducing pain recurrence and improving prognosis which targeted killing of OC stem cells while normal stem cells are not harmed. The focus is to further research on the cell surface markers of ovarian stem CSCs (14, 15).

In general, OC is damaging the health of women, and its incidence is gradually increasing (13). With the development of medicine, the research and early screening of tumor markers in recent years have increased the early detection and diagnosis rate of OC (16). The purpose of this experiment is to determine the level of regulated miRNA-145 in OC cell lines and explore its effect on the proliferation and invasion of OC cells.

Materials and methods

Animal Grouping

A total of 60 healthy male SD rats were randomly divided into 4 groups, namely the saline control group (group A), the model group (group B), the treatment group with 30 mg miR-145 (group C), and the treatment group with 100mg miR -145 (group D).

Establishment of Ovarian Fibrosis Model

After weighing the rats in groups B, C and D, the rats were completely anesthetized by intraperitoneal injection of 10% chloral hydrate (3 ml/kg). The rat is fixed on the rat rack behind. After preparing the experimental drugs, use compound iodine to disinfect the neck skin. Layer until the trachea is exposed insert a syringe (1 mL) into the heart and into the trachea through the cartilage ring of the trachea. After the bubbles are pulled back, the corresponding solution for each group will be injected. Group A was infused with an equal volume of saline, and the other group was infused with 0.3 ml of bleomycin saline (5 mg/kg), and continued to infuse 0.3 ml of air. Immediately after injection, the rat platform was assembled and rotated left and right 5 times to distribute the injected drug solution evenly in the ovaries on both sides of the animal, and then sterilized and sewn. During the experiment, each group of rats eats freely.

Method of Administration

Starting from the first day of model building, groups C and D were given 50mg/kg and 100mg/kg miR-145 dissolved in normal saline (2mL) by gavage once a day; while groups A and B were given equal volumes of Physiological saline (2mL) was intragastrically administered.

Collection and Processing of Specimens

On the 7, 14, and 28 days of modeling, each group of rats were randomly sacrificed, and the rats were randomly divided into 3 groups, each with 5 rats, a total of 20 rats. Take out the ovaries from the blood flow of the carotid artery, wash them quickly with normal saline, absorb the surface water with gauze, and use 5% formaldehyde for HE staining, Masson staining and immunohistochemical staining of the ovarian tissue; the ovarian tissue is cut into small pieces in a liquid nitrogen environment, Coexist in the EP tube treated with DEPC water, seal the tube, and immediately transfer it to -70°C freezer for RT-PCR detection of mRNA.

Immunohistochemical Staining Method to Detect the Expression of MMP-2 and MMP-9 Protein in Ovarian Tissue

(i) Dewaxing and dehydration of paraffin sections: routinely dewax with xylene and xylene for 10

minutes; in the order of 100% ethanol, 95% ethanol, 90% ethanol, 80% ethanol, 75% ethanol Dehydrate for 5 minutes respectively and then wash with distilled water for 10 minutes;

(ii) Enzyme inactivation: Incubate in 3% H2O2 deionized water for 10 minutes at room temperature to inactivate endogenous peroxidase, and rinse with distilled water 5 times;

(iii) Recover antigen: Place the slices in citrate buffer (PH6.0, 0.01mol/L), heat in a microwave oven (100°C) for 20 minutes, and rinse with PBS solution 3 times after cooling;

(iv) Perform immunohistochemical reaction according to the PV method, drop the primary antibody, overnight at 4°C, and rinse 4 times with PBS solution for 2 minutes each;

(v) Remove the PBS solution, add 1 drop of polymer enhancer (reagent A) to each slice, incubate at room temperature for 20 minutes, rinse with PBS solution 3 times, 2 minutes each time; remove the PBS solution, and add 1 drop of the enzyme to each slice Standard anti-mouse/rabbit polymer (reagent B), incubate at room temperature for 50 minutes and rinse with PBS solution 4 times, 2 minutes each time;

(vi) DAB color development: use DAB color development kit. Add 2 drops of freshly prepared DAB solution to the slices. Observe the color development under a microscope for 2−5 minutes, and wash with tap water to stop the reaction;

(vii) Adding Harris hematoxylin for light counterstaining, dehydration with alcohol, transparent xylene, and sealing with neutral resin;

(8) After each group of slides was taken under a light microscope (400 times) with different fields of view, the integrated optical density value was calculated using Image-Pro Plus 6.0 software and then statistically analyzed.

