Expression of lncRNA MALAT1 in cervical squamous cell carcinoma and its correlation with radiotherapy dose

Huarong Zhao
the First Affiliated Hospital of Xinjiang Medical University
Ruirui Ren
The First Affiliated Hospital of Xinjiang Medical University
Yang Zhang
the First Affiliated Hospital of Xinjiang Medical University
Longge Suo
the First Affiliated Hospital of Xinjiang Medical University
Rui Chen
the First Affiliated Hospital of Xinjiang Medical University
Chen Chen
Xinjiang Medical University
Jiazaila ZaKai
the fifth Affiliated Hospital of Xinjiang Medical University
Lei Zhang (ffaz125@163.com)
the First Affiliated Hospital of Xinjiang Medical University
Huerxidan Niyazi
the First Affiliated Hospital of Xinjiang Medical University

Research Article

Keywords: MALAT1, Cervical squamous cell carcinoma, Radiotherapy

Posted Date: February 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1328896/v1

Expression of lncRNA MALAT1 in cervical squamous cell carcinoma and its correlation with radiotherapy dose

Running title:Expression of lncRNA MALAT1 and its radiotherapy dose

Huarong Zhao[1], Ruirui Ren[2],Yang Zhang[1], Longge Suo[1], Rui Chen[1], Chen Chen[3], Jiazaila·ZaKai[4], Lei Zhang[1*], Huerxidan·Niyazi[1*]

[1]Department of Oncology, the First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, Xinjiang, China

[2]Department of Radiotherapy, the First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, Xinjiang, China

[3]School of clincal medicine, Xinjiang Medical University, Urumqi 830054, Xinjiang, China

[4]Department of Critical care medicine, the fifth Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, Xinjiang, China

*Lei Zhang and Huerxidan • Niyazi are both of corresponding author

Lei Zhang:The First Affiliated Hospital of Xinjiang Medical University, No.137 Liyushan Road, Urumqi, Xinjiang830054, China phone:0991-4366344 email: ffaz125@163.com

Huerxidan • Niyazi:The First Affiliated Hospital of Xinjiang Medical University, No.137 Liyushan Road, Urumqi, Xinjiang830054, China phone:0991-4366344 email: Ailifu_zl@163.com

Word count:4625words

Figure number:5

Table number:7

Abstract

Background: To investigate the relationship between the expression of long-chain non-coding RNA (LncRNA) MALAT1 and radiotherapy effect in cervical cancer tissues.

Methods: Sixty cervical cancer patients with radiotherapy were selected and their tissue samples were collected. Siha cell lines and C-33A cell lines were used as MALAT1 high expression group and low expression group. These two cell lines were treated with 6 MV X-ray at 0, 2, 4, 6, 8, 10, and 12Gy, and the cell inhibition rate was detected by MTT assay at 24h, 48h, 72h and 96h after irradiation. Apoptosis rate was tested by flow cytometry and the percentage of autophagic cells was evaluated by the MDC method. Quantitative real-time quantitative polymerase chain reaction (qRT-PCR) was used to detect the expression of MALAT1.

Results: The expression level of MALAT1 was higher in cancer tissues than in paired normal tissues in patients with cervical cancer ($P < 0.05$). And the expression level of MALAT1 in cell line C-33A was significantly lower than that of cell line Siha. Besides, the cell inhibition rate in the MALAT1 low expression group was significantly higher than that in the high expression group, and the percentage of autophagic cells in MALTA1 low expression group was also higher ($P<0.05$). The percentage of autophagy cells in MALTA1 low expression group was higher than that in the high expression group ($P<0.05$).

Conclusion: MALAT1 plays an important role in cervical cancer and it is also a key point to interfere with the radiosensitivity of cervical squamous carcinoma cells.

Keywords：MALAT1; Cervical squamous cell carcinoma; Radiotherapy

Background

Cervical cancer is one of the common reproductive system tumors in women. There are about 100,000 new cases of cervical cancer in China every year, accounting for 1/5 of the total number of new cases in the world, and it has shown regional growth and early age in recent years [1]. Xinjiang is one of the high incidence areas of cervical cancer in China, and the incidence rate of cervical cancer among Uyghur women in Xinjiang was reported to be 459-527/100,000, which is significantly higher than the national rate (14.6/100,000) [2]. Although most early-stage cervical cancers can be cured by surgery or radiotherapy, 20%-25% of these cases fail to take the treatment due to recurrence and distant metastases[3]. Therefore, predicting new markers of cervical cancer and elucidating the molecular mechanisms of cervical carcinogenesis and metastasis are key issues to improve the survival rate of cervical cancer patients, while evaluating the efficacy of radiotherapy by tumor radiosensitivity testing before treatment is important to provide a strong basis for selecting rational and effective treatment. Therefore, more studies focus on the molecular markers related to the efficacy of radiotherapy.

Long-stranded non-coding RNA (lncRNA) is a new type of transcript discovered in recent years, which generally exceeds 200 nt in length and does not have the function of encoding proteins but can function by regulating physiological processes such as mRNA shearing, degradation and translation[4]. lncRNA is also gradually considered as a tumor-related biomolecule and can be used as a new diagnostic target for tumors. The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was named after its first discovery in human non-small cell lung cancer and was one of the first lncRNAs identified [5]. Studies have shown that MALAT1 is highly expressed in lung, gallbladder, breast, liver, stomach, bladder and cervical cancers [6-10] and is closely associated with tumor metastasis and recurrence [11]. Previous studies have confirmed that MALAT1 is highly expressed in HPV-positive cervical cancer cells and low expressed in HPV-negative cervical cancer cells [12-16].

Therefore, in this experiment, we selected stable and suitable cervical squamous carcinoma cell lines Siha (HPV-positive, HPV type 16) and C-33A (HPV-negative). After radiation treatment, the cell inhibition rate, apoptosis rate and autophagy percentage of cervical squamous carcinoma cells were examined to investigate the effect of MALAT1 expression on the sensitivity of radiotherapy for cervical squamous

carcinoma, and to provide a scientific basis for the treatment plan of cervical squamous carcinoma with MALAT1 as the target combined with radiotherapy.

Materials and methods

Patients and tissues

Seventy cervical biopsy tissue specimens of Han and Uyghur patients with cervical cancer attending the First Affiliated Hospital of Xinjiang Medical University from January 2017 to December 2019 were selected. Among them, 10 patients were not included in the study because the tissue specimens were not qualified, the patients did not complete radiotherapy, or lost visits. The patients who did not undergo surgery and did not receive chemotherapy or radiotherapy were recruited. All participants signed an informed consent form. All specimens were rapidly placed in liquid nitrogen after collection and then stored in a -80°C refrigerator for backup.

All participants were followed up for 48 months, and those who failed to come to the outpatient clinic for review on time were asked by telephone. Survival time was defined as the interval (number of months) from the date of admission to the date of death or last follow-up. The patients' disease progress and survival status were recorded.

Cervical squamous carcinoma cell line

Cervical squamous carcinoma cell line Siha and C-33A (ATCC Cell Bank, Shanghai) were cultured in MEM medium (Hyclone Co., Ltd., USA) containing 10% fetal bovine serum (FBS) (Hyclone Co., Ltd., USA), 1% penicillin (Hyclone Co., Ltd., USA) and 1% streptomycin (Hyclone Co., Ltd., USA) in an incubator with 5% CO2 and 95% humidity at 37°C. Cell passages were performed when cell fusion reached 80%-90%.

