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• *REVIEW* •

Current status of tumor radiogenic therapy

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Abstract

Although tumor gene therapy falls behind its clinical use, the combination of irradiation and gene therapy is full of promise in cancer therapy based on traditional radiotherapy, chemotherapy and surgery. We have termed it as radiogenic therapy. This review focuses on the following aspects of radiogenic therapy in recent years: improvement of gene transfer efficiency by irradiation, radiotherapy combined with cytokine gene delivery or enhancement of the immunity of tumor cells by transgene, direct stimulation by radiation to produce cytotoxic agents, increase of tumor cell radiosensitivity in gene therapy by controlling the radiosensitivity genes and adjusting the fraction dose and interval of radiation so as to achieve the optimum antitumor effect while reducing the normal tissue damage, radioprotective gene therapy enhancing radiation tumor killing effect while protecting the normal tissue and organs with transgene using transfer vectors.

Key words: Tumor; Radiation; Gene therapy

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INTRODUCTION

Among the therapies for malignant tumor, the main disease threatening human health, traditional radiotherapy, chemotherapy and surgery still dominate at present. Nevertheless, because the reaction of tumor cells is much severer to chemotherapy than to radiotherapy, the later is so vital to the therapy of tumors. During the past decade, the development in new radiotherapy technologies, such as modern radiotherapy equipment and tumor-shaped stereoadaptability treatment, enables more patients to accept radiotherapy. The therapeutic effect has been improved as well. On the other hand, radiotherapy still faces difficulties, such as recurrence or metastasis after radiation, damage of normal tissues around the tumor. Although the history of tumor gene therapy is not as long as radiotherapy, it has shown some promising results in many *in vitro* experiments. Because therapy of tumors covers many aspects and is quite difficult, gene therapy has not made any breakthrough and falls far behind its clinical application. Molecular biology has provided a theoretical basis for tumor radiotherapy at molecular level, and a breakthrough will be made in increasing the radiation sensitivity and decreasing the damage to normal tissues. Gene therapy technology is also introduced into radiotherapy. How to organically combine gene therapy and radiotherapy has become the new topic in these fields.

RADIATION IMPROVES GENE TRANSFER EFFICIENCY

In 1980s, Perez and Skarsgard[1] successfully used X-ray, ultraviolet radiation and 144 keV/μm of argon ion to increase DNA-mediated gene transfer efficiency, and found that the heavy ion was more noticeable in enhancing transfer efficiency and this enhancement was related to the radiation dose. It was also found that radiation could contribute to the conformity of exogenous DNA and host DNA, as well as to the expression of transferred genes and the expression time was also longer than that with no radiation. Stevens *et al*[2], found that 9 Gy of γ-ray radiation could increase the initial transfection efficiency of DNA mediated by plasmid vectors to 1 400 times. The copies of dissociated plasmid in cells from the γ-ray radiated group were 50% of those from the non-radiated group, but the cell quantity with conformed plasmids was much more than that from non-radiated group, and also the transfection efficiency of linear DNA was higher than that of ring DNA. Zeng *et al*[3], also found that low radiation dose of 3 Gy could increase the Ad5-CMVlacZ-mediated gene transfer efficiency to 40 times. Tang *et al*[4], used adenovirus vectors (AdCMVluc) to infect lung cancer cells of mice radiated by γ-ray, and found that the luc gene encoding products inside a cell increased in a dose-dependent manner, and the efficiency could increase as high as 24 times, which effectively controlled the tumor growth. The mechanism of ion radiation-mediated gene transfer might be as follows. Radiation makes receptors on cell surface damage and perforate, which change both transit of cytomembrane and its electrical level so that the exogenous DNA with negative electricity can go into the cells. The radiation results in DNA damage of cells and activation of their restoration, so the exogenous DNA and host cells DNA can be recombined and conformed[5]. In the later researches,

all the DNA base analogs, H_2O_2, ultraviolet radiation, X-ray, heavy ion had similar effects, indicating that cytotoxin or radiotherapy could make the exogenous DNA go into host cells easily. In addition, similar to the physically electrical perforation-mediated gene transfer, there is no cell specificity for the radiation-mediated gene transfer.

