MiR-143-3p Increases the Radiosensitivity of Breast Cancer Cells Through FGF1

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ABSTRACT

Breast cancer is a common malignant tumor in women. At present, the main treatment for breast cancer is radiotherapy. Due to the difference in radiosensitivity between individuals or tumor cells, the effect of radiotherapy is not good. Therefore, in radiotherapy, how to use various auxiliary means to reduce the radiation resistance of tumor, Therefore, it has become an important research topic to improve the radiosensitivity of the tumor. Fibroblast growth factor-1 (FGF1) plays an important role in tumor migration. Therefore, the study of miR-143-3p increasing the radiosensitivity of breast cancer cells through FGF1 is proposed in this paper. In this study, a control group experiment was set up to study. During the experiment, the relative expression of miR-143-3p was detected by fluorescent quantitative PCR of miRNA, and the cell irradiation experiment was created to analyze the radiosensitivity of breast cancer cells by comparing their survival fraction. The results of this study showed that when the radiation dose was 0, the survival scores of the three groups were all 1. The survival fraction of the experimental group decreased from 0.26 ± 0.045 to 0.068 ± 0.008 when the dose was added to 4Gy. The survival fraction of the experimental group was always greater than that of the two control groups. The results of this study show that miR-143-3p can increase the radiosensitivity of breast cancer cells through FGF1.

Introduction

Radiotherapy is an important treatment for breast cancer, which plays an important role in reducing tumor recurrence and improving the cure rate of breast cancer (1). However, the biological resistance of breast cancer to radiotherapy is still an important problem of radiotherapy (2). MiRNA plays an important role in tumor development and treatment, It has become a promising new way to improve the radiosensitivity of tumor cells by studying the miRNA pathway (3). MiR-143-3p has various functions. Some studies have shown that there is a regulatory relationship between miR-143-3p and FGF1 (4). Therefore, the study of miR-143-3p increasing the radiosensitivity of breast cancer cells through FGF1 may have an important reference value for clinical radiotherapy of breast cancer (4, 5).

Breast cancer is the most common cancer in American women, not only that but also the second leading cause of cancer death in women (6). In general, the traditional diet of Asian women is high in soy products and related low incidence of breast cancer. Soy-based diets are rich in phytoestrogens, one of which is ginseng. Rostoker *et al*. (7) believe that Genictein has a protective effect on breast cancer. They studied the protective effect of genistein on breast cancer in a rodent model of dimethylbenzoanthracene (DMBA). Their research results show that genistein treatment of newborn and pre-puberty changes the individual development of the breast and reduces the sensitivity of adult animals to chemically induced breast cancer (7). Based on the relevant research results, we can see that the rate of formation and the persistence of DMBA-DNA addition to the mammary gland did not change significantly with the treatment of the new ginseng (8, 9). On the other hand, the high concentration of ginsengine in the neonatal period has adverse effects on the development of ovarian follicles, but treatment with ginseng before adolescence has no adverse

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effects on the female reproductive tract and the endocrine system (10, 11).

At present, there are many researches on fibroblast growth factor, many scholars have explored fibroblast growth factor. For example, Gozali *et al*. (12) suggested that fibroblast growth factor (FGF) signal transduction is related to the formation of early mesoderm and neural lineage in vertebrates. In mice, FGF receptor 1 (FGFR1) is expressed in an appropriate spatiotemporal manner to coordinate these functions. The homozygous mouse embryo with FGFR1 allele mutation died in early development, showing abnormal growth and abnormal mesoderm pattern (13, 14). Gozali *et al*. (12) conducted a chimeric analysis to further study the role of FGFR1 in morphogenesis and pattern formation of mesoderm in gastrulation. They believed that the primary defect related to FGFR1 (delta TMK) mutation is the defect of the ability of the ectodermal cells to pass through the primary stripe. FGFR1 (delta TMK) / FGFR1 (delta TMK) cells aggregate in the primary bands of chimeric embryos to form secondary neural tubes. These secondary neural tubes are completely derived from FGFR1 (delta TMK) / FGFR1 (delta TMK) cells (15). The use of ectopic neural fate shows that normal morphogenesis through striation is not only essential for the correct formation of mesoderm patterns, but also for the correct determination of mesoderm/neuroectoderm cell fate (16). This study provides some reference value for the study of the formation of mesoderm and neural lineage in early vertebrates.