RT-PCR Method to Detect the Expression of MMP-2 and MMP-9mRNA in Lung Tissue

(i) Pipetting Tips, EP tubes, grinders and tissue scissors are all treated with 1‰ DEPC water to remove enzymes, sterilized in a high-temperature and high-pressure steam boiler for 30 minutes, and dried in an oven for later use;

(ii) Take out the ovarian tissue from the refrigerator at -60°C, cut the tissue with tissue scissors under liquid nitrogen and weigh it, take 0.1g, grind and homogenize it thoroughly under liquid nitrogen, and add 1ml TRIZOL reagent. Repeated pipetting to fully lyse the sample;

(iii) Transfer the lysate to the EP tube, add chloroform at the ratio of 0.3mL chloroform per 1ml TRIZOL reagent, quickly cover the tube cap, shake vigorously for 4s, mix well, and leave it at room temperature for 3 minutes;

(iv) Centrifuge at 12000rpm and 4°C for 15min; the solution is divided into three layers, and the RNA is mainly in the upper colorless water phase. Pipette the upper aqueous phase and transfer it to a new enzyme-free EP tube. Add an equal volume of isopropanol, mix well, and place at room temperature for 10 minutes;

(v) After centrifugation at 12000 rpm for 10 minutes at 4°C, you can see that the total RNA is deposited as a white precipitate at the bottom of the tube. Discard the supernatant and leave the white precipitate. Add 1 mL of freshly prepared 75% ethanol (prepared with 1‰ DEPC water), wash for 6 minutes at 7500 rpm and 4°C, and then blot the liquid with a small tip and settle. Dry it at room temperature for 10 minutes, and then add 50uL of 1‰ DEPC water, 12000rpm, and centrifuge at 4°C for 2 minutes, aliquot and store in a -70°C refrigerator for later use.

Routine preparation of 1.8% agarose gel: Weigh 1.8g agarose, transfer it into a 200ml triangular beaker, add 100ml0.5×TBE, heat it in a microwave oven for 1min, shake gently to escape bubbles as much as possible, add 3ulEB dye solution, shake well and pour it into the mold. After cooling, it will become a milky white gel. Put it in the electrophoresis tank, add 0.5×TBE, and the liquid level is 1mm above the gel surface to check the integrity of the sample RNA.

Statistical Processing

The GraphPad prism 6 software was used to process the experimental data, and the results were tested by t-test. $P<0.05$ indicates that the difference is statistically significant.

Results and discussion

MiR-145 Regulates the Expression of MMP-2 Protein

The miR-145 regulates the expression of MMP-2 protein to deposit in brown particles. The expression

of normal rat lung tissue is weak, and it can be found in a small number of alveolar macrophages, epithelial cells, vascular endothelial cells and another cytoplasm; brown particles in the ovarian tissue of ovarian fibrosis rats have strong expression, which is seen in ovarian macrophages. The results of this experiment show that compared with group A, the expression of MMP-2 protein in rats in groups B, C, and D increased to varying degrees on 7 days, 14 days, and 28 days, reaching the highest at 14 days, and still high at 28 days In the control group, the difference was statistically significant (P<0.05). The expression of MMP-2 protein in rat ovarian tissue at each period are shown in Table 1 and Figure 1:

Table 1. Expression of MMP-2 protein in rat breast tissue at different time periods

Group	n	7d	14d	28d
Group A	5	35.065±2.225	36.881±3.083	37.372±3.615
Group B	5	51.783±3.591	148.966±3.724	98.696±1.969
Group C	5	47.662±2.688	78.518±2.234	63.429±2.729
Group D	5	38.748±2.246	67.176±2.387	48.793±1.276

