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# The Influence of Different Single Radiation Dose on Delayed Growth of Transplanted Tumor in Athymic Mouse

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**OBJECTIVE** To reveal the biological effects and effective dosage in radiotherapy model which applies high single-dose irradiation by animal experiment.
**METHODS** We inoculated subcutaneouly human pancreatic carcinoma cell line (MIA PaCa-2) in the lateral of the right lower extremity of the athymic mouse to grow transplantation tumor. While the median diameter of transplantation tumor reached 10 mm approximately, the animals were randomly divided into 7 groups (6 animals per group) and fixed with consciousness for irradiation by different dose in one fraction (0, 2, 5, 10, 17, 25, 35 Gy). All were kept on to be bred for observation of the change in gross tumor volume, calculation of delayed growth time and delayed growth curve.
**RESULTS** With increased dose per fraction, cutaneous reaction on the neoplasma surface of the animal, which was mainly moist yellow effusion was more and more severe. When dosage is less than 10 Gy, all animals showed similar effects, that's the delayed tumor growth was not obvious. Tumors receiving more than 10 Gy in one fraction showed very good biological effect and the delayed tumor growth was obviously related to dosage. The difference in delayed tumor growth between the 2 groups was statistically significant. The delayed tumor growth time in 10, 17, 25 Gy group was respectively 3 weeks, 6 weeks and more.
**CONCLUSION** The biological effect of the model which applies high single-dose irradiation (more than 10 Gy in one fraction) was very good. The effect of delayed tumor growth was obviously related to the dosage after transplantation tumor was radiated. Because of its higher dose per fraction and biological effects, the model of high single-dose irradiation can get better clinical effects.

**KEY WORDS: hypofactionation radiotherapy, delayed growth curve, MIA PaCa-2, oncosis, transplanted tumor.**



## Introduction

Malignant tumor has been regarded as the most important factor that influences severely human health. To decrease the rates of mobility and mortality of malignant tumor in the whole world has been a long way to go. For curing tumor, the work is heavy while the way is long. Now conventional radiotherapy mode (1.8-2 Gy/5 fractions/week) has been used for many years, but the cure rate of malignant tumor

with this mode is hard to be increased. Prognosis of cancer patients treated with radiotherapy is directly related to accumulated doses. According to radiation bioloGy, the dose, by which tumor cells are cured, is obviously higher than accumulated dose in conventional radiotherapy mode. But it is not wise to increase accumulated dose by prolonging the cumulative time in conventional radiotherapy mode. Based on the view of biological effect, the extreme pertinent choice includes raising radiation dose at each fraction and shortening cumulative time. In this study, we studied the biological effect and effective therapeutical dosage by animal-transplanted tumor in high-dose radiotherapy.

## Materials and Methods

### *Experimental animals*

Fifty (SPF) BALB/c nu-nu, 18-20 g, 5-week-age, male healthy athymic mouse were purchased from Beijing Weitong Lihua Experimental Animal TechnoloGy Limited Company [animal certificate: SCXK (Beijing) 2007-0001] (In this study, consistent with ethical norms animal experiments). All the experimental procedures and animal maintenance confirmed to the strict guidelines of institutional animal ethics committee.

### *Preparation of bearing cancer animals*

#### *Inoculation of transplanted tumor*

Conventional culture human pancreatic carcinoma cell line (MIA PaCa-2). It was inoculated in RPMI 1640 medium (contain 10% calf serum) and cultured in an incubator with 37°C, 5% $CO_2$. In exponential phase of growth, it was added with normal saline to prepare 1 × $10^7$/mL cell suspension. Two BALB/c nu-nu athymic mice were inoculated subcutaneously 0.2 mL/mouse MIA PaCa-2 cell suspension to raise transplantation tumor. Inoculated mice were bred on the sterilized consple in the lamina flow room and observed for the transplanted tumor every day. Transplanted tumor formed after 2 weeks (Fig.1).

**Fig.1. Formation of subcutaneously transplantation tumor in athymic mouse.**

#### *Passage*

After about 2 weeks, athymic mice with subcutaneously transplantation tumor were executed. The transplantation tumor was cut into little pieces (1 mm × 1 mm × 1 mm), which were inoculated subcutaneously into the lateral of the right lower extremity of 48 BALB/c nu-nu athymic mice. The achievement ratio was 95.83% (46/48). Athymic mice were fed until the diameter of transplantation tumor reached up to 10 mm (Fig.2). We measured weekly the maximum anteroposterior diameter (b) and transverse diameter (a) of transplantation tumor using a sliding caliper. According to formula $T = a^2 b/2$, we calculated gross tumor volume.