Treatment

Radiotherapy was performed by three-dimensional conformal irradiation or intensity-modulated irradiation (IMRT) with radiation energy of 6 MV-X lines, pelvic large field radiotherapy DT=45-50Gy/23-25f. The target areas included the upper border at the L4 and L5 levels; lower border at the inferior border of the pubic symphysis; both sides outside at 1/3c inside the femoral head, including the total iliac 1/2, internal iliac, external iliac, closed hole, presacral and other lymph node areas. After 3 weeks of total

pelvic irradiation, we started to use 192 Ir high dose rate rear-loader for intravaginal rear-load radiation therapy, with a dose of 600 cGy at each A site, for a total of 5 times, once a week, for a cumulative dose of 3000 cGy. Patients with intracavitary rear-load radiation therapy were not feasible for external radiation therapy on the same day, and patients were required to insist on daily vaginal douching during radiation therapy. Patients were given 1-2 cycles of FP chemotherapy according to the patient's tolerance, specifically: cisplatin (PPD) 75mg/m2 in 3 days + fluorouracil (5-FU) 1000mg/m2 in 24-hour micropump for 4 days, and symptomatic treatment such as protection of gastric mucosa and antiemetic during the treatment period.

Evaluation criteria of efficacy

The recent efficacy was judged according to WHO solid tumor efficacy assessment criteria, complete remission (CR): tumor completely disappeared for 4 weeks; partial remission (PR): tumor shrunk more than 50%; lasted for 4 weeks; no change (SD): tumor shrunk less than 50% or enlarged <25%; deterioration (PD): tumor enlarged >25% or appeared lesions. Pelvic examination and combined CT and ultrasound imaging were evaluated by two associate professors of oncology 4 weeks after the end of treatment. Total effective rate (RR) = CR + PR.

Evaluation of expression level of MALAT1

Total mRNA extraction was performed according to the operating instructions of the Trizol RNA extraction reagent (TaKaRa, Japan). Nanodrop was used to determine the content and concentration of mRNA to ensure the integrity of RNA (OD260/280 values of 1.8-2.0 were considered as high purity, and the general concentration of 1000ng/ml was more accurate). Total mRNA was reverse transcribed into cDNA according to TaKaRa reverse transcription kit instructions. Polymerase chain reaction (PCR) was performed using SYBR instant PCR kit (Tiangen, Beijing), according to the kit instructions.

After reverse transcription of RNA to generate cDNA, Quantitative real-time quantitative PCR (qRT-PCR) was performed and no spurious peaks appeared in the lysis curve analysis, indicating that the amplification products were single and there was no non-specific amplification. Using GAPDH as the internal control, the CT value was calculated automatically at the end of the reaction, i.e. the number of cycles that the fluorescence signal in each reaction tube had gone through when the fluorescence

signal reached the set threshold. The CT values of MALAT1 and GAPDH were obtained from the amplification curves, and their expression amounts were calculated by public display.

The MALAT1 and internal reference GAPDH primer sequences were as follows: MALAT1: upstream primer 5`-GAATTGCGTCATTTAAAGCCTAGTT-3`, downstream primer 5`-GGTTTCATCCTACCACTCCCAATTAAT-3`, GAPDH: upstream primer 5`- TGTTGCCATCAATGACCCCTT-3`, downstream primer 5`-CTCCACGACGTACTCAGCG-3`. The CT value of paired normal tissues was used as the standard to calculate the relative expression level of MALAT1, which was expressed by applying $2^{-\Delta\Delta ct}$. qRT-PCR was also performed to verify the relative expression of MALAT1 in two cervical squamous carcinoma cell lines. Cells at the logarithmic growth stage were collected by centrifugation.

Ionizing radiation

The irradiation model of the linear gas pedal for radiation treatment of cell lines is the American Varian-CX linear gas pedal. 6 MV X-ray vertical irradiation, 400 cGy/min dose rate, and 100 cm source skin distance were set. The irradiation field was defined to 10 cm × 10 cm and a 96-well cell culture plate at 15 cm × 15 cm was used.

MTT assay

Two cervical cancer cell lines were inoculated in 96-well plates at a concentration of 1×10^6 cells per well, while a blank control group was set up. 6 replicate wells were set up in each well and treated with 0, 2, 4, 6, 8, 10 and 12 Gy of X-ray respectively. Furthermore, the cells in the 96-well plates were incubated in a CO2 incubator for 24, 48, 72 and 96 h before MTT assay. We added 20 μL of 5 g/L MTT reagents (Solebro Co., Ltd., USA) to each well. And the cells were continued to incubate at 37 ℃ for 4 h. And then the culture solution was discarded and 150 μL of DMSO was added to each well. And it was shaken at room temperature for 5 min on a shaker, and finally, the absorbance (OD) value at 490 nm with an enzyme marker was measured to calculate the cell proliferation inhibition rate according to the following formula: cell proliferation inhibition rate = $1-(OD_{\text{experimental group}} - OD_{\text{blank group}})/ (OD_{\text{control group}} - OD_{\text{blank group}})$.

Apoptosis assay

Two cervical cancer cell lines were digested and counted (cell concentration was 1×10^6), and then inoculated into culture flasks. After being cultured for 24h, the cells were attached to the wall and then irradiated with 8 Gy of X-ray. The culture medium was discarded after 48h of further incubation at 37 ℃. The cells were digested, centrifuged, collected, and washed with PBS. According to the instructions of the Annexin V kit (BD Co., Ltd., USA), 1ml 1 × Binding Buffer was added firstly. And then 5 μL of Annexin V and 5 μL of 7-AAD were added. The cells were incubated for 15 min at room temperature without light and added 400 μl of 1× Binding Buffer to the flow tube. Apoptosis was detected using flow cytometry. Three independent replicate experiments were performed and the apoptosis rate was then calculated based on (Q2+Q3)/(Q1+Q2+ Q3+Q4).

Cell autophagy assay

Pre-treated was in the same way as Apoptosis assay. After digesting and being centrifuged, 100μl of 1×Wash buffer was added to resuspend the cells according to the instructions of the MDC kit (Solebro Co., Ltd., USA). We aspirated 90μl of the cell suspension into a new Ep tube, added 10μl of MDC stain, mixed gently and stained them at room temperature for 15~45min. The precipitated cells were centrifuged and collected, and then washed twice with 400 μl of 1×Wash buffer. We discarded the supernatant and the cells were resuspended by adding 100 μl of Collection buffer, added dropwise to a slide and coverslip, and the two cell lines were placed under a confocal microscope for observation. The experiment was repeated three times independently. And the percentage of autophagic cells (blue-purple fluorescent cells) in the original field of view of 100 cells was counted by two laboratory teachers who were familiar with the confocal microscopy technique and had been operating it for 5-10 years.

Statistical analysis

SPSS 23.0 statistical software was used for data analysis. The expression level of MALAT1 in cervical cancer tissues was compared with the baseline data and the characteristics of each clinical case and other count data by X2 test. The correlation between MALAT1 expression level and efficacy was tested by rank sum test. The Kaplan-Meier test was used to compare the survival rates of different expression levels. Cox proportional risk model was used to identify independent risk factors for indicators

such as expression level on survival. $\alpha=0.05$ was used as the test level and $P<0.05$ was considered a statistically significant difference.

