

## Progress of curcumin on esophageal cancer radiotherapy sensitization

Shengrong Lv, Xinjia He, Yongheng An\*

Department of Oncology, Affiliated hospital of Qingdao University, Qingdao, 266000, China

**Abstract:** Esophageal cancer is one of the top ten tumors in the world and radiotherapy is an important treatment method. Due to the interference of many factors, increasing the radiotherapy sensitivity of tumor cells has become an important part of the treatment. Curcumin is a traditional Chinese medicine. Many studies showed that curcumin has radiosensitivity by inducing apoptosis, regulating cell cycle, increasing DNA damage in tumor cells and restraining repair in hypoxic cell. The study of radiotherapy sensitivity of curcumin on esophageal cancer will bring a new opportunity for clinical application.

**Keywords:** Esophageal cancer; Curcumin; Radiotherapy sensitivity; Apoptosis

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\*Corresponding Author: Yongheng An, anyongheng@126.com

### 1. Introduction

Esophageal cancer is a malignant tumor originating from the mucosal epithelium, which is one of the top ten tumors. Globally, there are about 314,000 new cases of esophageal cancer, the sixth leading cause of death in the tumors[1]. The occurrence of esophageal cancer is obvious regional, especially Hebei and Henan province in China. Esophageal cancer was the fifth most malignant tumor in China in 2010, ranking the fourth cause of death in malignant tumor[2]. Bad eating and habits will lead to esophageal cancer, such as smoking, drinking, eating too hot food. The most patients were diagnosed at middle-later stage. And the five-year survival rate is only about 30%. So the radical operation of esophageal cancer is necessary for the patients. Radiotherapy and surgery are the most important ways for esophageal cancer. Located in the middle and upper segment of esophagus cancer, radiotherapy is effective treatment. Because of interference of many factors, increasing the radiotherapy sensitivity of tumor cells has become an important part of the treatment. Seeking drugs of radiotherapy sensitization is important.

Curcumin is a kind of acid polyphenol which is extracted from the roots of turmeric, turmeric and calamus. Its molecular formula is  $C_{21}H_{20}O_6$  and the molecular weight is 368.37. It has extensive pharmacological effects because it can control many signal transduction molecules, such as chemokines, transcription factors, growth factors, inflammatory cytokines, cell cycle regulatory proteins, enzymes, drug resistant protein, receptor, DNA, RNA. Because of molecular biological characteristics, curcumin has anti-inflammatory, anti-atherosclerosis, anticoagulant, anti-oxidative, anti-rheumatism and other functions[3]. The United Nations World Health Organization approved curcumin as a natural food additive. The National Institute of Medicine classified it as a third-generation anticarcinogen. Dattan[4] proposed the

anti-tumor effect of curcumin in 1985. In recent years, it has been found that curcumin has a synergistic effect with radiotherapy. It can increase the radiation injury to the tumor.

This paper reviews the mechanism of curcumin on the radiation sensitization of esophageal cancer cells. It provides basis for the further study and clinical application.

### 2. Basic Principle and Progress of Radiotherapy

Radiation therapy is the process of killing tumor cells using high-energy photons or charged particles. High-energy photons mainly include X-rays and  $\gamma$ -rays. The high-energy charged ions are mainly electrons. The using of clinic is the X-ray and electronic wires are produced by the linear accelerator. Due to the poor penetration of the electronic thread, it is mainly used in the treatment of superficial tumors. X-ray penetrate power which is mainly used for the treatment of deep tumors. Radiation has radiant energy to kill tumor cells. Radiation can induce cancer in the natural environment, but it can be used as an effective means to kill the tumor[5]. There is a lot of evidences that DNA is a key target for a range of radiobiological effects. Ionizing radiation can directly or indirectly damage DNA in tumor cells which changes cell structure and activity and kills tumor cells. Ionizing radiation can cause double-stranded DNA damaging and indirect DNA damaging. It is also considered an important mechanism to kill tumor cells. The indirect effect is mainly due to the radiation acting on atoms or molecules in cells and producing free radicals which can be spread far enough and make the key target DNA damage. It leads to the death of cancer cells. Indirect radiation damage can be modified by chemical means such as protective agent or sensitizer. And the direct radiation damage cannot be modified.

Radiation injury is not specific for tissue and cell damage. However, due to the difference of the biological characteristic between normal tissues and

tumor cells, the effect of radiation on normal tissue is not as large as tumor. First, tumor cells have less self-healing ability than normal tissue. Normal tissue are able to repair the damaged DNA when exposed to the right amount radiation, but tumor cells has poor ability in this respect which is not repair in time. Secondly, due to the rapid division of tumor cells, most tumor cells are in the sensitive stage of radiotherapy, which makes tumor cells more sensitive than normal tissues[6,7].Because of this, radiation has larger effect on normal tissue than tumor. It is the foundation of radiotherapy.

