OVEREXPRESSION OF p53, MDM2 PROTEINS IN SOME ATR RADIATION-IMDUCED SKIN ULCERS*

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ABSTRACT An animal model of radiation-induced skin ulcer was set up with 140 rats, which were locally irradiated with 35~55Gy γ-rays. The pathological changes were observed for 1 year. Immunohistochemical studies were performed in 72 rat radiation skin ulcer specimens using anti-p53 and anti-MDM2 protein polyclonal antibodies. The results showed that the positive rate for overexpression of p53 protein was 9.7%, and for that of MDM2 was 19.4%. The overexpression of p53 was mainly seen in the nuclei of activated squamous epithelial cells, and in fibroblasts, endotheliocytes in deeper part of the skin ulcers. The overexpression of MDM2 had the same localizations. It's suggested that the changes of p53 and MDM2, genes and proteins, may be related to the cancer transformation and poor healing of radiation-induced skin ulcers.

KEYWORDS Rat, Radiation skin ulcer, p53, MDM2

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1 Introduction

Radiation-induced skin ulcer is often seen and often complicated with radiation therapy of tumors. It is characterized by poor healing, stubborn relapse, and carcinogenesis. Only a few reports were found about the changes of genes and their products in radiation-induced skin ulcers. Previously we reported that there were overexpression of c-erbB-2 and p21 genes, and an elevation of telomerase activity in chronic radiation ulcer of human skin[1]. The MDM2 gene is located on human chromosome 12q13-14. Its product MDM2 protein has the function of binding p53 protein and inhibiting the function of wild-type p53. The alteration of MDM2 gene was associated with most of connective tissue tumors. Meanwhile, mutational p53 gene seems to play a key role in the process of carcinogenesis[2]. In the present study we have set up an animal model of radiation-induced skin ulcer with rats, and studied the changes and significance of p53

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and MDM2 genes in radiation-induced skin injuries.

2 Materials and methods

Altogether 140 female Wistar rats were locally irradiated with 35Gy (60 rats), 45Gy (40 rats), and 55 Gy (40 rats) ^{60}Co γ rays. The body weight of the rats was 250±10g. The irradiation field was focused on the buttocks, hind legs and tail, and the irradiation area was about 21cm^2. The rats were irradiated single-dosely, and the was 4.612Gy/min. There were 10 unirradiated normal rats served as controls. The pathological changes were observed for 1 year, and skin ulcer tissues were harvested at regular intervals. The specimens were formalin-PBS fixed, paraffin embedded, then the sections 4μm in thickness were placed onto poly-l-lysine-coated glass slides. H.E staining and immunohistochemistry were performed to detect the expression of p53 and MDM2 genes.

Methods for immunohistochemistry: Specimen sections were deparaffinized, and rehydrated before incubation for 30 min with 0.3% hydrogen peroxide-methanol to inhibit exogenous peroxidase. After treatment for retrieving antigens in a microwave oven[3] and in blocking serum to reduce any nonspecific binding of conjugated second antibody, the samples were incubated overnight at 4°C with anti-p53 polyclonal antibody (goat, Santa Cruz Biotechnology, California, USA), and anti-MDM2 polyclonal antibody (rabbit, Santa Cruz Biotechnology, California, USA) at a dilution of 1:100 following the steps indicated in the SP kit (Zymed, San Francisco, USA). After washing with PBS, immunohistochemical staining was carried out using Zymed histostatin-SP kit. The reaction products were visualized by immersing the slides in diamine-benzidine tetrachloride (DAB) and finally counterstaining with Mayer's hermetoxylin. The overexpression of the two gene products was evaluated in the area with maximum of staining, and the cytoplasmic and nuclear staining was recorded. Related positive tumors (stomach cancer) served as positive controls, and normal skin tissue was used for normal control.