IMMUNE GENE THERAPY AND RADIOTHERAPY OF TUMORS

Human tumor immunity is mostly weak and easy to escape from body's immune system monitoring. Additionally, tumor cells can produce various immuno-suppressive factors so as to suppress the host cellular and humoral immunity. In aid of the molecular biology technology, some immunity-relevant factor genes are transferred to cells and expressed in the body constantly or in tumor cells to stimulate the host immune system or enhance the immunity of tumor cells so that the body immune system can recognize them and take certain killing actions. The combination of immune gene therapy and radiotherapy in tumor treatment can theoretically enhance antitumor immune effect while providing a quick external killing radiotherapy. This kind of therapy can obtain a better result than any single therapy as mentioned above. The facts also proved that clinical use of single cytokines for tumor therapy is not only expensive, but also brings severe toxic and side effects in a large dose, and most clinical applications did not achieve good results[6,7].

Radiotherapy combined with IL-2, INF, TNF cytokines, has obtained preferable antitumor effects. The gene expression of cytokines induced by radiation was first reported by Hallahan *et al*[8], and Sherman *et al*[9]. They found that radiation not only could make DNA damage but also might be related to the tumor killing effect caused by the increase of cytokine secretion. After plasmid pEgr-TNF transfected human tumor cells HL525, Weichselbaum *et al*[10], injected it into squamous cancer cell line SQ-20B of nude mice combined with 20 Gy of radiation. The outcome showed that, in the simple radiotherapy group, the tumor itself shrank by 1.1% on the 36th d and on the 50th d all the other nude mice died except one (1/7); whereas in the combined therapy group, all the other tumors in nude mice were completely vanished on the 20th d, except recurrence in only one (1/7) on the 36th d. It indicates that the combination of TNF and radiotherapy can achieve better therapeutic effects than either one alone. As indicated in some animal experiments[11], radiation combined with Ad-EGR-TNF α vector was carried on human glioma cell line D54, and resulted in complete tumor regression in 71% of xenografts. Its histopathological results showed pronounced vessel thrombosis and tumor necrosis, but no obvious effect was found on the live D54 cells after being treated with TNF α or radiation. Raben *et al*[12], proved that expression of human carcino-embryonic antigen (CEA) could be induced on D54MG human glioma cell line infected with adenovirus vector with CEA gene *in vitro*, and could be recognized by COL-1 antibody of CEA marked by radionuclides. In China, Zhang and Cao[13] and Wei *et al*[14], used adenovirus vectors co-expressing the heterodimer of human IL-12 combined radiation to treat mice with liver cancer. Compared with the methods mentioned above, this combination achieved complete disappearance of 50% cancers. If the mice in combined therapy group were inoculated again with tumor cells, tumor would not form anyway. Moreover, the INF-γ level was notably increased in mice blood serum from the combined group, and induced specific and restricted T cell activity.

On the basis of large experiments *in vitro* and *in vivo*, TNF was also applied to some clinical therapies for solid tumors, such as breast cancer, lung cancer, rectal cancer, pancreatic cancer, melanoma, head and neck tumors. No obvious effect of simplex radiotherapy or chemotherapy was found. During phase I clinical experiment, Hanna *et al*[15], observed seven patients who received Ad-EGR-TNFα injection at doses of 4×10^{9}-$4\times10^{9.5}$ particle units with concurrent radiotherapy. Of the seven patients, two had complete responses, two partial responses, two minor shrinkage and one no change. Meanwhile, the tumors which accepted radiotherapy only at the same dose were not controlled. In the above experiment, complete responses occurred in breast and rectum tumors and the partial responses were observed in pancreatic and lung cancers. There were no obvious toxic and side effects except pain on the injection site and slight chills. No virus was detected in blood or urine from any of the patients. All these have shown the importance, effectiveness and safety of radiogenic therapy.

