Keratinocyte Growth Factor (KGF) Gene Therapy Mediated by an Attenuated Form of Salmonella typhimurium Ameliorates Radiation Induced Pulmonary Injury in Rats

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KGF/Pulmonary injury/Radiation/Gene therapy/Attenuated Salmonella typhimurium.

The aim of this study is to investigate the effect of KGF (Keratinocyte growth factor) gene therapy mediated by the attenuated Salmonella typhimurium Ty21a on radiation-induced pulmonary injury in rats model. Sprague–Dawley rats were divided into three groups: TPK group (treated with TPK strain, attenuated Salmonella typhimurium Ty21a-recombined human KGF gene); TP group (treated with TP strain, attenuated Salmonella typhimurium Ty21a-recombined blank plasmid); and Saline group (treated with saline). After intraperitoneal administration for 48 h, the thoraxes of the rats were exposed to X-ray (20 Gy), and the rats were administered again two weeks after radiation. On the 3rd, 5th, 7th, 14th and 28th day after radiation, the rats were sacrificed and lung tissues were harvested. Histological analysis was performed, MDA contents and SOD activity were detected, mRNA levels of KGF, TGF-β, SP-A and SP-C were measured by Real-time RT-PCR, and their concentrations in the BALF were quantified with ELISA. Administration of TPK strain improved the pathological changes of the lung on the 28th day. In the TPK group, KGF effectively expressed since the 3rd day, MDA contents decreased and SOD activity increased significantly, on the 7th day and 14th day respectively. SP-A and SP-C expression elevated, whereas TGF-β expression was inhibited in the TPK group. These results suggest that this novel gene therapy of KGF could ameliorate radiation-induced pulmonary injury in rats, and may be a promising therapy for the treatment of radiative pulmonary injury.

INTRODUCTION

Radiotherapy is one of the most important treatments for thoracic neoplasms, such as lung cancer and breast cancer.1–3) The cell cytotoxicity induced by radiation is not tumor specific, thus radiation therapy sometimes induces severe acute or chronic normal tissues damage in cancer patients.4,5) As reported, the lung is a very radiosensitive organ which is prone to be damaged in radiation treatment for thoracic neoplasms,6) and clinically significant radiation pneumonitis usually develops in 10–20% of patients.5) The radiotherapy for thoracic neoplasms is limited by the radiation pulmonary injury, however, there is still not established treatment which definitely prevents or repairs this injury. One of the most popular strategies, which mediates the physical dosimetric factors in radiotherapy involving radiation dose, irradiated volume, and dose per fraction,7–9) is insufficient to inhibit the radiation pulmonary injury.

Keratinocyte growth factor (KGF), discovered by Rubin et al. from M426 lung fibroblasts,10) is a paracrine growth factor mainly synthesized in mesenchymal cells, and specifically stimulates epithelial cell proliferation and differentiation, such as skin, lung, breast, corneal and intestinal epithelial cells.11–13) Keratinocyte growth factor (KGF) could promote alveolar type II cells proliferation,13,14) and protect the lung from hyperoxia, radiation, bleomycin, and acid instillation induced injury.15–18) In addition, KGF has been reported to prevent the epithelial cells from radiation damage,19,20) and the recombinant protein of human KGF (Palifermin) has
been used to treat the acute mucositis induced by chemoradiotherapy.21) However, the recombinant protein agent is inconvenient to be used for patients because of the short half-life, adverse side effects and highly cost.22) The gene therapy of KGF may be a potential therapy for the radiative pulmonary injury. Thus, the aim of the present study was to examine whether the gene therapy of human KGF mediated by attenuated Salmonella typhimurium could ameliorate the radiation pulmonary injury in the rats and analyze the changes of cytokine levels in this process.

MATERIALS AND METHODS

Materials

TP strain (attenuated Salmonella typhimurium Ty21a-recombined blank plasmid), TPK strain (attenuated Salmonella typhimurium Ty21a-recombined human KGF gene) were preserved by our laboratory. Female Sprague-Dawley rats, aged 8–12 weeks, were purchased from the Animal Experimental Center of Lanzhou University. Superoxide Dismutase (SOD) Detection Kit and Malondialdehyde (MDA) Detection Kit were purchased from Nanjing Jiancheng Bioengineering Institute. Human Keratinocyte growth factor (KGF) ELISA kit, Rat Transforming growth factor β (TGF-β) ELISA kit, Rat Surfactant protein A (SP-A) ELISA kit and Rat Surfactant protein C (SP-C) ELISA kit were purchased from R&D. RNAiso Plus, PrimeScript RT reagent Kit and SYBR® Premix Ex Taq™ II (Perfect Real Time) were purchased from TaKaRa.