**Fig.2. Inoculalation of subcutaneously transplantation tumor in athymic mice.**

### *Radiation therapy*

#### *Subgroup*

When athymic mice were fed until diameter of transplantation tumor reached up to 10 mm, they were irradiated. Inoculated athymic mice were randomly divided into 7 subgroups (6 mice/subgroup). After irradiated, they were continued to be fed and observed delayed growth.

#### *Radiation approach*

When transplantation tumor was irradiated by 6MV-X ray (SSD = 100 cm), athymic mice were fixed with self-made fixture and 1cm thick medical glycerin was applied on the tumor. The width of irradiation field was 1.5 cm. Irradiation procedure is hsown in Fig.3.

#### *Tumor growth observations*

After irradiation, athymic mice continued to be bred on the sterilized consple in the lamina flow room. We observed their general animation: weight, consciousness, activity, drink and food intake, etc. Whether cutaneous reaction surrounding the tumor, ulcer or necrosis, and metastasis occurred were observed. We measured weekly the maximum anteroposterior diameter (b) and transverse diameter (a) of transplantation tumor using the sliding caliper. According to the formula $T = a^2 b/2$, we calculated gross tumor volume.

(1) The average weight of the athymic mice in each group was compared to evaluate the change of their nutritional status.

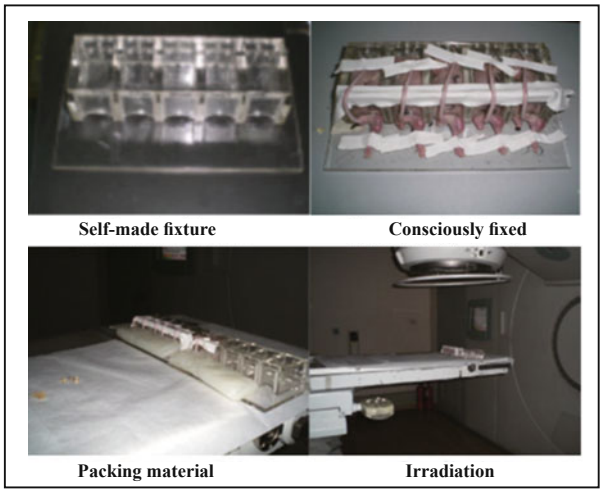


**Fig.3. Fixation and irradiation procedure of inoculated athymic mice.**

(2) According to measured average volume, tumor growth curve was drawn and then the different growth rate and tumor delayed growth time (tumor volume decreased first and then re-increased to original volume) among all groups were analyzed. The formulae which calculated the tumor volume inhibition rate and reduction rate were as follows:

Tumor volume inhibition rate= $[1 - (V_0 - V_i)_{\text{test group}} / (V_0 - V_i)_{\text{cntrol group}}] \times 100\%$

Tumor volume reduction rate = $(1 - V_0 / V_i) \times 100\%$

***Statistics analysis***

All measurement data showed as mean ± SD. SPSS11.5 was used, and the mean between the 2 sets was compared using pairing data for *t* test. Pairwise comparison for the mean among multiple sets used ANOVA. When $P < 0.05$, the differences were considered significant.

## Results

***Observation of changed weight***

The mouse's weight were measured weekly. Four weeks after irradiation, 1 mouse died of fight and it was excluded in statistics procedure. During the whole period, weight in each group trended to change at the same rate. At the second week and the fourth week, weight of the mice in certain group deviated from mean value and snapped back. The weight of the mice in the rest groups had no significant difference ($P > 0.05$).

***Observation of cutaneous reaction***

As irradiation dose in one fraction was raised step by step, cutaneous reaction surrounding the tumor presented primal moisture reaction with yellow effusion, and then was gradually aggravated. In high dose group (25, 35 Gy), the cutaneous reaction presented in all animals lasting for 2 weeks and then tumor volume was reduced obviously. In median dose group (10, 17 Gy), some showed cutaneous reaction, others demonstrated ulcer and necrosis. In low dose group (2, 5 Gy), cutaneous reaction was few. See typical appearance in Fig.4.