Results

The actual number of patients who participated in the statistical analysis in this study was 60, with patients ages 40-70 years old and an average of 55 years old. Among them, 30 were Han Chinese and 30 were Uyghur.

MALAT1 expression in cervical cancer tissues

The qPCR technique was applied to detect the expression of MALAT1 in 60 cases of Han and Uyghur cervical cancer tissues, and MALAT1 was highly expressed in tumour tissues compared to normal tissues and the difference was statistically significant ($P<0.05$).

The relationship between MALAT1 expression and clinicopathological characteristics of patients with cervical cancer

Among the 60 patients with cervical cancer, 36 tumour tissues showed low expression of MALAT1 and 24 tumour tissues showed high expression of MALAT1; while there was no correlation with age, ethnicity, degree of tumour differentiation, tumour stage and SCC index ($P>0.05$), see Table 1.

Relationship between MALAT1 expression and recent outcome of cervical cancer

The results showed that there were 16 cases of CR, 10 cases of PR, 27 cases of SD, and 7 cases of PD. 4 cases (16.7%) of CR and 2 cases (8.3%) of PR were found in 24 cases of cervical cancer with high MALAT1 expression. 12 cases (33.3%) of CR and 8 cases (22.2%) of PR were found in 36 cases of cervical cancer with low MALAT1 expression. The efficacy of radiotherapy for cervical cancer with low MALAT1 expression was better than that for high expression, and the difference in efficacy between the two groups was statistically significant ($P<0.05$), and the expression of MALAT1 was negatively correlated with the efficacy of cervical cancer, see Table 2.

Relationship between MALAT1 expression and patient survival

The 1- and 2-year survival rates were 80.0% and 59.2% for high MALAT1 expression and 96.7% and 85.9% for low expression, respectively. The survival rate of cervical

cancer patients with low MALAT1 expression was higher than that of patients with high expression, as shown in Table 3 and Figure 1.

Univariate analysis of prognostic relevance：

Age, ethnicity, MALAT1 expression, recent outcome, SCC index classification, lymph node metastasis, tumor stage, and tumor differentiation were used as independent variables, and univariate analysis was performed on the dependent prognostic variables. The results showed that the patients' recent efficacy, lymphatic metastasis and MALAT1 expression were statistically significant, while other indicators were not (Table 4).

Multifactorial analysis of prognostic relevance：

A multifactorial analysis of recent outcome, lymph node metastasis, ethnicity and MALAT1 expression (Table 5) showed that outcome was an independent protective factor for the prognosis of cervical cancer patients at 2-year follow-up (OR=0.452, 95% CI: 0.209-0.979, p= 0.044). Lymph node metastasis (OR=4.231, 95% CI: 1.085-16.506, P= 0.038) and expression status (OR=4.742, 95% CI: 1.338-16.805, P= 0.016) were independent risk factors affecting the prognosis of patients with cervical cancer at 2-year follow-up.

Validation of the relative expression of MALAT1 in cervical squamous carcinoma cell lines Siha, C-33A

The relative expression of MALAT1 was significantly lower in the cervical squamous carcinoma cell line C-33A than in Siha cells ($P < 0.05$, Table 6). It shows that MALAT1 is highly expressed in the HPV-positive cervical squamous carcinoma cell line Siha and lowly expressed in the HPV-negative cervical squamous carcinoma cell line C-33A.

According to the relevant literature and the preliminary experiments of our group, the cell concentration of 1×10^6 can cover 80% of the 96-well plate, and the growth is stable and suitable for experimental research. The cell concentration of 1×10^6 Siha and C-33A cells were treated with 2Gy, 4Gy, 6Gy, 8Gy, 10Gy and 12Gy radiation respectively, and the cell inhibition rate showed an increasing trend at 24h, 48h and 72h after irradiation, and the difference of cell inhibition rate at 72h and 96h after irradiation was not statistically significant (see Figure 2). It is reasonable to assume that the cervical squamous carcinoma cell line Siha, C-33A, undergoes irreversible cell death between

48h and 72h after irradiation, so 48h was chosen as the time point for subsequent experimental testing. Siha and C-33A cells were treated with 2 Gy, 4 Gy, 6 Gy, 8 Gy, 10 Gy and 12 Gy radiation at a cell concentration of 1×10^6. 48h after irradiation, there was no significant change in the cell inhibition rate after 2Gy irradiation compared to the control group. Except for the 10 Gy irradiation dose, the cell inhibition rate tended to increase with increasing irradiation dose, among which there was no significant difference between the 6 Gy and 10 Gy irradiation doses ($P>0.05$), and the difference in cell inhibition rate between the 10 Gy and 12 Gy irradiation doses was significantly higher than that between the remaining adjacent radiation dose groups (see Figure 3), which does not exclude that 12 Gy exceeds the tolerance dose of the cervical squamous carcinoma cell line. In summary, we chose 8 Gy as the irradiation dose for the follow-up experiment.

The cell concentration of 1×10^6 cervical squamous carcinoma cell line C-33A was significantly higher than that of cervical squamous carcinoma cell line Siha at 48h after X-ray irradiation at 8Gy, i.e. the cell inhibition rate of MALAT1 low expression group was significantly higher than that of MALAT1 high expression group, the difference was statistically significant ($P<0.05$), see Table 7.

Flow cytometry detection of apoptosis in two cervical squamous carcinoma cell lines, Siha and C-33A cells

Two cervical squamous carcinoma cell lines Siha and C-33A at a cell concentration of 1×10^6 were irradiated by X-ray at 8Gy and the apoptosis rates were $(55.99\pm0.38)\%$ and $(64.70\pm0.38)\%$, respectively, after continued incubation at 37 ℃ for 48h. The apoptosis rate was significantly higher in the MALTA1 low expression group (C-33A cells) than in the MALAT1 high expression group (Siha cells, Figure 4).

Autophagy percentage of two cervical squamous carcinoma cell lines Siha and C-33A cells by MDC method

Two cervical squamous carcinoma cell lines Siha and C-33A with a cell concentration of 1×106 were irradiated by X-ray at 8Gy and the percentage of autophagic cells detected after 48h of incubation was $(9.67\pm4.16)\%$ and $(32.67\pm6.02)\%$ respectively. The percentage of autophagic cells in the MALTA1 low expression group (C-33A cells) was significantly higher than that in the MALAT1 high expression group (Siha cells)

298 ($P < 0.05$). Representative images of C-33A cells under ordinary microscope and
299 confocal microscope were shown in Figure 5.

300

301 **Discussion**

302 **Correlation of long-lncRNA MALAT1 expression and different ethnic groups**

303 Cervical cancer is the most common malignant tumor among women worldwide, and
304 Xinjiang is one of the high incidence areas of cervical cancer in China. The incidence
305 rate of cervical cancer among Uyghur women in this region is 3-4 times higher than
306 that of Han women in the same region, and the death rate takes first place among ethnic
307 minorities in China. Most of the cervical cancer patients from ethnic minorities in
308 Xinjiang come from remote farming and herding areas with poor economic and medical
309 environments, and the clinical stage at diagnosis is more advanced and the prognosis is
310 worse. Due to the influence of Xinjiang's ethnic customs, early marriage, premature
311 birth and multiple births of Uyghur women, cervical cancer patients in Xinjiang are
312 characterized by an early age of onset and late stage at diagnosis [17]. Because of these
313 characteristics of cervical cancer patients in Xinjiang, this study conducted statistical
314 analysis by the expression of lncRNA MALAT1 between two different ethnic groups,
315 and the results found that there was no difference between ethnic groups. Because there
316 are no studies on the specificity of different ethnic groups, and the sample size of this
317 study was small, etc., we cannot fully affirm that there is no correlation between the
318 expression of lncRNA and ethnic groups Further studies are needed.