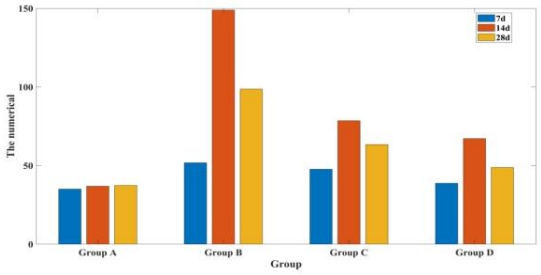

Figure 1. Expression of MMP-2 protein in rat breast tissue at different periods

The expression of MMP-2mRNA in group A rats is weak. Compared with group A, the levels of MMP-2mRNA in groups B, C, and D all increased to varying degrees on 7 days, 14 days, and 28 days, with the highest level at 14 days, and it was still higher than the normal group on 28 days (P<0.05). The expression of MMP-2mRNA in rat lung tissue during the period is shown in Figure 2:

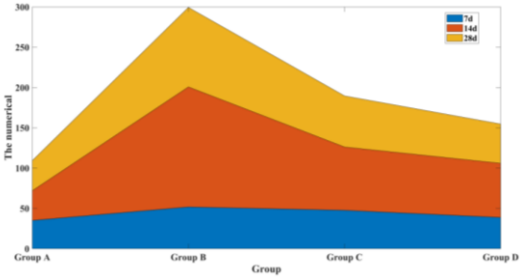

Figure 2. Expression of MMP-2 mrna in rat lung tissue at the period

MiR-145 Regulates the Expression of MMP-9 Protein in Rats

The miR-145 regulates the weak expression of MMP-9 protein in the normal group, which can be found in a small number of macrophages, neutrophils, and ovarian cell cytoplasm. The expression is strong in the ovarian tissue of ovarian fibrosis rats, which is seen in the middle among ovarian cells dominated by sex granulocytes, as well as ovarian epithelial cells, bronchiolar epithelial cells and fibrotic areas. The results of this experiment show that compared with group A, the expression of MMP-2 protein in rats in groups B, C, and D increased to varying degrees on 7 days and 14 days (P<0.05), the most obvious in 7 days, and then gradually decreased. Compared with the control group at 28 days, the difference was not statistically significant (P>0.05). Compared with group B, miR-145 regulated the expression of MMP-9 protein in ovarian tissue of each treatment group decreased on 7 and 14 days, and it was statistically significant (P<0.05). Table 2 and Figure 3 show the period Expression of MMP-9 protein in mouse ovarian tissue.

Table 2. Expression of MMP-9 protein in rat ovarian tissue at different time periods

Group	n	7d	14d	28d
Group A	5	40.587±1.560	38.177±2.184	40.788±2.382
Group B	5	142.588±4.177	95.676±2.858	43.678±2.788
Group C	5	102.587±2.331	88.489±1.257	42.254±3.399
Group D	5	90.225±4.987	62.878±2.873	42.765±2.451

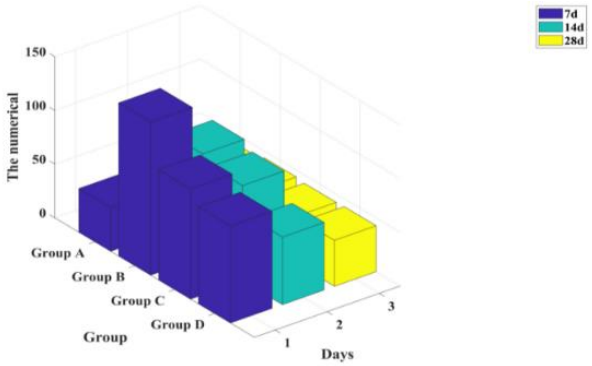

Figure 3. Expression of MMP-9 protein in rat ovarian tissue at different periods

Consistent with the results of MMP-9 protein expression, the expression of MMP-9 mRNA in group A rat was weak. Compared with group A, the expression of MMP-9mRNA of rats in groups B, C, and D increased to varying degrees on 7 days and 14

days (P<0.05), the most obvious was 7 days, and then gradually decreased. Compared with group B, miR-145 regulated the expression of MMP-9mRNA in ovarian tissues of each treatment group and decreased on 7 days and 14 days, and it was statistically significant (P<0.05). The expression of MMP-9mRNA in rat ovarian tissue at each period is shown in Figure 4:

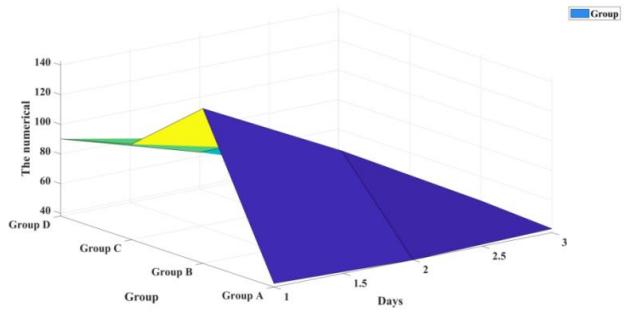

Figure 4. Expression of MMP-9mrna in rat ovarian tissue at each period

Ovarian Cancer (OC) is damaging the health of women, and its incidence is gradually increasing (17). With the development of medicine, the research and early screening of tumor markers in recent years have made the early detection and diagnosis rate of OC more effective. However, the survival rate of OC has not increased significantly in the past 30 years (18). Due to the deep anatomical position of the ovary, its manifestations in the early stages of the disease are often not obvious. Most patients are already in the advanced stage at the time of diagnosis, with a low 5-year survival rate and high mortality.

Matrix metalloproteinase 2 (MMP2) degrades various protein components of the extracellular matrix, destroys the histological barrier of tumor cell invasion, and plays an important role in tumor invasion and metastasis (19, 20). The nucleus then regulates its expression and promotes the proliferation and invasion of tumor cells. This suggests that PinX1 may regulate the expression of c-myc and MMP2 by participating in the regulation of Wnt/β-catenin signal activation to promote the proliferation and invasion of OC, and how PinX1 targets its related molecules to mediate this process is still It is not clear and needs further exploration (21, 22).

PinX1, which is low expressed in OC, participates in the occurrence and development of OC by promoting the proliferation and invasion of OC cells. It is believed that with the improvement of PinX1 gene function research, it will definitely become the diagnosis and treatment of OC (22).

Studies have shown that the Notch pathway not only plays an important role in the self-renewal and proliferation of OC stem cells but also plays a role in maintaining the stemness of OC stem cells. Notch pathway blockers can significantly inhibit the self-renewal and proliferation ability of OC stem cells, and can Down-regulating the expression of surface markers of OC stem cells and the expression of Oct4 and Sox2 proteins and mRNA, secretase inhibitors, as a kind of Notch pathway blockers, may bring new ways for treatment (23).

Studies have shown that wnt can maintain hematopoietic stem cells in the quiescent phase, inhibiting the wnt pathway can interfere with stem cell dormancy, and therapeutic monoclonal antibodies against wnt pathway can inhibit tumor growth in vivo (24).

Cyclopamine can inhibit the self-renewal of cancer stem cells by blocking Hedgehog signaling, and it also inhibits various Hedgehog signaling-dependent pathways, such as epithelial-mesenchymal transition. Combining cyclopamine with chemotherapeutic drugs can simultaneously kill tumor stem cells, thereby achieving the purpose of treating tumors (25).

The activation of oncogenes, the inactivation of tumor suppressor genes and the imbalance of their interactions are the main causes of tumors (26, 27). The expression of PinX1 in OC tissues is significantly lower than that of normal ovarian epithelial tissues and is closely related to tumor lymph node metastasis and FIGO staging. However, its specific molecular mechanism is largely unknown (28, 29). In order to further explore its effect on the biological behavior of OC cells and its related molecular mechanisms, people constructed a pCMV-N-Flag-PinX1 overexpression recombinant vector, which was transiently transfected into human OC cell SKOV3 by liposome, and The expression changes of PinX1 in cells were detected at the mRNA and protein levels, and the results showed that the expression level of PinX1 was significantly up-regulated and the PinX1 gene could be successfully expressed. However, the successful construction of a stable over-expressed PinX1 vector will likely become a gene target A powerful tool for treatment (30, 31). In addition,

CCK8, research results show that after overexpression of PinX1, SKOV3 cell viability is inhibited, and cell invasion ability is significantly reduced, suggesting that in OC cells, low-expression PinX1 may promote the malignant proliferation of OC, and infiltration and metastasis of tumors closely related (32, 33).