**Fig.4. Typical ulcer appearance of transplantation tumor in athymic mouse.**

***Observation of tumor volume***

Delayed tumor growth were seen in each experimental group, but tumor continued to grow in control group. Tumor growth in 2, 5 Gy group slowed down for 1-2 weeks and then soon turned back and even exceeded that in control group. In the group in which the mice received more than 10 Gy, delayed tumor growth was obviously and correlated to irradiated dose. Table 1 shows changed volume of tumor and mean value of the tumor volume in each group according to the measured data. As we compared the differences among each group, all groups were divided into 2 parts: one part included 0, 2, 5 Gy group, and the other included the rest gorups. The difference of the tumor volume was marked between the 2 parts ($P < 0.05$). Fig.5 shows the delayed tumor growth curve according to the measured data.

The time of delayed tumor growth was defined as the time when tumor volume decreased first and then re-increased to original volume. In 2, 5 Gy group, tumor volume did not decrease obviously. The time of delayed tumor growth lasted for 3 weeks, 6 weeks and more time separately in 10, 17 and 25 Gy group.

***Tumor volume inhibition rate and tumor volume reduction rate***

According to the measured data, we calculated tumor volume inhibition rate and tumor volume reduction rate which could directly reflect changed tendency of tumor volume. See below Table 2 and 3, and Fig.6 and 7.

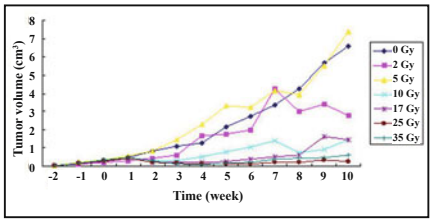


**Fig.5. Tumor growth curve of transplantation tumor in athymic mouse after different dose radiation in one fraction.**

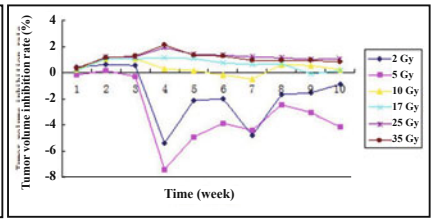


**Fig.6. Tumor volume inhibition rate of transplantation tumor in athymic mouse after different dose radiation in one fraction.**

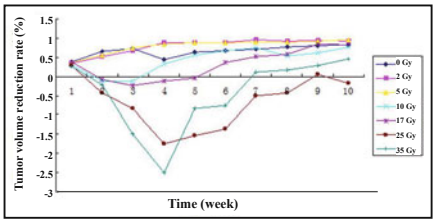


**Fig.7. Tumor volume reduction rate of transplantation tumor in athymic mouse after different dose radiation in one fraction.**

**Table 1. Changed tumor volume and mean value in each group according to the measured data (w: week, $cm^3$, $\bar{x} \pm s$).**

| Groups | 0 Gy | 2 Gy | 5 Gy | 10 Gy | 17 Gy | 25 Gy | 35 Gy |
|---|---|---|---|---|---|---|---|
| INOC 1 w | 0.04 ± 0.02 | 0.04 ± 0.02 | 0.05 ± 0.01 | 0.04 ± 0.03 | 0.03 ± 0.01 | 0.04 ± 0.03 | 0.05 ± 0.02 |
| INOC 2 w | 0.16 ± 0.07 | 0.13 ± 0.08 | 0.21 ± 0.04 | 0.15 ± 0.12 | 0.14 ± 0.05 | 0.18 ± 0.12 | 0.17 ± 0.07 |
| IR | 0.30 ± 0.13 | 0.21 ± 0.14 | 0.37 ± 0.12 | 0.35 ± 0.39 | 0.26 ± 0.09 | 0.33 ± 0.22 | 0.35 ± 0.13 |
| IR 1 w | 0.48 ± 0.20 | 0.32 ± 0.20 | 0.58 ± 0.20 | 0.50 ± 0.50 | 0.42 ± 0.11 | 0.46 ± 0.36 | 0.47 ± 0.17 |
| IR 2 w | 0.84 ± 0.43* | 0.44 ± 0.24 | 0.85 ± 0.51* | 0.31 ± 0.22 | 0.24 ± 0.06 | 0.23 ± 0.23 | 0.29 ± 0.17 |
| IR 3 w | 1.11 ± 0.68* | 0.62 ± 0.40 | 1.44 ± 0.87* | 0.31 ± 0.27 | 0.21 ± 0.06 | 0.18 ± 0.18 | 0.13 ± 0.05 |
| IR 4 w | 1.29 ± 0.89 | 1.68 ± 1.72* | 2.30 ± 1.66* | 0.52 ± 0.29 | 0.23 ± 0.15 | 0.12 ± 0.11 | 0.10 ± 0.15 |
| IR 5 w | 2.18 ± 1.49* | 1.79 ± 1.73* | 3.32 ± 2.71* | 0.78 ± 0.44 | 0.25 ± 0.19 | 0.13 ± 0.15 | 0.19 ± 0.34 |
| IR 6 w | 2.74 ± 1.78* | 1.98 ± 1.52* | 3.23 ± 2.50* | 1.07 ± 0.62 | 0.41 ± 0.28 | 0.14 ± 0.17 | 0.20 ± 0.35 |
| IR 7 w | 3.36 ± 2.19* | 4.26 ± 3.76* | 4.15 ± 2.57* | 1.41 ± 0.77 | 0.53 ± 0.39 | 0.22 ± 0.27 | 0.39 ± 0.77 |
| IR 8 w | 4.24 ± 2.52* | 2.99 ± 2.26* | 3.93 ± 2.62* | 0.74 ± 0.51 | 0.62 ± 0.55 | 0.23 ± 0.23 | 0.42 ± 0.84 |
| IR 9 w | 5.67 ± 3.69* | 3.41 ± 3.74* | 5.51 ± 3.38* | 0.91 ± 0.73 | 1.63 ± 2.10 | 0.35 ± 0.25 | 0.49 ± 0.94 |
| IR 10 w | 6.57 ± 4.28* | 2.80 ± 2.13 | 7.40 ± 4.34* | 1.47 ± 1.84 | 1.44 ± 1.27 | 0.28 ± 0.19 | 0.64 ± 1.13 |