319 **Correlation between age and prognosis of cervical cancer**

320 Cervical cancer is a disease that predominantly affects middle-aged and older women,
321 but still occurs in younger patients in censuses. Most of the current domestic and
322 international studies illustrate that the influence of age factors in the prognosis of
323 cervical cancer is uncertain, with some reporting that both youth and old age are
324 unfavorable prognostic factors, especially in advanced cervical cancer, and some
325 studies showing that younger patients have a better prognosis than older ones [18-20]. Our
326 study showed no difference between MALAT1 expression and age of cervical cancer
327 patients ($P>0.05$), and it can be considered that the age factor is not significantly related
328 to the prognosis of cervical cancer, and age does not affect the radiotherapy efficacy

and prognosis of cervical cancer, which may be related to the small number of cervical cancer samples in the elderly group of different ethnic groups in our data (only 15% of all cases).

Correlation between lymph node metastasis and prognosis of cervical cancer

The prognosis of patients with cervical cancer has been greatly improved by combined radiotherapy and chemotherapy, but the overall survival rate of patients has not been significantly improved, mainly because most patients with cervical cancer have developed micrometastases. How to effectively increase the survival rate and improve the quality of patients' survival is a concern. Therefore, in this study, statistical analysis was also performed on the degree of tumor differentiation, tumor stage and SCC indexes. and the difference in the incidence of lymph node metastasis between the two groups was found to be statistically significant ($P<0.05$). Therefore, adjustment for lymph node metastasis was again analysed by COX regression and the results showed that lymph node metastasis was an independent prognostic influence on cervical cancer ($p<0.05$) with a relative risk of OR=4.742 (1.338-16.806).

Correlation between MALAT1 expression and radiotherapy efficacy

LncRNA, as a novel tumor marker, plays an important role in the development of cervical cancer. Therefore, predicting new markers for cervical cancer and elucidating the molecular mechanisms of cervical carcinogenesis and metastasis are key issues to improve the survival rate of cervical cancer patients and are also current hot issues in oncology research. Lu et al [21] found that MALAT1 expression was significantly higher in radiation-resistant cancer cases than in radiation-sensitive examples by controlling tissue specimens from 50 cervical cancer patients with 25 healthy individuals in radiation-sensitive cases. However, there are no similar studies about the correlation between MALAT1 and radiotherapy efficacy of cervical cancer at home and abroad. Therefore, in this study, the expression of MALAT1 in the tissues of cervical cancer patients of different ethnic groups was statistically analyzed simultaneously with the radiotherapy efficacy and survival rate. And it was found that the survival rate of cervical cancer patients with low expression of MALAT1 was higher than that of patients with high expression, and the difference was statistically significant ($P<0.05$). This indicates that there is a correlation between the expression level of MALAT1 and radiotherapy efficacy.

Correlation between MALAT1 expression and other

It was reported that the expression of MALAT1 was associated with HPV. In our current project, HPV-positive cervical squamous carcinoma cell line Siha was in the high MALAT expression group and HPV-negative cervical squamous carcinoma cell line C-33A was in the low MALAT expression group.

Radiation prevents tumour cells from cloning due to loss of proliferative capacity, and several studies have used cell proliferation activity as an indicator of sensitivity to radiotherapy [22-23]. In this study, the cell inhibition rate of two cervical cancer cell lines irradiated with 8Gy X-rays for 48h was also measured by MTT. It was confirmed that the cell inhibition rate of MALAT1 low expression group was significantly higher than that of MALAT1 high expression group after irradiation, indicating that MALAT1 low expression could reduce the radiation-induced cell proliferation activity. Radiotherapy can simultaneously activate multiple receptor signalling pathways within tumour cells, altering cell cycle progression and affecting apoptosis [24]. Rugan et al [25] found that the apoptosis of tumor cells did not change significantly after down-regulation of lncRNA MALAT1, however, when combined with irradiation treatment, the apoptosis rate of tumor cells increased significantly. This suggests that MALAT1 is not directly involved in the apoptotic process, but that after radiation-induced apoptosis, lncRNA MALAT1 indirectly regulates the function of Bcl-2/Bax gene, thus indirectly affecting the radiosensitivity of tumor cells through the apoptotic pathway. Meanwhile, our results confirmed that the apoptosis rate in the MALTA1 low expression group (HPV-negative cervical squamous carcinoma cell line C-33A) was significantly increased compared to the MALAT1 high expression group (HPV-positive cervical squamous carcinoma cell line Siha) 48h after irradiation with 8Gy in both cervical squamous carcinoma cell lines, and the difference was statistically significant ($P<0.05$). We suggest that high expression of MALAT1 inhibits apoptosis and interference with MALAT1 expression inhibits the proliferation of cancer cells and promotes their apoptosis, and we speculate that MALAT1 may regulate the sensitivity of radiotherapy for cervical cancer by affecting apoptosis. Furthermore, MALAT1 is an important gene that regulates radiation-induced apoptosis. We found that by promoting radiation-induced apoptosis, we can increase the sensitivity of tumour cells to radiotherapy, improve prognosis, and thus improve patient survival and quality of life. However, due to the variety of signalling pathways involved in apoptosis and the complexity of the

relationships, we have only investigated the possible regulatory mechanisms between MALAT1 and apoptosis rates. In-depth studies on the role of MALAT1 in relation to radiation-induced apoptotic factors and their related pathways could provide new approaches to the treatment of tumours. Cell death is divided into programmed and non-programmed death, non-programmed death and cell necrosis, and programmed cell death including apoptosis and cell autophagy. Radiation affects autophagic flow through the fusion of autophagosomes with lysosomes, and several studies have shown that several factors regulate radiation-induced autophagy and thus the sensitivity of tumour cells to radiotherapy[26-27] . In this study, after irradiation of two cervical squamous carcinoma cell lines with 8Gy, the percentage of autophagic cells was significantly increased in the MALTA1 low expression group (HPV negative cervical squamous carcinoma cell line C-33A) compared to the MALAT1 high expression group (HPV positive cervical squamous carcinoma cell line Siha)., indicating that MALAT1 low expression promotes radiation-induced autophagy in cervical cancer cells. Therefore, by promoting radiation-induced autophagy in tumour cells, the sensitivity of tumour cells to radiotherapy can be increased.

However, there are still shortcomings in our study, for example, our study subjects are only cell lines and we have not studied the development process of malignant tumour in human body dynamically, and pure cell experiments cannot meet all aspects of human microenvironment, so we can adjust these shortcomings in the next step and further explore the regulatory mechanism of sensitivity to radiotherapy for cervical cancer, so as to provide effective therapeutic measures for the treatment of cervical cancer as early as possible and make the experiment more rigorous to provide a basis for clinical treatment.