With the development of computer technology, radiation physics, radiation biology and radiotherapy equipment, radiation oncology has achieved many theoretical and technological breakthroughs[8]. The radiotherapy equipment is gradually developed into three-dimensional radiotherapy system, such as three-dimensional conformal radiotherapy technology and three-dimensional intensity modulated radiotherapy technology. Their clinical application of the treatment of tumor has achieved the curative effect. In the 1990s, with the development of the digital image technology[9,10], the shape of space tumors in the human body can be accurate positioning and description. Three-dimensional conformal radiation therapy technology was two-dimensional planar pattern in transition to the three-dimensional model. In the processes of radiation therapy, radiation field can be changed according to the form of tumor. It makes that the shape of dose distribution in three dimensional directions have same direction about tumor of three-dimensional. In this way, when the total amount of radiation in the target area is obviously increased, the radiation of normal tissues and organs are reduced. It has been reported that this technique has been used in prostate cancer and breast cancer treatment[11,12]. In recent years, the three-dimensional intensity modulated technology has become the mainstream of radiotherapy technology. Three-dimensional intensity modulated technology not only requires the radiation field in three dimensional directions which are consistent with the tumor. But at the same radiation field gives the uneven of radiation intensity. This technology can be suitable for the radiation dose distribution in the form of three-dimensional. The biggest advantage that dose distribution in three-dimensional direction is consistent with the shape of the target area. Soincreasing the effective dose and reducing peripheral dose protect normal organs and kill tumor cells, which improving the survival[13].

### **3. The Application of Radiotherapy in Esophageal Cancer**

Today the treatment of esophageal cancer is mainly surgery and radiotherapy. Some indications as a result

of the surgery or medical reasons not surgery and surgery patients need to be consolidated, the conventional choice is radiation therapy. But the effect is not ideal. Clinical staging is the main factor that affecting the prognosis and uncontrolled locality, which is the main cause of treatment failure. In the early stage (0- I stage), the five year survival rate of esophageal cancer is 83.3-92.2% and the radiation treatment is 67.6-75.0%. The five year survival rate is 46.3-53.5% after the simple operation of esophageal cancer in the stage II. The five year survival rate in the stage III esophageal cancer is only 6.7-15.1%. The five year survival rate in the stage IV is 0.

#### **3.1. Simple Radiotherapy**

External exposure is the conventional choice of esophageal cancer radiotherapy, but the five year survival rate is only 8%. The main failure reason is local recurrence that account for 60-80%[14]. Thus reducing the local recurrence rate of esophageal cancer is the key to improve the curative effect of esophageal cancer radiotherapy.

The innovation of radiotherapy technology, such as three-dimensional conformal radiotherapy that can accurately irradiate the tumor, can improve the local control rate and the survival rate. Xiao Zefen[15] experimental research proved that the conventional radiation field and expand the radiation field failed to include all the tumor, but the three dimensional conformal radiotherapy can appropriate coverage of tumor when the dose of lung and spinal cord not increase.

#### **3.2. Comprehensive Treatment of Radiotherapy and Chemotherapy**

The effects of radiotherapy combined chemotherapy and simple radiotherapy on esophageal cancer were studied. Cooper[16] according to a study combined chemotherapy in patients with esophageal cancer radiotherapy five year survival rate was 14% and the five year survival rate for patients with simple radiotherapy is 0. The conclusion is that the effect of radiation and chemotherapy is better than pure radiotherapy on esophageal squamous carcinoma and adenocarcinoma. Zhang Jianyu study[17] showed that radiotherapy and chemotherapy has a higher survival rate than chemotherapy.

#### **3.3. Comprehensive Treatment of Radiotherapy and Surgery**

Chinese academy medical sciences compared esophageal cancer operation and preoperative radiotherapy with simple treatment. It shows that the preoperative radiotherapy add the opportunity of surgery. It reduced the rate of lymph node metastasis, narrowed tumor significantly, reduced the local and

regional recurrence, improved the resection rate and survival rate. Studies had shown[18] that postoperative radiotherapy for esophageal cancer can elevate the survival rate of patients with positive lymphadenopathy.

### **3.4. Comprehensive Treatment of Chemoradiotherapy and Operation**

Fiorica[19] research showed that preoperative chemotherapy plus surgery significantly reduced mortality and postoperative pathology compared with simple surgery. Because radiation therapy can control the local and regional of esophageal lesions, and chemotherapy can control the local and distant metastasis, preoperative chemoradiotherapy can improve the survival rate of resectable esophageal cancer.