*, marked difference among the group and other corresponding groups at some time; INOC, Inoculation; IR, irradiation therapy.

**Table 2. Data about tumor volume inhibition rate of transplantation tumor in athymic mouse after different dose radiation in one fraction (w: week).**

| Groups | 1 w | 2 w | 3 w | 4 w | 5 w | 6 w | 7 w | 8 w | 9 w | 10 w |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 Gy | 0.39 | 0.58 | 0.50 | -5.39 | -2.16 | -2.00 | -4.80 | -1.70 | -1.56 | -0.89 |
| 5 Gy | -0.17 | 0.13 | -0.30 | -7.39 | -4.90 | -3.85 | -4.41 | -2.46 | -3.11 | -4.13 |
| 10 Gy | 0.17 | 1.07 | 1.05 | 0.26 | 0.14 | -0.22 | -0.51 | 0.62 | 0.55 | 0.18 |
| 17 Gy | 0.11 | 1.04 | 1.06 | 1.13 | 1.02 | 0.75 | 0.61 | 0.65 | -0.10 | 0.14 |
| 25 Gy | 0.28 | 1.18 | 1.18 | 1.91 | 1.40 | 1.32 | 1.16 | 1.10 | 0.98 | 1.04 |
| 35 Gy | 0.33 | 1.11 | 1.26 | 2.09 | 1.32 | 1.25 | 0.94 | 0.93 | 0.89 | 0.79 |

**Table 3. Data about tumor volume reduction rate of transplantation tumor in athymic mouse after different dose radiation in one fraction (w: week).**

| Groups | 1 w | 2 w | 3 w | 4 w | 5 w | 6 w | 7 w | 8 w | 9 w | 10 w |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 Gy | 0.38 | 0.65 | 0.73 | 0.43 | 0.63 | 0.66 | 0.7 | 0.77 | 0.81 | 0.82 |
| 2 Gy | 0.34 | 0.52 | 0.66 | 0.88 | 0.88 | 0.89 | 0.95 | 0.93 | 0.94 | 0.93 |
| 5 Gy | 0.36 | 0.56 | 0.74 | 0.84 | 0.89 | 0.89 | 0.91 | 0.91 | 0.93 | 0.95 |
| 10 Gy | 0.3 | -0.13 | -0.13 | 0.33 | 0.55 | 0.67 | 0.75 | 0.53 | 0.62 | 0.76 |
| 17 Gy | 0.38 | -0.08 | -0.24 | -0.13 | -0.04 | 0.37 | 0.51 | 0.58 | 0.84 | 0.82 |
| 25 Gy | 0.28 | -0.43 | -0.83 | -1.75 | -1.54 | -1.36 | -0.5 | -0.43 | 0.06 | -0.18 |
| 35 Gy | 0.26 | -0.21 | -1.5 | -2.5 | -0.84 | -0.75 | 0.1 | 0.17 | 0.29 | 0.45 |

## Discussion

Now conventional radiotherapy mode (1.8-2 Gy/5f/w) has been used for many years. The cure rate of tumor is only 18% and hard to get marked breakthrough. Because the conventional radiotherapy is based on 2D image to define and treat the gross target volume (GTV) of tumor, which includes much normal tissue, fractionated dose and accumulated dose are limited. This mode results in lower biological effect, poorer local control rate and serious side effects (45%-70%)[1].