Conclusion

The study showed that MALAT1 plays an important role in regulating the sensitivity of cervical squamous carcinoma cells to radiotherapy, and further investigated its regulatory mechanism. Low MALAT1 expression inhibited tumor cell proliferation, promoted apoptosis and autophagy, suggesting that MALAT1 is involved in regulating the sensitivity of cervical squamous cancer cells to radiotherapy and may be a useful biological marker for predicting radiotherapy efficacy and prognosis.

Authors' contributions

HR-Z, RR-R and YZ designed the study and wrote the first draft of the manuscript and conducted the statistical analysis. LS ,RC and CC performed the data collection and took part in statistical analysis. JZ ,LZ and HN provided critical input into the data analysis and interpretation of the results. HR-Z participated in conception, designed of the study and revised it critically for important intellectual content. All authors have read the draft critically to make contributions and also approved the final manuscript.

Acknowledgments

Not applicable.

Funding

Youth Fund of Natural Science Foundation in Xinjiang Uygur Autonomous Region in 2019

Ethical approval

This study was approved by the Research Ethics Committee at the the First Affiliated Hospital of Xinjiang Medical University

Consent for publication

Not applicable

Availability of data and materials

The data is available upon reasonable request

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

452

453

454

455

456

457

458

459

460

461

462

463

464 **References**

465 1. Cai DH., Li Y, Guo RM, et al. Correlation study of HPV typing with Th17 and
466 Treg cells in cervical cancer. Chinese Clinical Oncology, 2017, 43 (63): 1099-1102.

467 2. Brianti P, DE Flammieis E, Mercuri SR. Review of HPV-related diseases and
468 cancers. New Microbiol, 2017, 40 (2):80-85.

469 3. Lee J, Lin JB, Sun FJ, et al. Safety and efficacy of semi extended field intensity-
470 modulated radiation therapy and concurrent cisplatin in locally advanced cervical
471 cancer patients: An observational study of 10-year experience. Medicine
472 (Baltimore) , 2017, 96(10): e6158.

473 doi: 10.1097/MD.0000000000006158

474 4. Barker HE, Paget JT, Khan AA, et al. The tumour microenvi-ronment after
475 radiotherapy: mechanisms of resistance and recurrence. Nat Rev Cancer, 2015,
476 15(7): 409-425. doi: 10.1038/nrc3958

477 5. Powell ME. Modern radiotherapy and cervical cancer. Int J Gynecol Cancer, 2010,
478 20(6):49-51.

479 doi: 10.1111/igc.0b013e3181f7b241

480 6. Gien LT, Beauchemin MC, Thomas G. Adenocarcinoma: a unique cervical cancer.
481 Gynecol Oncol, 2010, 116(7):140-146. doi:10.1016/j.ygyno.2009.09.040

482 7. Luo YH, Ye L, Kong DL, et al. Correlation between highly expressed lncRNAs
483 and tumorigenesis development and its clinical application value. Modern medical
484 oncology, 2016, 24(22): 3654-3659.

485 8. Zhou XL, Wang WW, Zhu WG, et al. High expression of long non-coding RNA
486 AFAP1-AS1 predicts chemoradio resistance and poor prognosis in patients with
487 esophageal squamous cell carcinoma treated with definitive chemoradiotherapy.
488 Mol Carcinog, 2016, 55 (12): 2095-2105. doi:10.1002/mc.22454

489 9. Jing L, Yuan W, Ruofan D, et al. HOTAIR enhanced aggressive biological
490 behaviors and induced radioresistance via inhibiting p21 in cervical cancer.
491 Tumour Biol, 2015, 36(5):36 11-3619. doi: 10.1007/s13277-014-2998-2

492 10. Jiao Y, Liu C, Cui F M, et al. Long intergenic non-coding RNA induced by X-ray
493 irradiation regulates DNA damage response signaling in the human bronchial
494 epithelial BEAS-2B cell Line. Oncol Lett, 2015, 9(1):169-176.
495 doi: 10.3892/ol.2014.2622

11. Zhang Mengmei. Changes of serum MALAT1 expression in non-small cell lung cancer patients before and after radiotherapy and its clinical significance. Zunyi Medical College, 2018.1-43.

12. Rugan. Study on the effect of LncRNA-MALAT1 and Hsa-miR-1 interaction mechanism on radio sensitivity of colorectal cancer. Suzhou:Soochow University, 2015.1-66.

13. Li Z, Zhou Y, Tu B, et al. Long noncoding RNA MALAT1 affects the efficacy of radiotherapy for esophageal squamous cell carcinoma by regulating Cks1 expression. J Oral Pathol Med, 2016. doi: 10.1111/jop.12538

14. Liberal V, Martinsson-Ahlzen HS, Liberal J, et al. Cyclindependent kinase subunit (Cks) 1 or Cks2 overexpression overrides the DNA damage response barrier triggered by activated oncoproteins. Proc Natl Acad Sci U S A, 2012, 109 (8): 2754-2759. doi: 10.1073/pnas.1102434108

15. Jin C, Yan B, Lu Q, et al. The role of MALAT1/miR-1/slug axis on radio resistance in nasopharyngeal carcinoma. Tumour Biol. 2016 Mar; 37 (3): 4025-33. doi: 10.1007/s13277-015-4227-z

16. Liu S, Song L, Zeng S, et al. MALAT1-miR-124-RBG2 axis is involved in growth and invasion of HR-HPV-positive cervical cancer cells. Tumour Biol, 2016, 37 (1): 633-640. doi: 10.1007/s13277-015-3732-4

17. Lu H, He Y, Lin L, et al. Long non-coding RNA MALAT1 modulates radiosensitivity of HR-HPV+ cervical cancer via sponging miR-145.Tumour Biol, 2016, 37 (2):1683-1691. doi: 10.1007/s13277-015-3946-5

518 18. Sun R, Qin C, Jiang B, et al. Down-regulation of MALAT1 inhibits cervical cancer
519 cell invasion and metastasis by inhibition of epithelial-mesenchymal transition.
520 Mol Biosyst, 2016, 12 (3):952-962. doi: 10.1039/c5mb00685f

521 19. Yang L, Bai HS, Deng Y, et al. High MALAT1 expression predicts a poor
522 prognosis of cervical cancer and promotes cancer cell growth and invasion. Eur
523 Rev Med Pharmacol Sci, 2015, 19(17):3187-3193.

524 20. Jiang Y, Li Y, Fang S, et al. The role of MALAT1 correlates with HPV in cervical
525 cancer. Oncol Letters, 2014, 7(6):2135-2141. doi: 10.3892/ol.2014.1996

526 21. Zhang JY, Feng LY, Rui JC, et al. Study on the correlation between high-risk HPV
527 infection and cervical cancer and precancerous lesions in Jiangsu women. Modern
528 Biomedical Progress, 2016, 16(36):7135-7139.

529 22. Zhang L, Hulsidan N, Dilidhar S, et al. Differential expression of long-stranded
530 non-coding RNA MALAT1 in serum and tissues of cervical cancer. Journal of
531 Xinjiang Medical University, 2018, 41(4):478-485.

532 23. Lin S, Meng LN, Shan D, et al. Effect of different targeted drugs on the sensitivity
533 of non-small cell lung cancer cell lines with abnormal EGFR expression . Clinical
534 misdiagnosis and misdiagnosis, 2019, 32(9): 87-94.