## **4. Radiotherapy Sensitization Mechanism of Curcumin**

Radiotherapy sensitization study is a hot topic in tumor research, which is great significance for improving the radiotherapy of clinical tumor. Radiosensitizer, a kind of chemical or drug sensitizing agent, combined with radiotherapy, which can reduce the radiation resistance of tumor cells and improve the killing effect of radiation on tumor cells[20]. Although the perfect radiotherapy sensitizer has not been found, there are still many drugs that have radiotherapy sensitization effect, such as arsenic trioxide and paclitaxel[21].

Basic experiments have shown that curcumin has the effect of radiotherapy and sensitization on a variety of tumors. Experiments show that different doses of radiation alone role with the joint action of curcumin can inhibit Hela cells formation of tumor. Combined using single application of curcumin and radio inhibitory effect is more apparent[22]. The growth inhibition rate of colorectal cancer cell HT-29 cells was significantly higher than that of the radiotherapy group[23]. The radiotherapy sensitization of curcumin of different tumors maybe have the following mechanisms.

### **4.1. Inducing Apoptosis and Enhance Radio-sensitivity**

Cell apoptosis, the programmed death of the cell, is the process of self-inflicted death after the cell receives a certain signal in certain conditions. Zhang[23]found that the cell apoptosis of radiotherapy combined with curcumin on Hela cell lines was more obviously. Compared with radiotherapy group, the growth inhibition rate of rectal carcinoma HT-29 cell was significantly increased. Multiple studies have shown that the combination of curcumin and radiotherapy increase radiation sensitization, which is associated

with apoptosis in malignant tumors. The mechanism may be related to the following factors.

#### **4.1.1. Cytochrome C and Apoptosis**

There are various complex mechanisms of apoptosis. Currently, there are three apoptosis pathways, but mitochondrial mediated apoptosis regulation is the main mechanism. In various apoptotic factors, mitochondrial damage and membrane permeability change, then cytochrome C releases from mitochondria to cytoplasm. So cytoplasm C appears in cytoplasm. Subsequently, cytochrome C activate Caspase apoptosis, then apoptosis cascade reaction occurred. Cytochrome C release is a key event of apoptosis, and cytochrome C is a key molecule for mitochondrial involvement in apoptosis. Studies have shown that[24], after curcumin effect on cervical cancer Hela cell line, Caspase-9 and cytochrome C expression levels is high. It shows that the apoptosis of cervical cancer cells may be related to the expression of Caspase-9 and cytochrome C. Cell apoptosis pathways are divided into membrane receptor mediated pathways and mitochondrial receptor mediated pathways. Membrane receptors mediated pathways is that the activation of Caspase-8 through a series of reactions activate Caspase-3 to induce cell apoptosis. At the same time it can significantly increase the apoptotic protease caspase-8 and caspase-3, so curcumin induced apoptosis of cervical cancer Hela happen. This process may be apoptosis of caspase dependent way realization. The apoptotic protease caspase-8 and caspase-3 played an important role in apoptosis. JNK pathway has always been considered related to regulate cell apoptosis. It through the mitochondrial level activates endogenous induced apoptosis pathway to promote apoptosis protein and cytochrome C release, then start caspase cascade cause apoptosis[24]. Gan[25] found that after curcumin treatment for 24h, the activity of MKK4 and MKK7 rises on cervical cancer Hela cells, which makes silk crack the original activated protein kinase JNK pathway. Therefore curcumin radiotherapy sensitization mechanism has relationship with the JNK pathway activation.

#### **4.1.2. Bcl-2 and Apoptosis**

The cytochrome C in mitochondria is regulated by the Bcl-2 family. The Bcl-2 family is an important signaling molecule in apoptosis. According to its functions, it can be divided into anti-apoptotic protein, such as bcl-2, bcl-xl, and apoptotic protein, such as Bax, Bak[26]. VDAC is the channel of cytochrome C release, then the anti-apoptotic protein bcl-2 and bcl-xl are combined to close the channel. Apoptosis protein Bax and Bak can accelerate the opening of VDAC channel, then inducing the release of cytochrome C from mitochondria to cytoplasm and activating Caspase-9 to accelerate apoptosis process. Bcl-2 has highly expression in the most tumors. Bcl-2 and Bcl-xl

can bind the apoptosis protein Bax and Bak to prevent the activation of the Bak, so that the cells can survive. Wang Jingpeng[27]works showed that Bax protein expression can increase after curcumin treatment of cervical cancer Hela cells, while the Bcl-2 protein expression does not have obvious change. This may be a mechanism of curcumin induced apoptosis. This is consistent with the research by Singh M[28]. Compared with the control group, the Bcl-2 protein decreased and Bax protein content increased in Hela cells in drugs and radiation group. The important results demonstrate that the ratio of the Bcl-2/Bax reduce radiation sensitive effect of curcumin on cervical cancer cells is one of the possible mechanisms.