3 Results

3.1 Pathological changes of rat radiation skin ulcers

After irradiation, all of the irradiated skin was injured. Skin swelling and edema (8 days after irradiation), erosion and trichomadeis (12 days after irradiation) could be seen in some areas of the irradiation field. On 12 to 14 days after irradiation, small skin ulcers occurred in all of theirradiated rats, but obvious infection was not seen. The ulcers got bigger and deeper by days past, meanwhile tissue swelling and edema became lighter, and ulcers were complicated with infection gradually. 4 months after irradiation, 63% of the hind legs were broken or lost due to big and deep ulcers, 21% ulcer healed (small and shallow ulcers, 1~7mm in diameter, healed comparatively easily), 33% of the healed ulcers relapsed 6 months after irradiation. 54 of the irradiated rats died of exhaustion, and all of the rats were sacrificed 1 year after irradiation, and their skin ulcer specimens were harvested.

By microscopy, the basic pathological changes of rat radiation skin ulcer were as follows: (1) Necrosis and bleeding. Necrosis was observed in the irradiated squamous

epithelial cells and fibroblasts in dermis in the early period (20 days) after irradiation. Bleeding occurred in the deeper part of skin ulcers, where the endothelial cells of small blood vessels might drop off , and the structure of the wall of blood vessels was damaged in different extents. (2) Collagen fibers in dermis appeared swelling and denatured. And the leiomyocytes in media of the arterioles began to proliferate, while the wall of the arterioles became thicker 5 months after irradiation. (3) Muscle cell atrophy occurred in deeper part of the skin ulcers. The irradiated epithelial cells were found atrophic too. (4) Many neutrophils, macrophages, and a few lymphocytes were exuded into the skin ulcer tissues. (5) Repair and regeneration including the proliferation and migration of epithelial cells, regeneration of hair follicles and sebaceous follicles occurred. Fibroblasts migrated and accumulated in the deeper part of the ulcers, sometimes several newly formed capillaries may be seen. But the repair process was not active, compared with the normal wound healing. (6) Cell injury. Large, stellate "radiation fibroblasts" were present[4] (Fig.1). (7) No tumorigenesis was found.

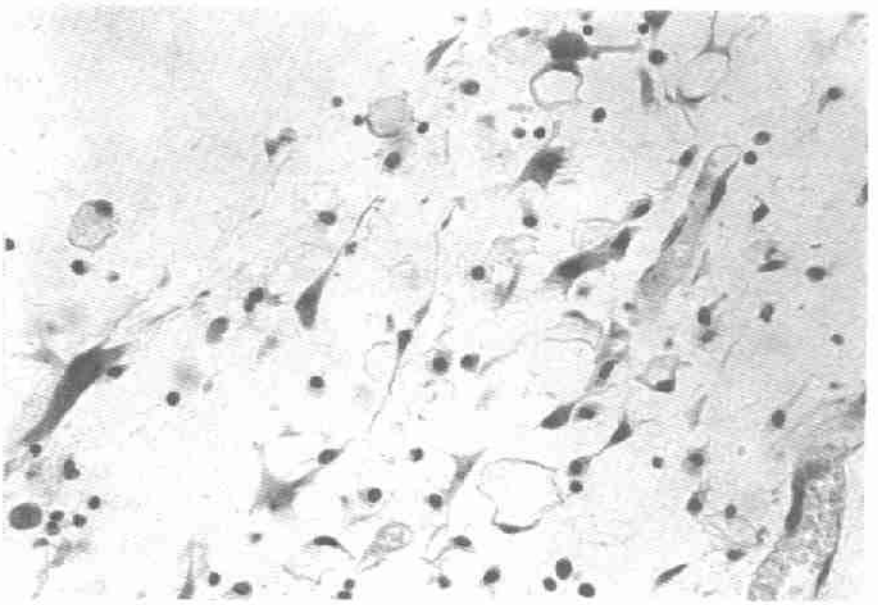

Fig.1 Large, stellate "radiation fibroblasts" were present in the deeper part of radiation-induced skin ulcer (by H.E. staining, ×400)