The incidence is between benign adenoma and cancer, and malignant differentiation may occur. It usually develops on both sides, with early abdominal metastases, and the prognosis is poor (31). Therefore, exploring new molecular markers related to the development and development of serous OC and applying them to the early diagnosis of OC effectively and conveniently will reduce the mortality of patients with OC and improve their quality of life. MicroRNA (Mirna) is a highly conserved non-coding single-stranded small RNA, consisting of 18-25 nucleotides, which can participate in gene expression by targeting specific mRNA (32). MiR-145 is located on human chromosome 5q32. Its expression level is reduced in OC and inhibits the growth and angiogenesis of OC cells. In addition, miR-145 can be used as a tumor suppressor gene to regulate tumor cell growth, apoptosis, invasion, metastasis and other relationships, and has potential tumor protection effects (31).

Abnormally expressed miRNA molecules are involved in the development of many diseases, and they play different roles in each stage of the disease (33). The biological functions of miRNAs are diverse, and the expression levels of miRNA molecules are also different in different tumors (such as cell differentiation, proliferation, apoptosis, invasion and metastasis). Some miRNAs are overexpressed in tumors, some are abnormally inactivated, and some are tumor proto-oncogenes and some tumor suppressor genes. Differentially expressed miRNAs can participate in the entire tumorigenesis and development process by regulating target genes. At present, the research hotspots of miRNA mainly focus on miRNA molecules, miRNA molecules and their therapeutic targets are used as indicators for early tumor diagnosis, and new methods of tumor biotherapy and the regulation of miRNA target genes are studied (32). There are various methods for miRNA quantitative research, but the most commonly used methods at this stage include colorimetry, fluorescence, chemiluminescence, surface-enhanced Raman spectroscopy, single-molecule detection and electrochemical analysis. Most of them now use the real-time Quantitative PCR detection method. The continuous development of miRNA hypersensitivity detection methods has led to the deepening of miRNA research (33).

Researchers have conducted a lot of research on the relationship between miR-145 and OC (32). The relevant literature investigated in this article shows that miR-145 is a tumor suppressor gene in OC, which can be achieved by increasing p53 in SKOV3 cells and reducing the malignancy of cell tumors. The up-regulation of miR-145 inhibits the invasion, metastasis and proliferation of OC cells and enhances the cytostatic effect induced by paclitaxel (34). The main mechanism is that miR-145 inhibits the MUC1/E-cad signaling pathway and blocks the epithelium caused by the invasion and metastasis of SKOV3 cells (33).

This study confirms through research that microRNA has an extremely important function in the natural progression of tumors, and it mainly binds to the bases of target messenger RNA to act in many ways. This article proves through experimental studies that miR-145 can inhibit the occurrence and development of several human malignant tumors by regulating the signal pathways of the body; this s shows that miR-145 can inhibit the metastasis of gastric cancer, but it is rarely reported in OC. In this study, it was detected by qRT-PCR that the expression of miR-145 was found to be higher in pIRES-miR-145 SKOV3 cells than in the wild-type and cells that only transferred the pIRES empty plasmid, and the experiment observed that pIRES- miR-145 SKOV3 grows more slowly, which inspires us that miR-145 may have a function similar to tumor suppressor genes in OC.

The experimental results in this article show that, compared with group A, the expression of MMP-2 protein in rats in groups B, C, and D increased to varying degrees on 7, 14, and 28 days, and reached the highest at 14 days. At present, the specific mechanism of miR-145 in tumors is still uncertain, and there are a lot of experiments to explore it. This experiment confirmed that the regulation of MMP-2/MMP-9 by miR-145 can significantly reduce the proliferation and invasion of OC cells. However, the effects of miR-145 on OC in vivo need to be further explored.

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None.

Interest conflict

None.

