ISSN 2706-6819 Vol.3, Issue 5: 21-26, DOI: 10.25236/IJFM.2021.030503

Changes of Expression and Function of Tumor Infiltrating Lymphocytes in Esophageal Cancer Induced by Heavy Ion Beam Radiation

Fan Ding[1,a], Juntao Ran[2,b], Ziying Dai[2,c], Qiulan Wang[3,d*]

[1]Teaching Experiment and Training Center, Gansu University of Chinese Medicine, Lanzhou City 730000, China
[2]Department of Radiotherapy, the First Hospital of Lanzhou University, Lanzhou City 730000, China
[3]Department of Diagnostics, Gansu University of Chinese Medicine, Lanzhou City 730000, China
[a] lzdingfan@sina.com, [b] ranjt@lzu.edu.cn, [c] daizy16@lzu.edu.cn, [d] qiulwang@163.com
**Corresponding author*

Abstract: *Due to the characteristics of dietary structure, esophageal cancer is a malignant tumor of human digestive system with high incidence, while esophageal cancer is a heterogeneous malignant tumor with high mortality and can not be treated early. Although there are many diagnostic methods at present, the side effects are great and have a great impact on the body. Its development is mainly affected by factors such as immune dysfunction, antioxidant system damage and apoptosis. In recent years, clinical studies have shown that the study of lymph tissue infiltration, proliferation and differentiation has an important contribution to cancer. Heavy ion beam radiation is an important targeted therapy and has broad prospects. This paper mainly studied the expression and functional changes of hepatoma cells and lymphocytes induced by heavy ion radiation. Firstly, the concept, characteristics and characteristics of heavy ion beam radiation were introduced. Then, the tumor infiltrating lymphocytes of esophageal cancer were studied. Then, the expression and functional changes of tumor infiltrating lymphocytes of esophageal cancer induced by heavy ion radiation were observed. Finally, the experimental results show that heavy ion beam radiation has a strong ability to inhibit the proliferation of tumor infiltrating cells. The inhibitory effect of heavy ion beam irradiation on the proliferation of tumor infiltrating cells was positively correlated with dose time ($P < 0.05$). The invasion rate of cancerous cells was different between the two groups in different environments. Under the irradiation of heavy ion beam radiation, the invasion and metastasis rate of cancer cells in the experimental group was maintained at 0.1%. At the same time, the metastasis rate of the control group was the highest at 0.95% without the interference of heavy ion beam radiation.*

Keywords: *Heavy Ion Beam Radiation, Esophageal Cancer, Tumor, Invasive Lymphocytes, Cancerous Expression*

1. Introduction

Tumor is one of the common diseases endangering human health. With the development of social economy, people pay more and more attention to cancer treatment. At present, scholars at home and abroad have done a lot of research on the interaction mechanism and function between cancer cells and tissues [1-2]. In recent years, a large number of reports have shown that esophageal cancer has become the most serious and teratogenic cause of death threatening human health, life safety and physical health [3-4]. Heavy ion beam irradiation can effectively inhibit cell proliferation and enhance the effect of target tissue on tumor receptor-mediated effect and drug activity. It is one of the better methods in clinical practice.

Many scholars have studied tumor infiltrating lymphocytes. At present, the research on intracellular tumor at home and abroad mainly focuses on its pharmacological activity and drug action mechanism. Among them, cell indirect polysaccharide is of clinical value. In recent years, with the rapid development of genetic engineering, chemical synthesis and biomedicine [5-6]. Some scholars have conducted extensive experiments on the dissolved lymphoid tissue in water environment. It is found that substances related to conventional cirrhosis can reduce the incidence rate, improve immunity and reduce the risk of death through direct induction of esophageal lesions. Other scholars used X-ray diffraction and electron microscope scanning to characterize the tumor tissue structure and cell

ISSN 2706-6819 Vol.3, Issue 5: 21-26, DOI: 10.25236/IJFM.2021.030503

morphology, fluorescence analysis and determination of drug release and distribution characteristics [7-8]. The above research has laid the foundation for this paper.

In this paper, heavy ion beam radiation was used as the starting point to observe the infiltration and cell morphological changes of tumor tissues, and to explore the inhibitory development rate and metastasis rate of lymph node cells in cancer lesions under the influence of heavy ion pressure.