Accumulated dose is directly related to prognosis. According to radiation bioloGy, the dose by which tumor is cured is obviously higher than accumulated dose of conventional radiotherapy mode at present. For example, it is calculated based on Martel's clinical study of increasing radiation dose progressively that under conventional radiotherapy mode (1.8-2 Gy/5f/w) 50%, 60%, 70% and 84% 30-months-no-recurrence-survival-rate of non small cell lung cancer required in abstracto respectively for 84.5, 90, 100, 110 Gy[2]. Now non small cell lung cancer only gets 60 Gy that is hard to cure the cancer.

At invariable accumulated dose, prolongation of total therapeutic time is not a proper way to get supposed biological effective dose (BED). Elevation of fractionated dose is an effective and safe way. As repopulation in the following course of the treatment (total therapeutic time exceed 30 days), accumulated dose will reduce 0.6-1 Gy and survival rate of the patients will decrease 1.6% when therapeutic time is prolonged for 1 day[3,4]. Therefore, it is not wise to elevate the accumulated dose by prolonging total therapeutic time in the conventional radiation therapy. Based on the biological availability, elevating fractionated dose and reducing total therapeutic time may be the most pertinent way to increase biological effects in radiotherapy.

BED is defined as the measurement of actual extent of biological radiation reaction. It is commonly used in clinic to evaluate the post-treatment effects of radiotherapy. Physical dosage is another distinct concept. According to Fowler's formula[4], fractionated dose is higher,, and results in biological effect increased, especially in late reaction tissue. For example, 70% physical dosage (70 cGy) can convert to biological dosage of 74.2 cGy and 50% physical dosage can convert to biological dosage of 40.5 cGy when 100 cGy is irradiated.

Hypofractionation radiotherapy mode[5-10] can concentrate high dose to tumor volume and cause mild damage to normal tissue. It has some obvious advantages: shortening total therapeutic time from 6-7 weeks to less than 2 weeks, no tumor cell accelerating re-increment as treatment finished in short time, no dosage waste, elevation of BED. Above advantages are difficult to get in conventional radiotherapy mode because it includes much normal tissue in GTV. Newly modern radiation therapy technology, especially stereo comfort technic, developed. But the injury caused by late reaction is increased in conventional radiotherapy mode, therefore, the relationship between dosage and biological effects is needed to further research. In conclusion, it is believed that biological effects of radiotherapy in cancer treatment will be much improved in the near future.

It is by animal experiment that can provide more actual conclusion closely to clinical practice. Cancers under control directly correlate to biological effect. BED can be calculated by linear quadric- equations: BED = d* n (1＋d/α/β), α/β = 10. It has clinical significance to evaluate dosage-effect relationship. BED is significantly related with the local control rate and the survival rate. In literature[11,12], when BED ≥ 100 Gy, the local control rate and the survival rate of the patients are higher than those when BED < 100 Gy. However, hhe local control rate and the survival rate are no longer to elevate when BED > 140 Gy. For small size of tumor (< 4 cm), local control rate is over 90% when BED > 100 Gy. Ideal BED is between 100Gy and 130 Gy. Whether or not elevate BED, depends on fractionated dose.

We calculated the irradiated dosage for each group according to formula BED = dn (1 + d/α/β). Comparison of physical dose and biological dose is shown in Table 4.

According to table 4, we can predict clearly the difference in biological effects among each group. When physical fractional dose is less than 17 Gy in hypofactionation radiotherapy, biological effects was obviously weaker than conventional radiotherapy. 17 Gy in one fraction is a reversal point. Because its biological dose (physical dose 17 Gy) is close to accumulated dose in clinical conventional radiotherapy, and its biological effects are supposed to be the same as clinical radical effects. According to the literature, clinical effects will continue to elevate in the groups which the dosage is higher than 17 Gy.