References

1. Sarwar HA, Iftikhar J, Yasmeen S et al. Factors Favoring Long Term Survival in Patients with Stage IV Epithelial Ovarian Cancer: An Institutional Research. Asian Pac j cance care 2020; 5(1): 15-18.
2. Ikram D, Masadah R, Nelwan BJ, Zainuddin AA, Ghaznawie M, Wahid S. CD133 Act as an Essential Marker in Ovarian Carcinogenesis. Asian Pac J Cancer Prev 2021; 22(11): 3525-3531.
3. Dong Y, Liu R-L, Deng Y-Z, Wang W-J, Li W-D. Erratum to: Tumor suppressor miR-145 reverses drug resistance by directly targeting DNA damage-related gene RAD18 in colorectal cancer. Tumor Biol 2016; 37(5): 7003-7003.
4. Du J, Cheng X, Shen L et al. Methylation of miR-145a-5p promoter mediates adipocytes differentiation. Biochem Biophys Res Commun 2016; 475(1): 140-148.
5. Sing SL, An J, Yeong WY, Wiria FE. Laser and electron-beam powder-bed additive manufacturing of metallic implants: A review on processes, materials and designs. J Orthop Res 2016; 34(3): 369-385.
6. Carter TE, Smith TE, Osteen PJ. Gender comparisons of social work faculty using h-index scores. Scientometrics 2017; 111(3): 1547-1557.
7. Pourahmad S, Foroozani S, Nourelahi M, Hosseini A, Razmkhah M. Evaluation of Twenty Genes in Prognosis of Patients with Ovarian Cancer Using Four Different Clustering Methods. Asian Pac J Cancer Prev 2021; 22(6): 1781.
8. Hannan J, Brooten D, Youngblut JM, Galindo AM. Comparing mothers' postpartum concerns in two clinical trials 18 years apart. J Am Assoc Nurse 2016; 28(11): 604-611.
9. Yamasaki T, Seki N, Yoshino H et al. MicroRNA-218 inhibits cell migration and invasion in renal cell carcinoma through targeting caveolin-2 involved in focal adhesion pathway. J Urol 2013; 190(3): 1059-1068.
10. Winarno GNA, Pasaribu M, Susanto H et al. The Platelet to Lymphocyte and Neutrophil to Lymphocyte Ratios in Predicting Response to Platinum-based Chemotherapy for Epithelial Ovarian Cancer. Asian Pac J Cancer Prev 2021; 22(5): 1561-1566.
11. Gomes SE, Simões AE, Pereira DM, Castro RE, Rodrigues CM, Borralho PM. miR-143 or miR-145 overexpression increases cetuximab-mediated antibody-dependent cellular cytotoxicity in human colon cancer cells. Oncotarget 2016; 7(8): 9368.
12. Zhan M, Zhao X, Wang H et al. miR-145 sensitizes gallbladder cancer to cisplatin by regulating multidrug resistance associated protein 1. Tumor Biol 2016; 37(8): 10553-10562.
13. Lee SS, Park JS, Lee KB, Jeong DH, Byun JM, Lee SM. Diagnostic Performance of F-18 FDG PET/CT Compared with CA125, HE4, and ROMA for Epithelial Ovarian Cancer. Asian Pac J Cancer Prev 2021; 22(4): 1123-1127.
14. Wang W, Ji G, Xiao X et al. Epigenetically regulated miR-145 suppresses colon cancer invasion and metastasis by targeting LASP1. Oncotarget 2016; 7(42): 68674.
15. Zhang H, Mao F, Shen T et al. Plasma miR‐145, miR‐20a, miR‐21 and miR‐223 as novel biomarkers for screening early‐stage non‐small cell lung cancer. Oncol Lett 2017; 13(2): 669-676.
16. Ercisli MF, Kahrizi D, Aziziaram Z. Environmental factors affecting the risk of breast cancer and the modulating role of vitamin D on this malignancy. 2020.
17. Temtanakitpaisan A, Kleebkaow P, Aue-aungkul A. Epithelial Borderline Ovarian Tumor: Clinicopathological Features, Outcome and Prognostic Factors. Asian Pac j cance care 2018; 3(4): 75-75.
18. Kazemi E, Zargooshi J, Dehpahni MF et al. Androgen regulated protein and pyruvate dehydrogenase kinase 4 in severe erectile dysfunction: A gene expression analysis, and computational study of protein structure. Cell Mol Biol 2021; 67(2): 89-94.
19. Takai T, Yoshikawa Y, Inamoto T et al. A novel combination RNAi toward Warburg effect by replacement with miR-145 and silencing of