2. Study on Tumor Infiltrating Lymphocytes Induced by Heavy Ion Beam Radiation in Esophageal Cancer

2.1 Heavy Ion Beam Radiation

2.1.1 Concept

Heavy ion beam refers to the use of ion accelerator to accelerate charged particles heavier than protons to close to the speed of light, and then use micro beam technology to electromagnetically focus the newly increased amount of heavy ions or align through micron technology holes, so as to produce electron beam in micron scale. Then according to the different energy, the heavy ion beam is divided into low-energy ion beam, medium energy ion beam and high-energy ion beam. Compared with X-ray, X-ray, y-ray and neutron irradiation, heavy ion radiation, as a higher energy LET radiation, has more physical characteristics such as energy deposition, inverse dose distribution, low dispersion and large damage cross section, and has a higher and more complex biological effect on biological radiation. Due to the interaction between the radiation particles and the small molecules that make up the living body in the tracking process, there will be more intense energy order transfer, chemical bond destruction and ionization in the biological system, and produce many free radicals. Heavy ion radiation can be obtained through the cooperation between tumor cells. Its mechanism is mainly based on the selective stimulation of target molecules in this region by tumor tissue to specifically bind them to drugs in the tumor region, which improves the effectiveness of drugs [9-10].

2.1.2 Advantages

Heavy ion beam is a beam with high linear energy transfer, and its energy presents an inverted depth dose distribution, which means that heavy ions have high relative biological efficiency (RBE). In contrast, it can be concluded that heavy ion beam can treat deep malignant tumors. The principle is: heavy ion beam uses its own high RBE to destroy DNA double strand at the same time, so that it has no time to repair itself, so as to inhibit the growth of tumor tissue, which can not be achieved by X-ray. Therefore, compared with X-ray, heavy ion beam is more suitable for the treatment of malignant tumors. Heavy ion beam has been recognized all over the world. In some developed countries, heavy ion beam treatment of cancer has entered the stage of clinical treatment. The annual control rate is different. Therefore, the unique physical and biological effects of heavy ion beam are used to reach malignant tumors from various parts and sources, inhibit their proliferation, and achieve the purpose of treating malignant tumors [11-12].

2.1.3 Characteristics

(1) Physical properties

The heavy ion beam interacts with solute atoms in the process of penetrating matter, and bethe Bloch formula gives a good description of the heavy ion beam and its energy deposition in solute, that is, the so-called physical process of stopping power force:

$$-\frac{dE}{dx}=\frac{4\pi e^4 Z_{eff} ZN}{m_e c^2 \beta^2}\left(\ln\frac{2m_e c^2\beta^2}{1}+\ln\frac{1}{1-\beta^2}-\frac{c}{z}-\frac{\delta}{2}\right) \tag{1}$$

Where, e is the electron to electricity ratio; Z is the atomic number of the medium; N is the electron density of the medium; Me is the static mass of the electron; C is the propagation speed of light in vacuum. Zeff velocity can be calculated by barkas's empirical formula:

$$Z_{\text{eff}}=\left(1-\exp\left(-125\beta/z^{2/3}\right)\right) \tag{2}$$

Where Z is the atomic number of the incident ion. It can be seen from the above formula that the energy loss rate is inversely proportional to the square of velocity, and the ion beam (including protons and heavy ions) deposits at the end of range with energy attenuation to form a sharp energy deposition

ISSN 2706-6819 Vol.3, Issue 5: 21-26, DOI: 10.25236/IJFM.2021.030503

peak, namely Bragg peak, which presents an inverted depth dose distribution. Ion beam radiotherapy has obvious advantages in depth dose distribution. When the ion beam passes through the medium, Rutherford scattering occurs with the coulomb field of the atomic nucleus of the medium. The lateral scattering of the heavy ion beam is small, which is more conducive to the control of rays and the accurate irradiation of tumors.

(2) Biological characteristics

In addition to physical advantages, heavy ions have a series of biological advantages compared with other radiation. It can represent the change trend of cell survival rate with radiation dose of different quality radiation sources, which is described by linear quadratic model:

$$S = e^{-\alpha D-\beta D^2} \tag{3}$$

Where s is the cell survival rate and d is the total irradiation dose. Carbon ions have high relative biological effects in the peak area. The dose effect relationship of radiation is generally nonlinear and is affected by many factors at the same time. Therefore, the influencing factors of RBE value are also various, mainly including ion type, dose, energy, and cell tissue type, biological end point, etc.