535 24. Chen JY, Zhang F, Zhuang YX, et al. The effect of serpentine on the proliferation
536 and radiotherapy sensitivity of CNE2 stem cells in hypodifferentiated
537 nasopharyngeal carcinoma. Chinese Traditional and Herbal Drugs, 2018, 49
538 (16):3854-3860.

25. Jeggo PA, Geuting V, Lobrich M. The role of homologous recombination in radiation-induced double-strand break repair. Radiother Oncol, 2011, 101 (1):7-12. doi: 10.1016/j.radonc.2011.06.019

26. Guo D, Ma J, Yan L, et al. Down-regulation of lncRNA MALAT1 attenuates neuronal cell death through suppressing beclin1-dependent autophagy by regulating miR-30a in cerebral ischemic stroke. Cell Physiol Biochem, 2017, 43 (1):182-194. doi: 10.1159/000480337

27. Huang J, Yang Y, Fang F, et al. MALAT1 modulates the autophagy of retinoblastoma cell through miR-124-mediated stx17 regulation. J Cell Biochem, 2018, 119 (5):38 35-3863. doi: 10.1002/jcb.26464

Figure 1 Relationship between MALAT1 expression and long-term outcome of cervical cancer

Figure 2 Comparison of cell inhibition rates of Siha, C-33A cells with a cell concentration of 1×10^6 after 2Gy, 4Gy, 6Gy, 8Gy, 10Gy, and 12Gy irradiation

Figure 3 Comparison of cell inhibition rates of Siha and C-33A cells with a cell concentration of 1×10^6 after 48 hours of irradiation with 2Gy, 4Gy, 6Gy, 8Gy, 10Gy, and 12Gy

Figure 4 Effect of the expression of MALAT1 on apoptosis of cervical cancer cell lines Siha and C-33A cells

Note: The detection error in the upper left quadrant Q1 is within the permitted range; the upper right quadrant Q2 (7AAD + / PE-) shows non-viable cells, that is, necrotic cells and late apoptotic cells; the lower left quadrant Q3 (7AAD- / PE +) shows early decay. Dead cells; live cells (7AAD- / PE-) are shown in the lower right quadrant.

Figure 5 Taking C-33A cells as an example, comparing cell staining in two fields of view (×4)

Note: A is C-33A cells in the ordinary field of view; B. C-33A cells in the field of the confocal microscope; C is Siha cells in the field of common vision; B is Siha cells in the field of confocal microscope