GENE THERAPY OF TUMORS INDUCED BY RADIATION

The safety and effectiveness of gene therapy of tumors are the key factors in gene therapy, and its control mechanism affects the targets of gene therapy. At present there are two types of control mechanisms, one is called "transcriptional target control", which means to choose tumor-related antigens. For example, some cis-effect elements (such as promoter, enhancer) of AFP and CEA genes are formed into expression boxes with their corresponding target genes, and then are inserted into gene transferring vectors. In this case, the transgene only expresses in tumor cells that produce tumor-related proteins as mentioned above, thereby it can bring a specific killing effect to tumor cells. The other is called "exogenous control of transgene expression", which means to use the cis-effect elements, that can be induced by some factors to express genes, are formed into expression boxes with corresponding target genes and then are inserted into gene transferring vectors. In this case, the transgene will be directly controlled by corresponding inducible factors whether it expresses in the body or not. Among the first genes that were induced by radiation, the early growth gene (EGR)[8] is related to the early growing reaction, c-Jun, β-actin and interleukin-1[16]. These gene encodings can bind to the specific DNA sequences and control other gene expressions. For instance, by means of increasing transcriptional induction of the expression of c-Jun by ionizing, Jun protein could combine DNA at Ap-1 site and activate the downstream gene expression. The vectors constructed by radiation-induced EGR-1 gene and different therapeutic genes have been studied most. EGR-1 encodes a nuclear phosphoprotein containing 533 amino acids. The active oxygen generated

by ionizing can act on the CC (A+T rich) 6GG structural domain of this gene's promoter and promote EGR-1 gene expression[8].

Kawashita *et al*[17], constructed the plasmid vectors, pEGR-TK and pEGR-luc, which meant to insert herpes simplex virus thymidine kinase (HSV-TK) gene or report gene luc into the downstream of early growing gene (EGR-1) promoter. On the next day after the liver cancer cell line was transfected by these two plasmid vectors, 10 Gy of radiation was given and prodrug ganciclovir (GCV) was added as well. As shown in the final results, after transfected by pEGR-luc, the luc gene expression in radiated cells was 15-28 times of that in non-radiated cells. Additionally, the expression level was dependent on the radiation dose. The drug sensitivity of liver cancer cell line transfected by pEGR-TK and radiation to GCV increased by 10^3-10^4 compared with the non-radiated group. The therapeutic effects were good on other cancer cell lines when the vector contained EGR-1 and HSV-TK. Scott *et al*[18], and Marples *et al*[19], designed a type of molecular switch with a recombination system of bacteriophage to establish a radiation-induced gene expression model. EGR-1 enhancer/promoter regulated the expression of Cre recombinase. Through the recombination of loxP site-mediation, the molecule switch was activated and turned on, then was given 1 Gy of X-ray radiation to achieve the goal of completely suppressing the growth of tumor cells. The research on EGR-1 promoted other studies on radiation inducible promoters, such as P21 (WAF1)[20]. Worthington *et al*[20], used WAF1 as a promoter to construct a vector with NO synthetic enzyme gene, and then injected it into the rat tail artery. They found the quantity of nitric oxide synthase increased by four times after a radiation dose of 4 Gy was given.

The study on applying radiotracer combined with the gene therapy, to the tumor targeted diagnosis and therapy has made many progresses. The radiation-inducible gene encodes a type of ligands or transits agents, which can be taken as a target to cytotoxins radiolabeled. The functions are as follows: one is to use the new cytomembrane receptors induced by irradiation for receptor development, the other is to use the transgene products to metabolize the specific substances radiolabeled, and to retain the metabolism of products inside the cells for position display and target study. Tjuvajev *et al*[21], transfected tumor cells by Ad-HSV-TK and then injected a therapeutic dose of FIAU radiolabeled with radionuclide I^{131}, finally the transferring target was improved. Meanwhile, β-ray emitted by ^{131}I-FIAU was incorporated to DNA and the killing effect was enhanced on tumor cells. Auger emitting therapeutics can specifically deliver the ray to receptors bearing tumor cells. Many researches indicated that the ray emitted during radionuclide decay by low-energy Auger electron was highly cytotoxic. In the early experiments, 85 patients with unresectable somatostatin receptor-positive neuroendocrine tumor were given somatostatin analogs with ^{111}In-labeled, 62-69% of the tumors obviously shrank. It was found in research that the dose deposited by γ-ray emission from In^{111} did little damage to the cells, but low-energy Auger electron had a killing effect instead. However, γ-ray emitted by In^{111} could be used for tumor positioning diagnosis[22,23]. The sodium/iodine symporter (NIS) gene concentrates iodine in the thyroid gland, salivary gland, gastric mucosa, *etc*. Radioiodine (^{131}I) has been shown to selectively concentrate on tumor cells transfected with NIS gene and suppressed the growth of tumor cells *in vitro* and *in vivo*. Tissue-specific promoters combined NIS gene such as PAS promoter should be further studied[24,25].