3.2 Overexpression of p53 and MDM2

p53 gene was overexpressed in the skin ulcer tissues in 4 of 44 rats (9.1%) which were sacrificed or died from 1 to 6 months after irradiation. But out of 28 rats which were sacrificed or died from 6 to 12 months after irradiation, 3 cases were positive (10.7%). The total positive rate was 9.7% (7/72). The positive sites of overexpression were mainly observed in the nuclei of activated squamous epithelial cells in skin ulcers of 5 rats (Fig.2), and in the nuclei of fibroblasts in skin ulcers of 2 rats. The positive areas were stained in

a brown-yellow color. Other cells in the irradiated skin ulcers were negative as the normal tissue of the normal control group. Three cases of stomach cancers were all positive.

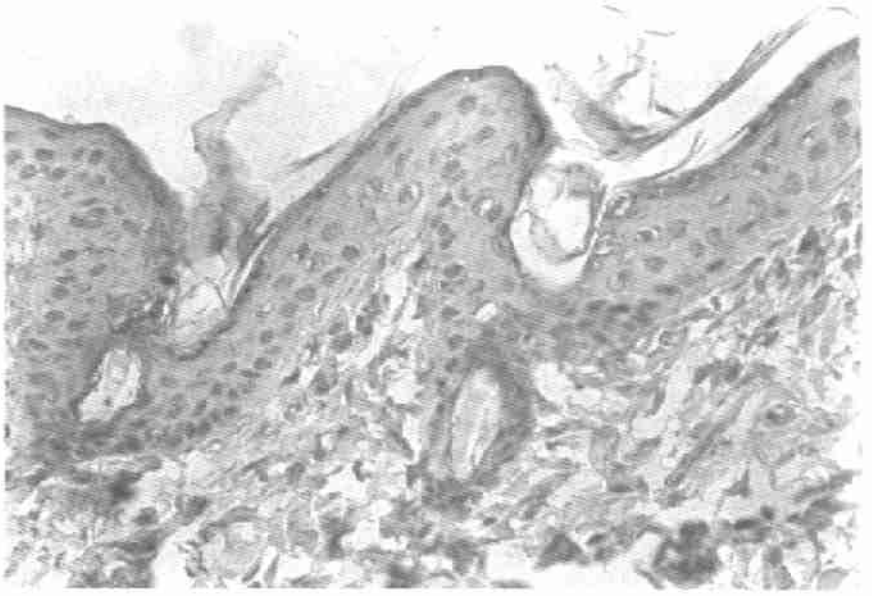

Fig.2 P53 positive in the nuclei of activated squamous epithelial cells in rat radiation skin ulcers (by immunohisto-staining, ×400)

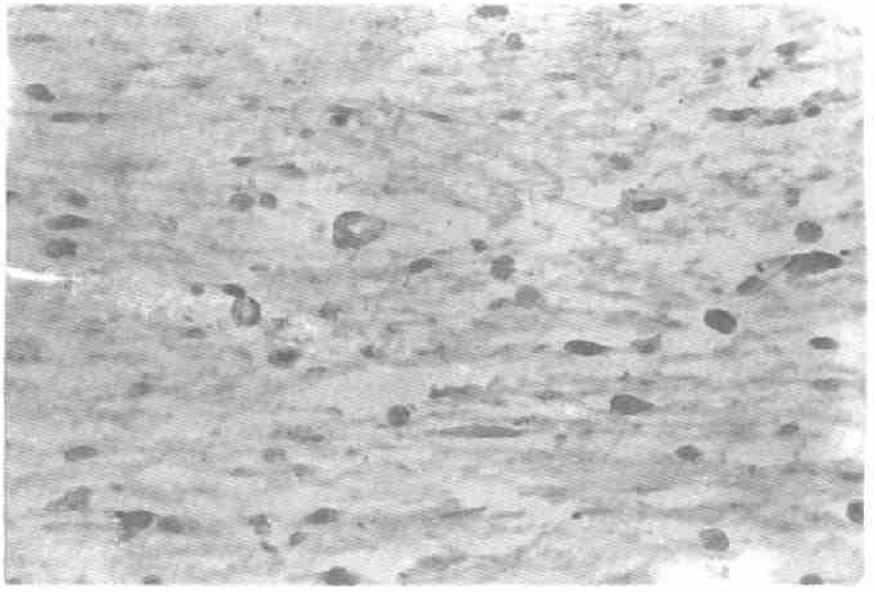

Fig.3 MDM2 positive in the nuclei and cytoplasm of fibroblasts, endotheliocytes in deeper part tissues of rat radiation skin ulcer (by immunohisto-staining, ×400)