2.2 Tumor Infiltrating Lymphocytes in Esophageal Cancer

2.2.1 Concept

The incidence of esophageal cancer ranks ninth in the incidence of malignant tumors in the world, and there is a distinct regional difference between high incidence areas and low incidence areas. China is also a high incidence area of esophageal cancer, which is one of the main causes of malignant tumor death. Although surgery is still the main treatment for esophageal cancer, supplemented by radiotherapy and chemotherapy, the prognosis of esophageal cancer is not ideal due to the limited medical effect. The proposed tumor targeted therapy and biological immunotherapy have opened up a new way for the clinical treatment of malignant tumors. Tumor infiltrating lymphocytes (TIL) are new anti-tumor cells after lymphokine activated killer cells (LAK). Due to the rapid development of modern immunology, the research on cancer and immunology is becoming more and more sophisticated. Therefore, immunology is becoming more and more important in the study of the origin, progress, prognosis and diagnosis of cancer.

2.2.2 Tumor infiltrating lymphocyte immunophenotype

Til is mainly composed of T cells, NK cells and B cells, and their structural changes are mainly affected by the number of dendritic cells (DC), tumor cell size and local tumor environment. But usually, til mainly exists in the matrix or around malignant tumor cells. T cells are mostly CD3 +, CD4 + and CD8 +, while B cells and NK cells are relatively small. With the heterogeneity of TIL cell surface, the composition of til is different in different tumor tissues. In general, CD 8 + in til cells is greater than or equal to CD4 +. However, due to the heterogeneity and variability of TIL tables, there are many factors affecting their expression, and there can be different immunophenotypes for different reasons.

3. Experiment

3.1 Experimental Materials and Equipment

Before the experiment, we need to select the materials radiated by heavy ion beam to a certain extent, because different substances have a great impact on tumor cells. The material of this experiment comes from self-made specimens in the laboratory. Five heavy ion beam radiation tube cancer samples were selected. Before the test, a beaker with a diameter of about 6mm and 2m needs to be prepared as the control group. After being cut into a circle by WEDM, it is put into the oven for drying treatment, made into a transparent shape, and evenly coated with adhesive on its surface, which can be used to observe the morphological changes of tumor tissue and cells. Put the powder on sterile paper (such as polyethylene cloth), add an appropriate amount of distilled water to dissolve it, and then store it in a vacuum suction filter bottle for 48h.

ISSN 2706-6819 Vol.3, Issue 5: 21-26, DOI: 10.25236/IJFM.2021.030503

3.2 Experimental Method

The random sampling method used in this study is based on XRF detection and MTT colorimetric analysis. Firstly, the canceration of tumor tissues and cells, the growth and development of lymphatic vessels and the change trend of lipid metabolites in patients under heavy ion radiation technology were collected. Then the treatment plan was determined according to the data results, and then the influencing factors (such as glucocorticoid) of lymph node metastasis time, injury degree and distribution were calculated by XRF signal processing method. The drug effect was judged by observing the infiltration of tumor cells at different dose concentrations and the same dose.

3.3 Experimental Process

Heavy ion beam radiation technology was applied to the tissue reconstruction and functional mutation of cancer cells. Pretreatment was carried out before the experiment to remove the bad environment of the tumor. During the experiment, the lymphoid infiltration and red blood cell growth between different dose groups and the control group were observed by direct irradiation. Under the whole blank drug loading concentration (5mg / L), observe the damage degree of liver tissue and t lymphatic system. The application of heavy ion beam radiation technology in cancer cell reconstruction can effectively improve the reconstruction efficiency and recovery speed and reduce the experimental operation time.

4. Discussion

4.1 Functional Changes of Tumor Infiltrating Lymphocytes

Table 1 shows the survival inhibition rate of heavy ion beam in different dose groups at different times.

Table 1: Cell survival inhibition rate

Group	12h	24h	48h
1	24.02±0.25	26.5±0.58	32.25±0.25
2	34.25±0.14	35.36±0.17	40.87±0.69
3	23.36±0.24	25.36±0.31	35.32±0.47
4	36.24±0.85	39.31±0.45	41.31±0.65
5	27.68±0.98	31±0.58	36.25±0.87