#### 4.1.3. ROS and Calcium Ion and Apoptosis

The increase of ROS production calcium ion intracellular flow also promote the release of cytochrome C, which lead to cell damage and apoptosis. ROS includes hydrogen peroxide, hydroxyl radical, superoxide anion. The content of reactive oxygen in normal cells is lower than that of tumor cells. Continuously high energy ROS can destroy oxidative stress balance and induce apoptosis. The increase of intracellular reactive oxygen causes the disturbance of oxidative stress, the cell membrane potential depolarization, the initiation of the endoplasmic reticulum release calcium ion and the intracellular circulation pathway, leading to the increases of intracellular calcium ion level. At the same time, the mitochondrial permeability transformation hole is open. It causes the decline of mitochondrial membrane potential, and result in irreversible cell apoptosis. Gan[25]has found that active oxygen increased in Hela cells with curcumin treated 4h. And it could be concluded that the increase of intracellular reactive oxygen was positively correlated with the radiation sensitization of tumor cells by curcumin.

#### 4.1.4. p53 and Apoptosis

Normal p53 gene (wild-type p53 gene) plays an important role in the growth of normal cells and inhibits cell transformation and growth. Change of normal gene, such as mutation or loss, can induce a variety of cancer. P53 gene influence cell apoptosis by adjusting the expression of bax and BCL-2 gene. P53 is bax gene transcription activation factor directly. The accumulation of P53 protein and activity are caused by cell apoptosis. Jiang[29]results showed that with the increase of curcumin, the concentration of p53mRNA increased. Curcumin can inhibit the proliferation of Hela cells and the inhibitory effect is dose-dependent. The mechanism may be achieved by increasing the expression of p53 gene. Experimental studies have shown that curcumin can increase the content of p53, Bax and PUMA in tumor cells of colon cancer. Choudhuri[24]showed that in tumor cells, curcumin

could selectively increase the expression of apoptosis gene p53 in G2 cell stage and promote apoptosis. In the effect of curcumin on the proliferation of Hela cells, curcumin could significantly inhibit the proliferation of tumor cells and promote the expression of mRNA of Hela cell p53.

#### 4.2. Regulating Cell Cycle and Enhance Radiosensitivity

The typical cell proliferation cycle has four phases, including G1, S, G2, and M, which depends on the specific signal transduction of cyclin-dependent kinase complex. In four phases, the most important is the transformation of G1 to S and G2 to M. The sensitivity of M and G2 stage to radiotherapy is the highest. And the lowest sensitivity to radiotherapy is S stage, especially late S stage. It is an important way to enhancement of radiosensitivity that regulates tumor cell proliferation cycle and makes tumor cells stay in G2 and M phase. Zhang[22] study showed that cervical cancer cell line Hela cells using curcumin had radiosensitization effect. The results also showed relationship of the retardant of cell cycle G2 and M stage. Experiments have confirmed that combination of curcumin group and radiation for esophageal cancer cell line Eca-109 cells increased apoptosis rate, reduced the G0/G1 ratio and increased G2/M ratio[30]. It showed that curcumin could suppress the accept rays by organizing the cell cycle of the growth of cells.

#### 4.3. Increasing the Damage of Tumor Cell Dna and Inhibiting the Repair of Hypoxic Cells and Enhance Radiosensitivity

Glutathione is necessary to repair damaged DNA. It is inversely proportional to the tumor radiotherapy sensitization. In normal conditions, glutathione is reducing form. Reduced glutathione has two roles in radiation. One role is the direct removal of free radicals caused by ionizing radiation. Other role is that adjusting the content of oxygen and other electrophilic sensitization material make the charge on the cellular target molecules shift. Gan[25] observed that the radiotherapy effect of curcumin on Hela cell in cervical cancer decrease the content of glutathione. It suggested that glutathione was related to the radiotherapy sensitization mechanism of curcumin.

#### 5. Conclusion

The occurrence of esophageal cancer is long-term, multifactorial and multi-phase processes, which is the result of multi-gene synergy. The progress of radiotherapy technology improves the survival rates of esophageal cancer. Protection of normal tissue and the important role of the tumor, finding suitable radiotherapy sensitization is important. It can improve

the survival rate and quality of life. Curcumin has different effects on multiple cell signaling pathways. It is understood that curcumin has a complex biological effect on tumor cells. We still have a long way to go for the purification and utilization rate of curcumin. Therefore, it is necessary to make further studies to produce curcumin with higher purity and utilization of curcumin. It will play a more important role in the radiotherapy of patients with esophageal cancer.

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