The overexpression rate of MDM2 was 18.2% (8/44 rats sacrificed or died from 1 to 6 months after irradiation) and 21.4% (6/28 rats sacrificed or died from 6 to 12 months after irradiation), and the overall positive rate was 19.4% (14/72). The positive sites were seen in the nuclei of activated squamous epithelial cells in 4 rat skin ulcers, and in the nuclei and cytoplasm of fibroblasts, endotheliocytes in the deeper part of skin ulcers in 12 rats (in 2 cases epithelial cells, fibroblasts and endotheliocytes were all positive) (Fig.3). But MDM2 was negative in the 7 cases in which p53 protein was positive. 2 of 3 cases of stomach cancers were positive. Other controls were negative.

4 Discussion

Research on structure and expression of p53 and MDM2 genes has become one of focuses in the field of oncology[2,5,6] but few reports about changes of the 2 genes were found in radiation skin ulcer researches. Po Zhao et al in our laboratory detected some human radiation skin ulcer samples using anti-p53 and anti-MDM2 antibodies. And their results showed that the positive rates were 8% and 36% respectively[7]. We have established a fairly typical animal model with radiation skin ulcer by local irradiation in Wistar rats, the pathological changes of which were very similar to those of human radiation skin ulcers. In this study we detected the expression of p53 and MDM2 genes in these samples, and found that the overexpression rates were 9.7% and 19.4%, respectively. Our results further supported that both p53 and MDM2 genes may be altered by radiation damage, and lead to some extent of overexpression.

Overexpression of p53 protein may be due to the mutation of p53 gene or declination of its degradation while combining to oncoproteins. Our results showed that the overexpression rate of p53 was 9.7%, which means that the abnormal overexpression of p53 does not occur often but incidentally in radiation skin ulcer. Because there is a difference between the mutation rate of p53 gene and its protein expression level[8], we consider that it is involved in the pathogenesis of radiation skin cancer. Gene analysis should be made to determine the mutation rate of p53 gene in radiation skin ulcers. The results also showed the rate of overexpression of MDM2 was 19.4%, which implicated a radiation-induced high level of MDM2 gene amplification leading to a high level of protein expression. MDM2 may also be involved in the molecular mechanism of cancer transformation in radiation skin ulcer, mainly by blocking the direct or indirect oncogenic effects of retro-activation induced by wild type p53 protein. Overexpression of MDM2 in fibroblasts may be correlated with the formation of radiation fibroblastoma, but needs more evidence.

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大鼠放射性皮肤溃疡组织中 p53, MDM2 蛋白高表达病理研究

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摘要 研究大鼠放射性皮肤溃疡组织中 p53, MDM2 表达。采用 140 只 Wistar 大鼠进行局部照射制备放射性皮肤溃疡动物模型，观察病变一年，然后采用 p53(Goat), MDM2(兔)多克隆抗体和 SP 免疫组化方法检测皮肤溃疡组织中 p53, MDM2 的表达情况。结果表明，p53, MDM2 的高表达阳性率分别为 9.7%(7/72) 、19.4%(14/72), 两者阳性部位主要见于增生肥大的鳞状上皮细胞核、间质成纤维细胞及血管内皮细胞核。 p53, MDM2 基因的变化及蛋白的高表达可能与放射性皮肤溃疡癌变及不愈合有关。

关键词 大鼠，放射性皮肤溃疡， p53, MDM2

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