Breast cancer has always been a female killer (2). In order to make the radiation effect of breast cancer better, this study has studied how to increase the radiosensitivity of breast cancer cells. In order to ensure the reliability of the research results, we set up a double control group, namely a blank control group and control group. In the experiment, we analyzed the relative expression of miR-143-3p and analyzed the radiosensitivity of the three groups by observing the survival fraction of cancer cells. After the study, we found that mRNA and protein levels in the experimental group were significantly lower than the levels of the control group and the blind control group, at this time, the two results were statistically significant (P <0.01).On the other hand, there was no significant difference in mRNA and protein levels between the control group and the blind control group (P> 0.0). In addition, we found that when the radiation dose was 2 g, 4Gy, 6gy, 8Gy, its survival fraction the control group was the highest, the lowest in the experimental group, and the survival fraction of the experimental group was always greater than that of the two control groups once the radiation dose was added.

Materials and methods
Experimental step

In this study, a control experiment was used to improve the accuracy of the results of this study. In this study, cancer cells were divided into three groups, one was control group mir-nc, the other was experimental group miR-143-3p, in addition, a group of the blank control group was set, the blank control group did not do treatment, the three groups of cancer cells were compared.

Cell culture and treatment

In this study, human breast cancer cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum and placed in a 37°C, 5% CO_2 concentration saturated humidity incubator. Observe the cytological morphology under a microscope and record the cell growth status. After the cells grow to 80%-90%, they are digested with 0.25% trypsin and centrifuged, and then passaged in a 1:2 ratio. The cancer cells were cultured in the incubator for 24h, and then the cells were transfected with miR-143-3p. For the method of transfection, please refer to the instructions of the LipofectamineTM2000 transfection reagent. Observe the transfection effect of the cells under a fluorescent microscope 48h after transfection.

Relative expression of miR-143-3p

Take the treated cells, add a proper amount of Trizol reagent, extract the total RNA of cells, then reverse the RNA into cDNA according to the instructions of miRNA first chain synthesis kit, prepare the PCR system according to the instructions of miRNA fluorescence quantitative PCR kit, carry out miRNA fluorescence quantitative PCR, and detect the relative expression of miR-143-3p.

Analysis of double luciferase reporter gene

Using bioinformatics online prediction technology, fgf13'utr mutant vector (3'utrmut) was constructed by point mutation. MiR-143-3pmimics and its control group, fgf13'utrwt and mut were transfected into HCT-116 / L respectively. 48 hours later, the cells were collected and the activity of firefly luciferase (FIR) and marine kidney luciferase was detected according to the instructions of the double luciferase reporter gene test kit.

Cell irradiation experiment

The 6MV X-ray of varian2100c linear accelerator was used as the radiation source, the source skin distance was irradiated, the dose rate was 400cgy / min, SSD = 100cm, PDD = 80%, and the irradiation field was 100mm × 100mm. According to the dose groups of 0, 2, 4, 6, 8Gy, the radiation was given to describe the dose survival curve, and the mean of the three times of irradiation survival fraction was analyzed.

Statistical analysis

Spss19.0 software was used to analyze the experimental data. In order to ensure the accuracy of the experimental results, the cell experimental results were repeated at least three times. The experimental data were expressed by $(x \pm s)$. The comparison between the two groups was a t-test, with $P < 0.05$ as the difference.

Results and discussion
FGF1 is the target gene of miR-143-3p

In this study, bioinformatics was used to predict the basic complementary relationship between miR-143-3p and 3'UTR of FGF1 on the Internet, and the results of the detection of the double clearance gene of a journalist were shown in Figure 1. From Figure 1, the fluorescence intensity of the miR-143-3pmimics and fgf13'utrwt co-transfection group was significantly lower than that of the control group ($P < 0.05$). However, there was no difference in fluorescence intensity between the miR-143-3pmimics and fgf13'utrmut co-transfection group and its control group ($P > 0.05$), which indicated that FGF1 was one of the miR-143-3p target genes.