The findings in our study are coincident with the prediction mentioned above. During the whole experiment, each animal grew well. Changed weight had no significant difference. After irradiation, tumor grew slowly in each group. Tumor growth rate in 2, 5 Gy group decreased for 1-2 weeks and then increased quickly and even exceeded that in the control group.

**Table 4. Comparison of physical dose and biological dose in each group according to formula BED = dn (1 + d/α/β).**

| physical dose | 0Gy | 2 Gy | 5 Gy | 10 Gy | 17 Gy | 25 Gy | 35 Gy |
|---|---|---|---|---|---|---|---|
| biological dose | 0 Gy | 2.4 Gy | 7.5 Gy | 20 Gy | 45.9 Gy | 87.5 Gy | 157.5 Gy |

Cellular radiation damage includes 3 subtypes: sublethal damage, latent lethal damage and lethal damage. Only lethal damage can completely deprive the reproductive capacity of the irradiated cells. It is not prothetic or retrieve, and is inconvertible. Cellular damage shows the processes of apoptosis and necrosis. Target of cellular radiation damage is DNA. DNA damage includes basio-damage, single-strand break and double-strand break. After irradiated 1-2 Gy, DNA damage in one cell can be detected immediately. As estimated, it included more than 1000 basio-damages, 1000 single-strand DNA breaks and 40 double-strand DNA breaks. Most of DNA damage can be repaired successfully. Certain amount of double-strand break is the key to cellular lethal damage. Because lethal cellular damage in 2, 5 Gy group was little and the most rest was repaired rapidly. Only temporary slow down of tumor growth is shown after irradiation. For a while, as absence of reproductive cells, cellular repopulation effect is switched on. Therefore, tumor cells grow up more quickly than before the irradiation.

In higher than 10 Gy group in our study, delayed tumor growth is obviously and correlates to irradiated dose. The time of delayed tumor growth was 3 weeks, 6 weeks and more time separately in 10, 17 and 25 Gy group. Tumor volume inhibition rate and tumor volume reduction rate directly reflected the tendency of changed tumor volume. It was illustrated that in 10 Gy group, the lethal damage post irradiation was increased markedly, but part of reproductive cells remained. In 17 Gy group, lethal damage continued to increase, and cellular repopulation kept on decreasing. In 25, 35 Gy groups, this tendency was more obvious. But the difference between the 2 groups was minified. The tendency of tumor growth curve showed that biological effects were increased gradually following elevation of single dose. Following elevation of single dose, the number of living cells decreased and cellular apoptosis correlated to increased lethal damage. Cellular apoptosis played a part in cell damage less and less. It was gradually substituted by quick, intense and dissolvable oncosis. Remnant living cells still continued the process of apoptosis for a while because of lethal damage. Tumor volume continued to contact.

In observation, cutaneous reaction surrounding the tumor, showing the moisture reaction with yellow effusion at first, gradually aggravated following elevation of single dose. At the same time, tumor volume continued to decrease. In low dose group (2, 5 Gy), cutaneous reaction was few. It was caused by necrosis induced by quick tumor growth.

Similar conclusions can be seen in literatures[13]. Walsh et al.[14] irradiated A498 renal carcinoma transplantation tumor with fractionated dose of 48 Gy (16 Gy × 3f, 1f/W). It showed that the tumor volume kept on decreasing and the cellular change was obvious .

Lotan et al.[15] irradiated C4-2 prostatic carcinoma transplantation tumor by accumulated dose 15, 22.5, 45 Gy (3f, 1f/W). Results showed that tumor responsed effectively to higher dose. Tumor volume in 15, 25 Gy group contracted respectively to 58%, 90%, but repopulation occurred after 1 week. Mean tumor volume in 45 Gy group contracted more than 90% and remained for more than 1 month. Ding et al.[16] established a model of rat CBRH-3 hepatoma carcinoma cell and irradiated randomly in one fraction by 0, 16, 24, 32 and 40 Gy. It showed that after 24 h tumor volume contracted, which directly correlated to radiation dose. It was coincident with our results that tumor growth was restrained obviously by over 15 Gy in one fraction.

In conclusion, good biological effects are aquired when tumor is irradiated by more than 10 Gy in one fraction. In 25 Gy group, treatment effect of the patients with conventional radiotherapy can be seen. It is not significant when dose continues to rise. Hypofractionation radiotherapy mode itself had many shortcomings. Following with technological development, hypofractionation radiotherapy mode come really true. Dose in every fraction and biological dose can rise so as to obviously improve clinical effects.

## Conflict of interest statement

No potential conflicts of interest were disclosed.

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