INCREASE OF RADIOSENSITIVITY IN GENE THERAPY

The same type of tumors, even with similar clinical phases, is different in radiosensitivity. In recent years, many genes related to radiosensitivity of tumor cells have been found. The current research focuses on, through controlling radiosensitive genes and adjusting the fraction dose and interval of radiation, how to realize individualization of radiotherapy and how to change tumor cell radiosensitivity, and how to reduce the normal tissue damage so as to achieve the optimum therapeutic effect. P53 and bcl-2 gene play an important role in the tumor generation and development, they have attracted researchers' attention most. Some results of *in vitro* experiments showed that the high expression of bcl-2 protein was related to cell apoptosis induced by radiotherapy and chemotherapy, while to suppress the expression of bcl-2 gene might increase tumor radiosensitivity. Kawabe *et al*[26], compared the ability of adenovirus-mediated wild-type P53 to radiosensitize non-small lung cancer and normal human lung fibroblasts. Ad/CMV/P53 increased the radiosensitivity of two types of non-small lung cancer cell lines and meanwhile the Bax gene expression of lung cancer cells rose, but there was no obvious change of these characteristics in normal lung fibroblast cells. In *in vivo* studies, tumor growth suppression was enhanced by this combination strategy in xenograft tumors in nude mice compared to Ad/CMV/P53 or radiation therapy used alone. Grunbaum *et al*[27], observed tumor cell apoptosis and change of radiosensitivity after P53 mutant and radioresistant soft tissue sarcoma cell line combined treatment with DNA transfection either with mdm2 antisense oligodeoxynucleotides or with a wild-type P53 plasmid and irradiation. At the same radiation dose, the sensitivity of tumor cells with wtP53-plasmid transfection was higher than those without transfection, and clone formation was reduced by two times. Forty-eight and 72 h after radiation, the percentage of apoptotic cells was 25% and 38.9% respectively. Compared with the control group, the apoptotic cell ratio of tumor cells transfected with mdm2 antisense oligodeoxynucleotides was 7.7%. A striking result was obtained with the combined treatment of wtP53 and 12 Gy irradiation, which produced 25% and 38.9% of apoptotic cells 48 and 72 h after transfection, respectively. Besides the regulation of P53 gene, people have also done a lot of work on enhancing the tumor cell radiosensitivity through combining regulation with other genes. Subtraction hybridization identified mda-7 gene as a gene associated with melanoma cell differentiation and growth, which could selectively suppress the growth of various tumor cells without much effect on normal cells. Su *et al*[28], transfected human glioma cell lines by mda-7 adenovirus vector (Ad.mda-7) and found that the expression