PTBP1 induces apoptotic cell death in bladder cancer cells. Int J Mol Sci 2017; 18(1): 179.
20. Yang L, Song X, Zhu J et al. Tumor suppressor microRNA-34a inhibits cell migration and invasion by targeting MMP-2/MMP-9/FNDC3B in esophageal squamous cell carcinoma. Int J Oncol 2017; 51(1): 378-388.
21. Tian J, Sun C, Yu B. GW28-e0394 IncRNA-CRNDE regulates the expression of MMP-2/MMp-9 and influences the function of macrophages in the atherosclerotic plaque rupture. J Am Coll Cardiol 2017; 70(16S): C96-C96.
22. Ivanovic RF, Viana NI, Morais DR et al. miR-29b enhances prostate cancer cell invasion independently of MMP-2 expression. Cancer Cell Int 2018; 18(1): 1-6.
23. Ren W, Liu Y, Wang X et al. The Complement C3a–C3aR Axis promotes development of thoracic aortic dissection via regulation of MMP2 expression. J Immunol 2018; 200(5): 1829-1838.
24. Yazdani Y, Rad MRS, Taghipour M, Chenari N, Ghaderi A, Razmkhah M. Genistein suppression of matrix metalloproteinase 2 (MMP-2) and vascular endothelial growth factor (VEGF) expression in mesenchymal stem cell like cells isolated from high and low grade gliomas. Asian Pac J Cancer Prev 2016; 17(12): 5303.
25. Dong M, Zhou C, Ji L, Pan B, Zheng L. AG1296 enhances plaque stability via inhibiting inflammatory responses and decreasing MMP-2 and MMP-9 expression in ApoE−/− mice. Biochem Biophys Res Commun 2017; 489(4): 426-431.
26. Huang Y-F, Chiu W-C, Chou S-H et al. Association of MMP-2 and MMP-9 expression with recurrences in primary spontaneous pneumothorax. Kaohsiung J Med Sci 2017; 33(1): 17-23.
27. Yin H, Wan Q, Tian Y, Zhao B, Deng Y. Female Hormone 17β-Estradiol Downregulated MMP-2 Expression and Upregulated A1PI Expression in Human Corneal Stromal Cells. Cell Biochem Biophys 2018; 76(1): 265-271.
28. Hu K, Liang M. Upregulated microRNA-224 promotes ovarian cancer cell proliferation by targeting KLLN. In Vitro Cell Dev Biol - Anim 2017; 53(2): 149-156.
29. Mahapatra L, Andruska N, Mao C, Le J, Shapiro DJ. A novel IMP1 inhibitor, BTYNB, targets c-Myc and inhibits melanoma and ovarian cancer cell proliferation. Transl Oncol 2017; 10(5): 818-827.
30. McDowell A, Afroze SH, Tobin R et al. Cinobufotalin as a novel agent to inhibit in vitro epithelial ovarian cancer cell proliferation, migration and invasion. Open J Obstet Gynecol 2016; 6(6): 343-351.
31. Xu Y, Su D, Zhu L et al. S-allylcysteine suppresses ovarian cancer cell proliferation by DNA methylation through DNMT1. J Ovarian Res 2018; 11(1): 1-8.
32. Kreitzburg KM, Landen CN, Gamblin T, Arend R, Alvarez R, Yoon K. Combination of FTY720 and tamoxifen inhibits drug-resistant ovarian cancer cell proliferation. AACR; 2017.
33. Alsina-Sanchís E, Figueras A, Lahiguera A et al. TGFβ controls ovarian cancer cell proliferation. Int J Mol Sci 2017; 18(8): 1658.
34. Wu Y, Mu X, Kahrizi D. Design of biological gene information collection system based on data mining technology. Cell Mol Biol 2020; 66(7): 93-102.