Figures

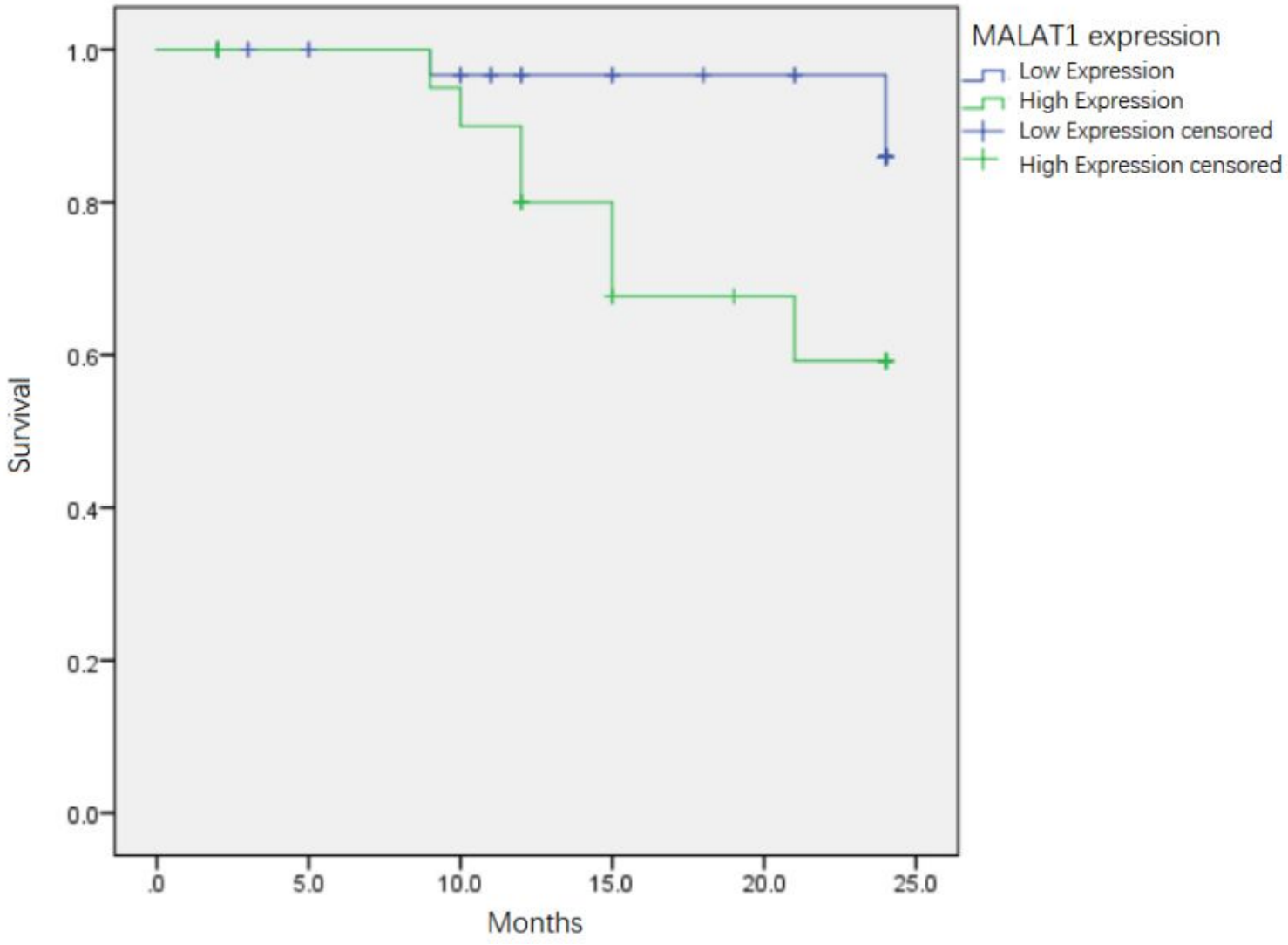

Figure 1

Relationship between MALAT1 expression and long-term outcome of cervical cancer

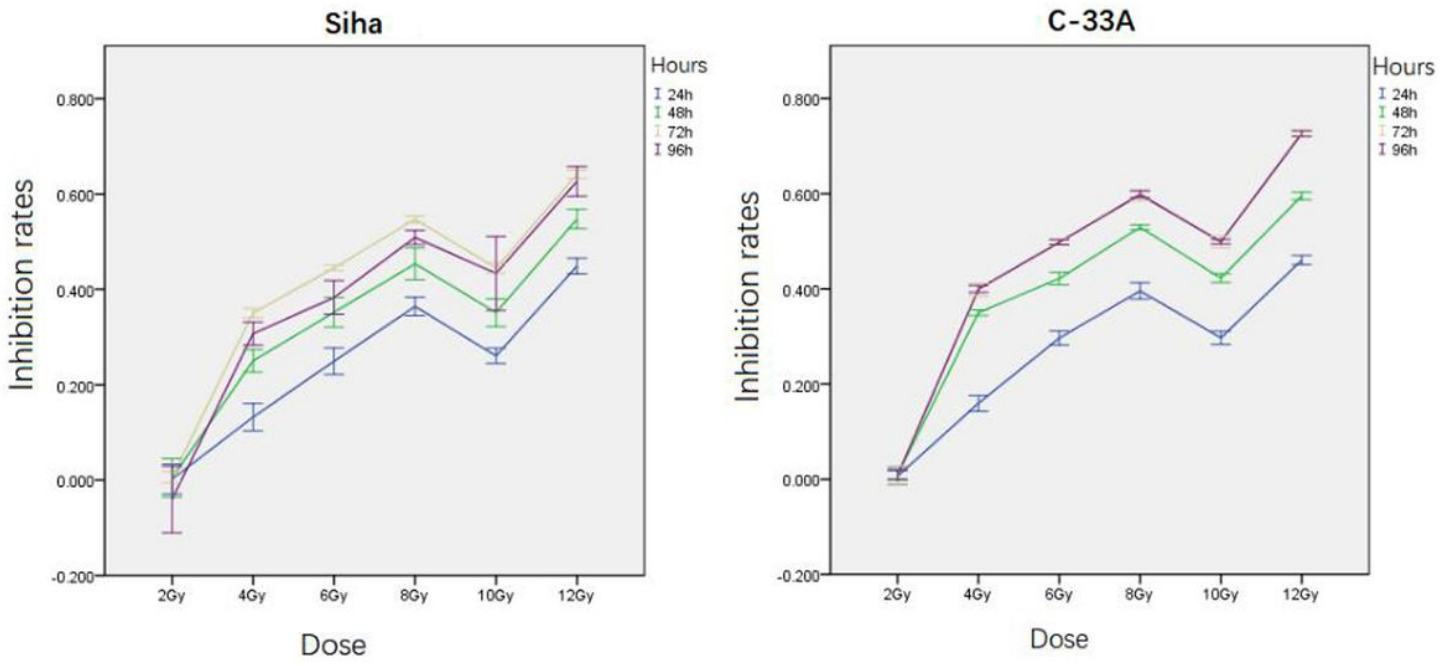

Figure 2

Comparison of cell inhibition rates of Siha, C-33A cells with a cell concentration of 1 × 106 after 2Gy, 4Gy, 6Gy, 8Gy, 10Gy, and 12Gy irradiation

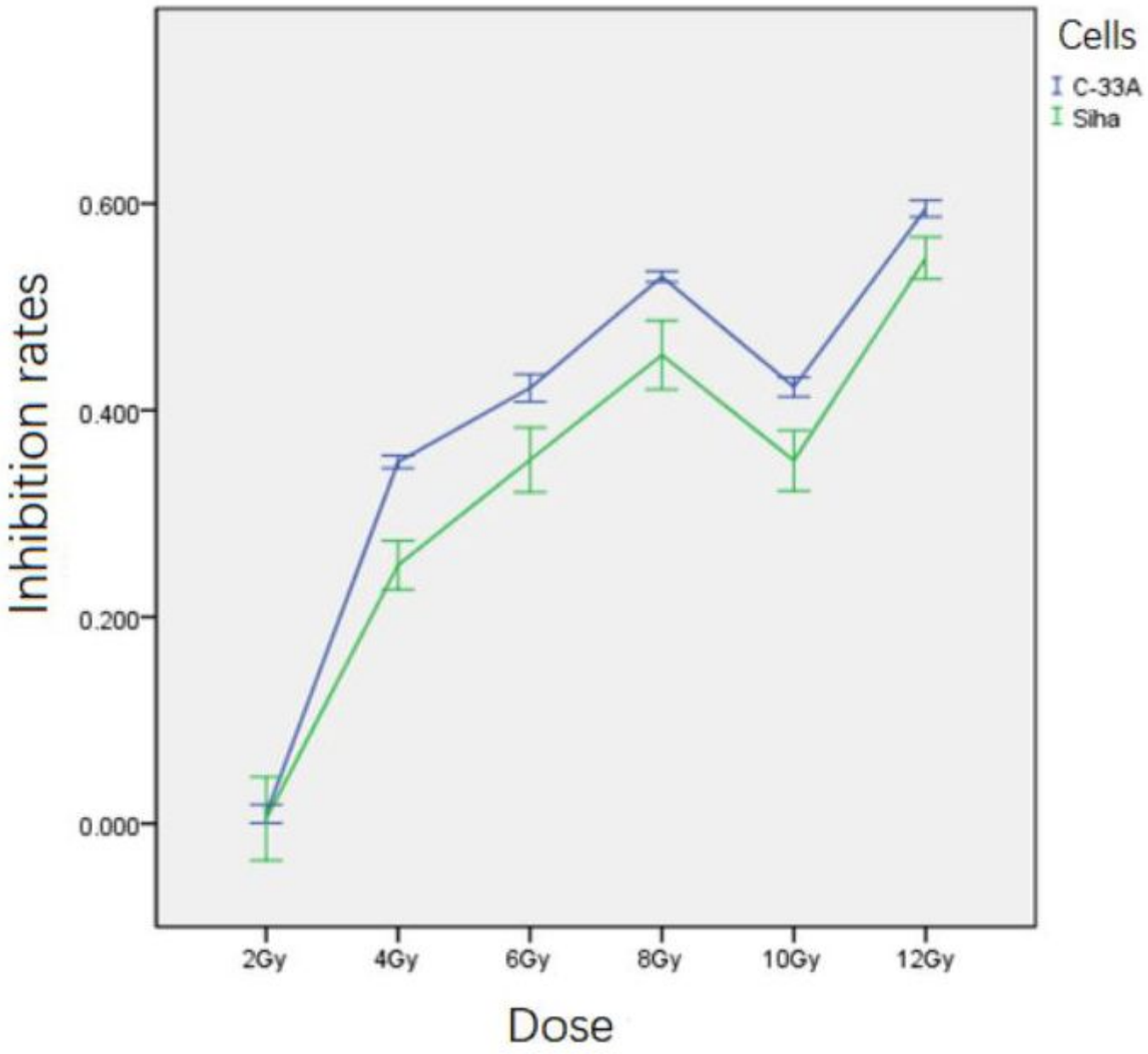

Figure 3

Comparison of cell inhibition rates of Siha and C-33A cells with a cell concentration of 1 × 106 after 48 hours of irradiation with 2Gy, 4Gy, 6Gy, 8Gy, 10Gy, and 12Gy