The transfection effect and the relative expression of miR-143-3p

The transfection effect of the two groups of cells is shown in Figure 2. Three mRNA and three protein molecules were selected as the tracking detection objects to analyze the relative expression of miR-143-3p. The results are shown in Figure 3(*Figure from WWW.baidu.com*).
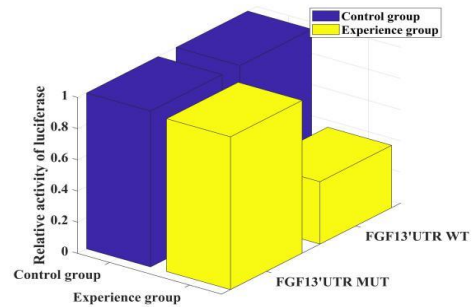

Figure 1. Luciferase reporter gene test results
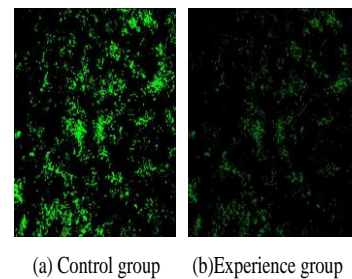

(a) Control group   (b)Experience group
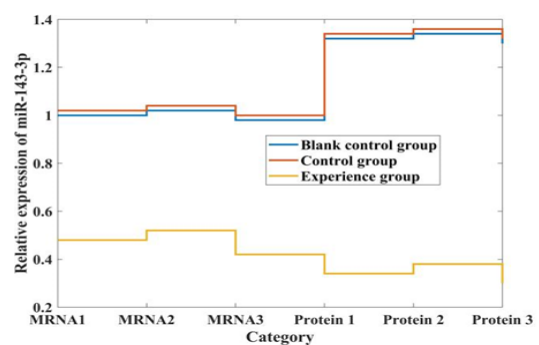**Figure 2.** Transfection results of two groups of cells

Figure 3. Relative expression of miR-143-3p

It can be seen from Figure 2 that the transfection effect of the cells is observed under a fluorescent microscope. From Figure 3, the relative expression level of miR-143-3p can be seen. In this Figure 3, we can see that the mRNA and protein levels in the cells of the experimental group are significantly lower In

the control group and the blank control group, the mRNA and protein levels between the two groups were statistically significant at this time (P<0.01), however, the mRNA and protein levels of the control group were not significantly different from the blank control group. Is not statistically significant (P>0.05).

Cell survival after irradiation

After cell irradiation, the survival fraction of three groups of cancer cells at different doses is shown in Table 1 and Figure 4.

Table 1. Survival fraction of three groups of cancer cells at different doses

Irradiation dose	Blank control group	Control group	Experience group
0Gy	1	1	1
2Gy	0.512 ± 0.046	0.621 ± 0.105	0.26 ± 0.045
4Gy	0.147 ± 0.031	0.192 ± 0.046	0.068 ± 0.008
6Gy	0.045 ± 0.018	0.046 ± 0.012	0.021 ± 0.004
8Gy	0.018 ± 0.016	0.019 ± 0.006	0.012 ± 0.003
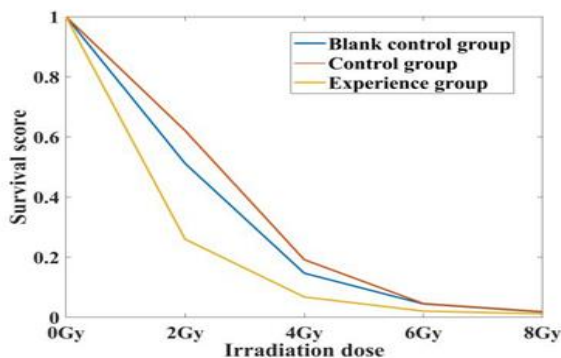
Figure 4. Survival fraction results of three groups of cancer cells at different doses