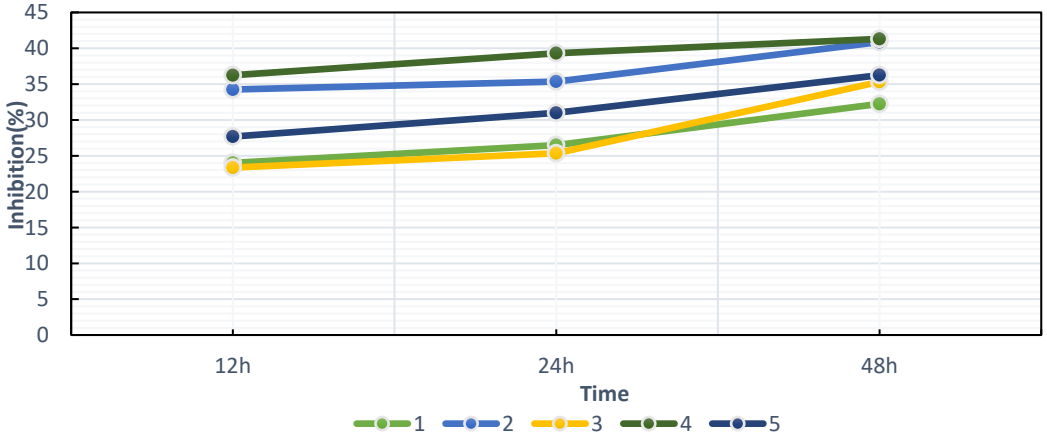

Figure 1: Medium ion beam radiation showed cell survival inhibition

In tumor tissue, different types of cell structures are produced due to the interaction of drugs. The with certain inducing force is formed by specific binding with the target molecule. Therefore, the corresponding sensitive receptors can be selected as mutagens according to the tumor tissue morphology, the pathological characteristics of lymphatic carcinoma and the surrounding environment, so as to stimulate treatment or directly inhibit the substances in the cancer focus, so as to reduce the inflammatory response and promote cell repair. It can be seen from Figure 1 that the cell survival

ISSN 2706-6819 Vol.3, Issue 5: 21-26, DOI: 10.25236/IJFM.2021.030503

inhibition rate was studied by MTT colorimetry at 12h, 24h and 48h after the cells were irradiated with heavy ion beams with different radiation doses. According to the experimental results, the following conclusions can be drawn: when the irradiation dose is constant, the cell inhibition rate increases with the increase of time; At a certain time point, the cell inhibition rate increased with the increase of irradiation dose, especially at 48h. Heavy ion beam radiation has a strong ability to inhibit the proliferation of tumor infiltrating cells. The inhibitory effect of heavy ion beam irradiation on the proliferation of tumor infiltrating cells was positively correlated with dose time ($P < 0.05$).

4.2 Analysis of Cell Invasion and Metastasis

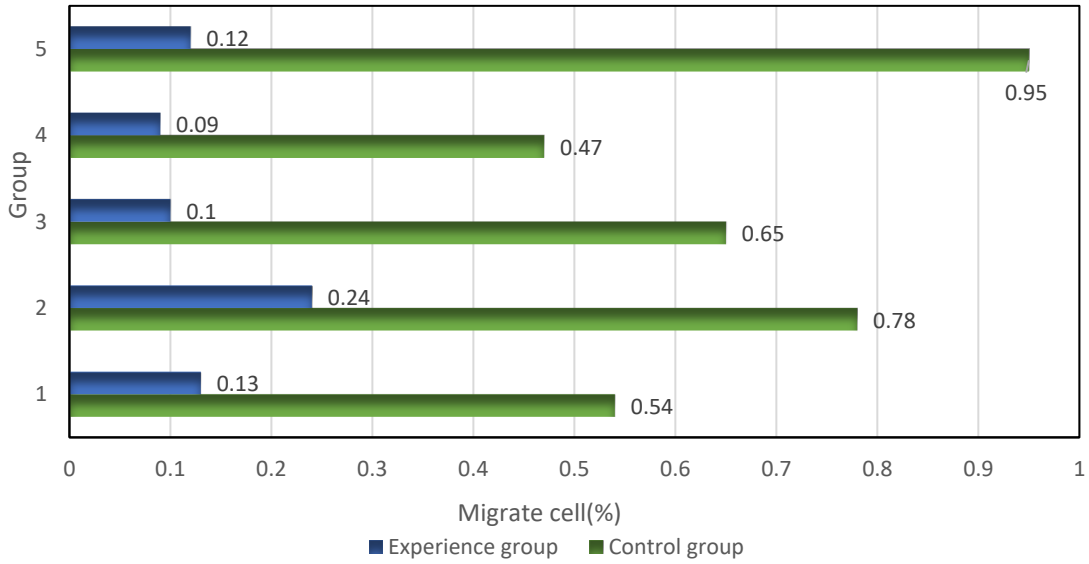

Figure 2: Comparison of cell invasion and transfer rates

The formation of cancer is caused by lipid peroxidation in tumor cells. There are a large number of tumor tissues with sensitization and immunity. Under the induction of heavy ion radiation, the proliferation, differentiation and apoptosis of lymphofibroblasts will be affected. The cells in the tumor tissue transmit lymph nodes to each other, and this transmission is inhibited by oncogenes, which affects the resection and metastasis of esophageal cancer. Because heavy ion radiation can effectively control and change tissues through receptor induction. It can be seen from Figure 2 that the invasion speed of cancerous cells is different in different environments. Under the irradiation of heavy ion beam radiation, the invasion and metastasis rate of cancer cells in the experimental group was maintained at 0.1%. At the same time, the metastasis rate of the control group was the highest at 0.95% without the interference of heavy ion beam radiation. Therefore, this shows that heavy ion radiation has a significant effect on tumor invasiveness and canceration level.