of mda-7 could induce growth inhibition and apoptosis in malignant human gliomas with mutants and wild P53, and these effects correlated with an elevation in expression of growth arrest and DNA damage genes. The growth of human glioma cells expressing mutant P53 was inhibited when transfected with AdP53 vector and the sensitivity of the cells increased when transfected with Ad.mda-7 vector. Since the heterogeneity in P53 expression is common in gliomas, it indicates that Ad.mda-7 may, in many cases, be more beneficial to gene-based therapy of malignant gliomas than administration of wild-type P53. Applying Ad-P53 injection to solid tumors, such as breast cancer, lung cancer, rectal cancer, pancreatic cancer, melanoma, head and neck tumors, has been licensed and is in clinic phase III now. Zhang *et al*[29], and Chen *et al*[30], reported that the results of rAd-P53 agent combined with radiotherapy in treatment of head and neck squamous cell carcinomas and nasopharyngeal carcinoma respectively. Randomized controlled study of patients with head and neck squamous cell carcinomas showed that the radio-sensitized enhancement rate was 1.72 times higher at 40 Gy time point and the CR rate of the combined treatment group at the validation point was 1.68 times higher than that of the radiotherapy group. The nasopharyngeal carcinoma was reduced by 95±10% and 80±17% (P<0.001) 8 wk after treatment and the rate of CR of tumor 12 wk after treatment was 75% in the combined treatment group and 15% in the radiotherapy group respectively (P<0.01). Swisher *et al*[31], evaluated the feasibility and mechanisms of apoptosis induction after Ad-P53 (INGN 201) gene transfer and radiation therapy in patients with non-small cell lung cancer in clinic phase II study. They found that 17 of 19 patients completed all planned radiation and Ad-P53 (INGN 201) gene therapy as outpatients with radiation alone. The most common adverse events were grade 1 or 2 fever (79%) and chill (53%). Three months after completion of therapy, pathologic biopsies showed that 12 of 19 patients (63%) had no viable tumor, 3 of 19 patients (16%) had viable tumor, and 4 of 19 patients (21%) were not assessed. Quantitative reverse transcription-PCR analysis of the four P53-related genes (p21 (CDKN1A), FAS, BAK, and MDM2) revealed that BAK gene expression was most closely related to Ad-P53 (INGN 201) gene transfer. In clinical trial no significant toxicity was observed in Ad-P53 patients with mild fever and flu-like symptoms during dose escalation studies. P53 gene can increase the radiosensitivity of tumor cells, and the mechanism has not been fully elucidated. It has been observed that the introduction of P53 induces apoptosis. The clinical trial of head and neck carcinoma treatment by Ad-P53 combined with radiotherapy and chemotherapy is undergoing in 34 centers of the world.

There are many radiosensitive relevant genes. In 1995, ataxia telangiectasia (AT) gene was cloned. The cell lines from AT patients (usually used fibrous cells) showed hypersensitivity to irradiation *in vitro*. The AT cells did not have clear fractionated radiation effect, and high LET ray made much less damage to AT cells than low LET ray. Further research revealed that the activity of DNA topoisomerase suppressor of AT cells was high and highly sensitive to the restricted endonuclease, which influenced its DNA damage repair. Moreover, there was no DNA synthesis suppression in radiated AT cells, so it was characterized with DNA synthetic radioresistance and easy to die from radiation. The high radiosensitivity of AT cells was related to the delayed raise of P53 protein. With the adenovirus vector, Fan *et al*[32], transferred antisense ATM RNA into P53 mutational prostate cancer cells and found that abnormal control of cell-cycle and the radiosensitivity were distinctly increased. Tribius *et al*[33], also proved that, compared with primary glioma cells, the established glioma cells gained adaptive characteristics during cell cultures. The expression of ATM gene products decreased and its radioresistance increased accordingly.

GENE THERAPY AND RADIOPROTECTION

Normal tissue damage around tumors caused by ionizing radiation limits the radiotherapy dose. In order to increase the radiation killing effect on tumor cells, while protecting the normal tissues and organs at the normal tolerant dose, people have been working a lot on physics, chemistry, and biology including the introduction of multileaf collimators, sophisticated immobilization techniques, and intensity modulated radiotherapy *vs* computer-controlled radiotherapy beam modulation, as well as some medicines used to protect the normal organs. However, the medical protection has let people think of tumor cells possibly escaping from radiation killing, so the therapeutic effect would decline[34]. When transgene therapy is used particularly for normal tissues, the radiation dose for tumor cells can be increased while the normal tissue generates tolerance to radiation. The candidate genes involved in radioprotection should have the function to inhibit the apoptotic pathway, repair the damage induced by oxidative stress and radiation, as well as neutralize cytopathic cytokine effects. The transgenic vector should target specific organs and has minimal pathological effects on normal cells. What was studied most was superoxide dismutase (SOD), an important enzyme that widely exists inside the body to clean out the free radicals. The function of this type of enzymes is to protect cells against oxidative stress. SOD can be induced by many environmental factors and chemical substances. Due to the induction to SOD, the ability of body to protect the organs against damage can be strengthened and the body can tolerate these exogenous toxic substances.