It can be seen from Table 1 and Figure 4 that the survival scores of three groups of cancer cells at 2gy, 4Gy, 6gy and 8Gy doses are statistically significant, and when the radiation dose is 0, the survival scores of the three groups are all 1. When the radiation dose was 2gy, 4Gy, 6gy, 8Gy, the survival fraction of the control group was the highest, and the experimental group was the lowest, which was statistically significant. When the dose was added to 4Gy, the survival fraction of the experimental group decreased sharply from 0.26 ± 0.045 to 0.068 ± 0.008, and the survival fraction of the experimental group was

always greater than that of the two control groups since the radiation dose was added.

Breast cancer is the most common malignant tumor of women, and it is already seriously endangering the health of women all over the world (1). Not only that, the incidence of breast cancer is increasing year by year in recent years, and it is gradually ranking first in female malignant tumors. Radiation therapy is one of the important means of comprehensive treatment of breast cancer, but many factors will affect the effect of radiotherapy in the clinical application (3, 17). Therefore, it is particularly important to improve the radiation sensitivity of tumors, and some studies have shown that miR-143-3p is related to cancer cell mechanisms (18). Therefore, in this paper, miR-143-3p was used to increase the radiotherapy sensitivity of breast cancer cells through FGF1.

Malignant tumor has become one of the main causes of the serious threat to the health of Chinese women, and in recent years, the incidence and death of malignant tumor are on the rise, and the situation of prevention and control is grim. Breast cancer is the most common cancer among women, and its incidence rate has increased every year (19, 20).

Breast cancer is a hormone-dependent tumor, which occurs in menopausal and perimenopausal women (21). The emergence of endocrine therapy has attracted people's attention to the field of hormone, however, most of the research is aimed at estrogen, progesterone and other sex hormones (22). It has been clear that gonadotropins affect the body by forming steroids. Most breast cancers are hormone-dependent. They express estrogen receptors and are affected by these hormone levels or therapies that interfere with their receptors, such as aromatase inhibitors or tamoxifen.

Many research results have proved that the incidence of breast cancer is related to the following aspects: birthplace, pathogenic virus, atypical breast hyperplasia, improper nutrition, long-term high dose ionizing radiation, long-term estrogen stimulation, genetic factors and so on (23). At present, the main treatment methods for breast cancer are radiotherapy, chemotherapy, surgical treatment, endocrine treatment and molecular targeted treatment (24).

Fibroblast growth factor family (FGFs) plays an important role in many cell processes, such as cell proliferation, cell survival, cell differentiation, tumor

metastasis and so on. FGFs are highly expressed in many tumors, such as kidney, bladder, prostate and testis. FGFs can promote tumor growth through different mechanisms, such as inducing angiogenesis, promoting mitosis of tumor cells, inhibiting apoptosis and so on. So far, about 23 kinds of FGF have been found, among which fibroblast growth factor 1 (FGF1) is one of them (25).

FGF1, also known as the acidic fibroblast growth factor, is one of the members of the fibroblast growth factor family. Compared with most members of the family, FGF1 lacks the secretory signal peptide sequence. Its secretory is not mediated by the classic pathways dependent on the endoplasmic reticulum and Golgi apparatus but occurs under stress conditions (26).

FGF1 is a non-glycosylated polypeptide composed of 155 amino acids (27). It is composed of 12 reverse parallel 6 chains and two ends of amino and carboxyl groups. FGF1 functions from endocrinology to extracellular. The N-terminal of free extension has no typical secretory signal sequence and is closely related to its biological activity. Therefore, FGF1 mutants or derivatives with different activities can be designed by N-terminal substitution or splicing modification (28). On the one hand, FGF1 itself is a growth factor, which can promote cell proliferation, mainly refers to the proliferation of vascular endothelial cells and smooth muscle cells, so as to promote the generation of blood vessels, reduce myocardial ischemia and improve myocardial function. On the other hand, FGF1 is involved in the regulation of glucose and lipid metabolism, and it can also regulate a variety of endocrine functions, providing a new method for the treatment of obesity, non-alcoholic fatty liver, T2DM and other metabolic diseases (29).