5. Conclusion

In this study, heavy ion radiation technology is of great significance to the invasive and functional changes of tumor tissues. This experiment mainly discussed the ability of different concentrations of drugs (anticancer gene drugs) to induce the production and proliferation of cellular lymph nodes under heavy ion beam irradiation. The results showed that there was a positive correlation between lymph node metastasis and cell proliferation in vitro. Heavy ion radiation technology can effectively improve tumor infiltration and proliferation rate, and has certain reference value and guiding significance for cancer treatment.

Acknowledgements

Innovation Fund Project of Gansu Education Department (No: 2021B-164).

ISSN 2706-6819 Vol.3, Issue 5: 21-26, DOI: 10.25236/IJFM.2021.030503

Reference

*[1] Sha Z , Hong Y M , Zhang J , et al. Changes in Indoleamine 2,3-Dioxygenase 1 Expression and CD8+ Tumor-Infiltrating Lymphocytes after Neoadjuvant Chemoradiation Therapy and Prognostic Significance in Esophageal Squamous Cell Carcinoma[J]. International Journal of Radiation Oncology*Biology*Physics, 2020, 108(1):286-294.*

[2] Katsuhiko S , Riki O , Shinsuke S , et al. Prognostic value of Cox-2 and PD-L1 expression and its relationship with tumor-infiltrating lymphocytes in resected lung adenocarcinoma[J]. Cancer Management & Research, 2017, 9: 741-750.

[3] Zhu X , Zhang Q , D Wang, et al. Expression of PD-L1 Attenuates the Positive Impacts of High-level Tumor-infiltrating Lymphocytes on Prognosis of Triple-negative Breast Cancer[J]. Cancer Biology & Therapy, 2019:1-8.

[4] Bianchini G , Smart C , Mansutti M , et al. Modulation by treatment of tumor infiltrating lymphocytes (TILs) and PDL1 expression in triple-negative breast cancer in the ETNA trial[J]. Journal of Clinical Oncology, 2020, 38(15_suppl):555-555.

[5] Chen K , Zhu Z , Nan Z , et al. Tumor-Infiltrating CD4+ Lymphocytes Predict a Favorable Survival in Patients with Operable Esophageal Squamous Cell Carcinoma[J]. Medical Science Monitor International Medical Journal of Experimental & Clinical Research, 2017, 23:4619-4632.

[6] Bai S , Yang X , Zhang N , et al. Function of tumor infiltrating lymphocytes in solid tumors - a review[J]. Sheng wu gong cheng xue bao = Chinese journal of biotechnology, 2019, 35(12): 2308-2325.

[7] Okugawa Y , Toiyama Y , Ohi M , et al. Comment on "Prognostic Nutritional Index, Tumor-infiltrating Lymphocytes, and Prognosis in Patients With Esophageal Cancer"[J]. Annals of surgery, 2019, 270(6):e104-e105.

[8] Kim J Y , Heo S H , Choi S K , et al. Glutaminase expression is a poor prognostic factor in node-positive triple-negative breast cancer patients with a high level of tumor-infiltrating lymphocytes[J]. Virchows Archiv, 2017, 470(4):381-389.

[9] Linette G P, Carreno B M. tumor infiltrating lymphocytes in the checkpoint inhibitor era [J]. Current psychological malignancy reports, 2020, 2019,14 (4): 286-291

[10] Andres f h, Valerie m, Yuri F, et al. Relationship of infiltrating internal T lymphocytes in the diagnosis of oral lichen plans versus, oral veterinary dysplasia: a pilot study [J]. Oral surgery, oral medicine, oral pathology and oral radiology, 2020, 2019127 (6): e123-e135

[11] Klampatsa A , Shaun M. O'Brien, Thompson J C , et al. Phenotypic and functional analysis of malignant mesothelioma tumor-infiltrating lymphocytes[J]. OncoImmunology, 2019, 8(9):1-12.

[12] Schirosi L , Saponaro C , Giotta F , et al. Tumor Infiltrating Lymphocytes and NHERF1 Impact on Prognosis of Breast Cancer Patients - ScienceDirect[J]. Translational Oncology, 2020, 13(2):186-192.