Manganese superoxide dismutase (Mn-SOD) gene is an ideal radioprotective gene at present because other genes have some toxic and side effects. The radioresistance mechanism of Mn-SOD gene might be related to the upregulated gene expression after ionizing radiation, probably caused by activating the Mn-SOD gene promoter through the radiation-induced NF-κβ transcriptional activator. Mn-SOD is mainly localized at the mitochondrial matrix of prokaryocytes and eukaryocytes. Because of the importance of mitochondria-mediated irradiation apoptosis, genes associated with stabilization of mitochondrial membranes were first evaluated *in vitro*. Epperly *et al*[35], proved the importance of Mn-SOD positioning in radioprotection of mitochondria. They transferred Cu/Zn-SOD gene and Mn-SOD gene into 32D cl3 cells respectively so as to overexpress the genes. Both groups showed similar antioxidant activity, but Cu/Zn-SOD gene transferring cells

did not have radioprotection effect and this effect was related to the mitochondrial localization signals. Recent data showed that cells which transduced Mn-SOD gene at mitochondria were radiated, the stable mitochondrial membrane could prevent cytochrome C from releasing and going to cytoplasm to activate the cell death pathway[36]. A herpes simplex viral vector containing the full-length human Mn-SOD gene has been successfully expressed on human hematopoietic progenitor cells. Further research will determine whether the hematopoietic progenitor cells transduced Mn-SOD gene has differentiation and self-renewal abilities. Currently the research on human umbilical cord blood transgene would provide experimental information on protecting normal hematopoietic stem cells during total body irradiation in patients receiving chemotherapy who might need marrow transplantation[37,38]. Epperly *et al*[39-41], applied C57BL/6J mouse model to the research on the relationship between radiated TGF, IL-I and TNF-α cell factors and the effect of lung tissue inflammation on MnSOD-PL protection. Before radiation, MnSOD-PL or intra-tracheal administration of adenovirus-mediated Mn-SOD could prevent acute or chronic radiation damage. Administrating 250 μg of MnSOD-PL 24 h before radiation could decrease radiation-inducible electron spin resonant signals. Lung tissue pathology showed that alveolus fibrosis was obviously reduced, compared with the group not given MnSOD-PL and the group given MnSOD-PL at different times after radiation. The mechanism might be as follows. Macrophages and macrophage progenitor cells of bronchoalveolar macrophages from bone marrow are recruited to the vasculature of radiated lung, then by means of the combination of adhesion molecules on its surface and VCAM-1 and VCAM-2 on endothelial cells, they differentiate into macrophages and produce TGF-β1 and TGF-β2 which recruit fibroblast progenitor cells of bone marrow origin to the lungs. Some experiments have proved that TGF-β1 and TGF-β2 produced by bronchoalveolar macrophages could mediate signaling from lung macrophages so as to cause fibroblast progenitor cells moving to the lungs[42]. Other hypotheses include fibroblast progenitor cells in circulation independent on TGF-β1 and TGF-β2, and can directly bind to the upregulated adhesion molecules in the lung tissue. Acute and chronic damages due to esophageal irradiation were similar to the lung damage, but with some differences[43]. Epperly *et al*[44], and Stickle *et al*[45], observed acute apoptosis of esophageal basal squamous stem cells 24 h after irradiation. The apoptotic cells gradually developed into microscopic ulceration and then macroscopic ulceration 10 d later. During the treatment, lots of mice had dehydration, weight loss, high-frequent esophagostenosis or even died. MnSOD-PL administration to mice 24 h prior to single radiation or intermittently during the fractionated radiation every 3-4 d could greatly help to reduce the radiation-induced cell apoptosis, dehydration, weight loss and death, and occurrence of esophagostenosis. At present, the research on esophageal irradiation toxicity is focused on the mechanism of MnSOD-PL radioprotection of esophageal stem cells. The above experimental results showed, compared with the simple radiated control group, giving MnSOD-PL 24 h before radiation could speed-up the recovery of esophageal stem cells.

SUMMARY

The role of genes in human diseases has been gradually identified with the development of human genome planning work. Gene therapy of tumors is becoming one of the important therapies for tumors. In radiogenic therapy, the current problems to be solved are high-efficient gene transferring vectors, efficient targeted genes and the safety of gene therapy.

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