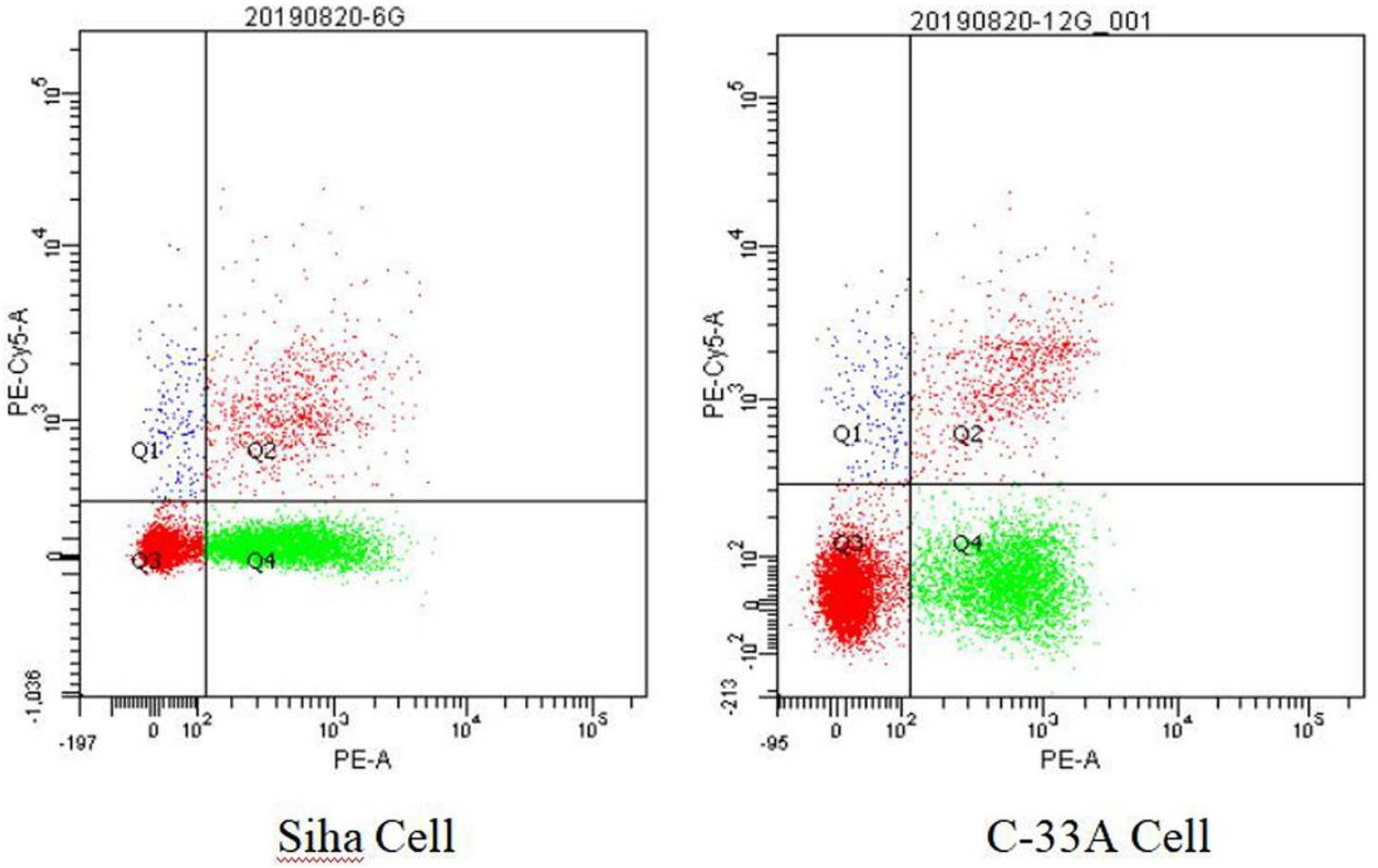

Figure 4

Effect of the expression of MALAT1 on apoptosis of cervical cancer cell lines Siha and C-33A cells

Note: The detection error in the upper left quadrant Q1 is within the permitted range; the upper right quadrant Q2 (7AAD + / PE-) shows non-viable cells, that is, necrotic cells and late apoptotic cells; the lower left quadrant Q3 (7AAD- / PE +) shows early decay. Dead cells; live cells (7AAD- / PE-) are shown in the lower right quadrant.

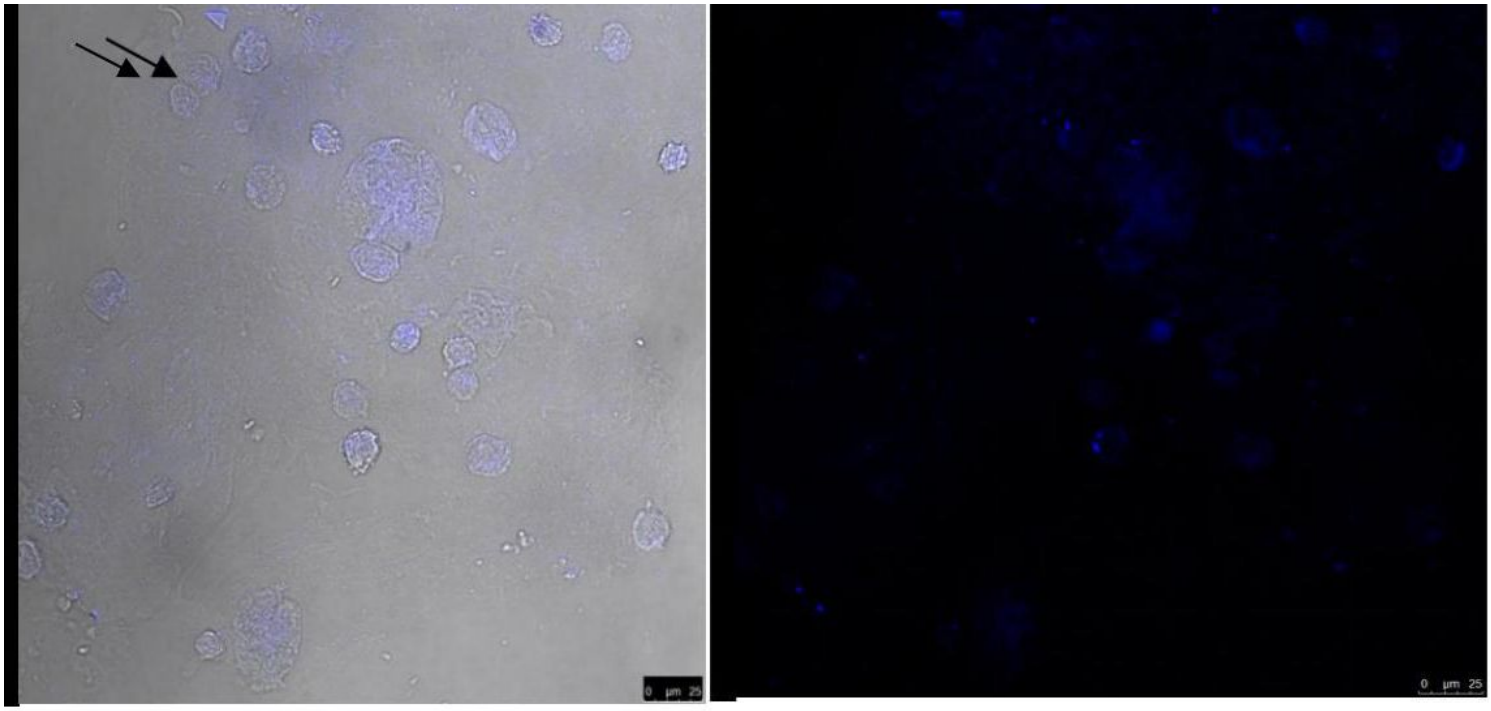

Figure 5

Taking C-33A cells as an example, comparing cell staining in two fields of view (×4)

Note: A is C-33A cells in the ordinary field of view; B. C-33A cells in the field of the confocal microscope; C is Siha cells in the field of common vision; B is Siha cells in the field of confocal microscope