FGF1 has three main functional areas: nuclear localization area, receptor binding area and heparin-binding area (26). FGF1 has a nuclear localization signal (NLS), NLS has a basic role: first, it can receive FGF-1 into the nucleus; second, NLS can also be used as a direct induction signal to promote FGF1 induced nuclear division. The receptor-binding region of FGF1 binds FGF-1 to its receptor and activates a series of downstream reactions. Heparin-binding region can enhance the stability of FGF1 (29). Because of the above three functional areas, FGF1 mainly functions through two signal pathways: one is

the classical way of ligand-receptor combination, which can activate receptor activity, and then cause a series of downstream changes; the other is through the way of endocrine. It has been proved that FGF1 enters into the nucleus through the endocrine pathway and combines with p53, thus inhibiting p53 dependent apoptosis pathway and cell cycle arrest (28). FGF1 is highly expressed in a variety of tumor cells, such as pancreatic cancer, breast cancer, liver cancer and glioma. It has been proved that FGF1 may be one of the factors that lead to the poor prognosis of tumors. FGF1 plays an important role in the occurrence and metastasis of tumors and may become a target in tumor treatment (25).

MicroRNA, also known as microRNA, is a noncoding RNA found in eukaryotes and encoded by endogenous genes with a length of about 22 nucleotides (25, 30). It plays an important role in the timing regulation of normal biological activities and the occurrence and development of diseases (25).

Mature miRNA has the following characteristics: first, it is a kind of non-coding RNA molecule, which can be located between genes or in introns, with a length of 21-25 NT in general, and 22-23 NT in animals in general (31). Second, it comes from an arm of the styloid ring precursor. Moreover, the. 3 'terminal hydroxyl group and the 5' terminal phosphoric acid group are its unique markers. The first base pair at the 5 'terminal is usually U (28). Finally, it has high conservation and tissue specificity. With the help of Argonaute protein (ago), miRNA can complement the target gene through the 3 ' non-coding region (UTR), thus guiding the RNA-induced silencing complex (RISC) to down-regulate the expression of the target gene through two different mechanisms. When the target mRNA is completely complementary to it, it can cause mRNA degradation; when the degree of complementarity is low, it can cause the inhibition of mRNA translation, and animals and people usually exercise the mechanism of translation inhibition (26).

MiRNA plays an important role in the process of tumor proliferation and apoptosis. At least 52.5% of miRNA expression changes in tumors (31). More and more evidences show that miRNA expression is related to tumor detection, recovery, classification and treatment. At present, two possible mechanisms of action are generally recognized as follows: the

inhibition of translation at the posttranscriptional level or the binding of specific sites in the 3'UTR of the target mRNA induces the degradation of the target mRNA, reduces the stability of the target mRNA, and thus leads to the decrease of protein expression. The expression of miRNA in different diseases is different (26).

The miR-143-3p has 20 target genes. However, there are only four closely related tumors, namely ASAP3, MSI2, CRELD1 and ITGA6. The expression of miR-143-3p may play a role in inhibiting OS cell metastases by inhibiting the expression level of target genes of ASAP3 and MSI2 (27).

In this paper, a dual control group is set up for the experiment. The blank control group and the control group are set to ensure the accuracy of the experiment in this study. On the one hand, after the cell culture and treatment in this study, the relative expression of miR-143-3p was analyzed. From this perspective, the results of this study showed that the 3'UTR of miR-143-3p and FGF1 There is a base complementarity relationship, that is, FGF1 is one of the miR-143-3p target genes.

In addition, this study also conducted a cell irradiation experiment and analyzed the radiosensitivity of cancer cells by analyzing and comparing the cancer cell survival analysis of the three groups. The results of the study show that the survival score of the experimental group is always greater than that of the two control groups after the addition of radiation dose. This research result shows that miR-143-3p can increase the radiotherapy sensitivity of breast cancer cells through FGF1. Radiotherapy provides a reference value for breast cancer.

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Interest conflict
The authors declare no conflict of interest.

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