Radiation procedure and administration schedule

Female Sprague-Dawley rats (aged 8–12 weeks, n = 185, 200–250 g) were placed in wire-bottom cages housed in a temperature-controlled room with a 12 h light-dark cycle. Rats were acclimatized to their environment for 7 days before the blinded study. All animal studies were approved by the Institutional Animal Case and Use Committee, which was certified by the Chinese Association for Laboratory Animal Care. The rats were anesthetized by intraperitoneal injection pentobarbital sodium (50 mg/kg) and divided into three groups: TPK group (n = 62), treated with TPK strain (10^8 cfu) by intratracheal instillation; TP group (n = 62), treated with TP strain (10^8 cfu) by intratracheal instillation; and Saline group (n = 61), treated with saline by intratracheal instillation. BALF was pooled from the rats in the three groups at same dose, respectively. And another Normal group (n = 10) with non-irradiated rats was set up.

Bronchoalveolar lavage fluid (BALF) collection

Rats, randomly selected from each group on the 3rd, 5th, 7th, 14th and 28th day after radiation, were sacrificed and bronchoalveolar lavage fluid (BALF) was carried out as previously described.23) Briefly, the lung was lavaged with 3 mL of isotonic sterile saline for three times. BALF was pooled and centrifuged at 1500 rpm for 5 min at 4°C, the supernatant was harvested and stored at −70°C for cytokine analysis. The cell pellet of BALF was resuspended in 1 mL phosphate buffered saline (PBS), and the total number of cells in BALF was counted using a hemocytometer. In another set, animals were sacrificed and their lungs were quickly removed, part of the lungs was freeze clamped and dropped in liquid N2 for mRNA analysis, MDA contents and SOD activity assay. The other part was fixed in 10% formalin for histological analysis.

Histological examination

Histological analysis from the rats’ tissues was performed on the 28th day after radiation. The lung tissues were fixed in 10% formalin, embedded in paraffin and sectioned at a thickness of 6 mm. Sections were then stained with hematoxylin and eosin (H&E) for evaluation with a light microscopy.

Measurement of oxidative stress

The lung tissues were homogenized with nine-fold volume of ice-cold PBS using a glass homogenizer at 4°C. The homogenate was then centrifuged at 3000 rpm for 10 min at 4°C to remove the cell debris and the obtained supernatant was used for the determination of MDA contents and SOD activity by a spectrophotometric method according to the manufacturer’s protocol. And MDA contents and SOD activity were expressed as per unit of protein.

RNA isolation and Real-time PCR

KGF, TGF-β, SP-A and SP-C mRNA levels in the lung tissues were measured using a Real-time PCR assay with SYBR-GreenII. Total RNA from lung samples at various time points was isolated using RNAiso Plus in accordance with the manufacturer’s protocol. Total RNA (1 mg) was reverse-transcribed with PrimeScript RT reagent Kit, and Real-time PCR was carried out in a total volume of 25 μL for 40 cycles of 5 seconds at 95°C and 31 seconds at 60°C. To compare relative expression levels in different samples, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control. The primers were used for Real-time PCR as showed in Table 1.

ELISA

The concentrations of KGF, TGF-β, SP-A and SP-C in BALF were quantified with ELISA kits according to the manufacturer’s instruction. The OD value was detected with an enzyme-linked analyzer at 450 nm.
Statistical analysis
The data from the different groups during various time points were analyzed by One-Way ANOVA with SPSS 11.5 software. P < 0.05 was considered as statistically significant. Data were presented as mean ± SD.

RESULTS

Histological assessment
For histological assessment, the histological sections were observed by two blinded observers. The remarkable inflammatory cell infiltrates and interstitial edema were observed microscopically in the alveoli and lung interstitium of the TP group and Saline group on the 28th day after radiation (Fig. 1B, C). Administration of TPK strain decreased radiation-induced acute inflammatory response, and may be useful to inhibit pulmonary fibrosis (Fig. 1A).

Changes in MDA contents and SOD activity in lung tissues
The effects of TPK strain on the MDA contents and SOD activity in lung tissues were shown in Table 2 and Table 3, respectively. MDA contents increased in the lung tissues after radiation injury compared to the normal group. Administration of TPK strain resulted in a significant decrease in MDA contents (P < 0.05, Table 2) compared to the TP group and Saline group on the 7th day, however, MDA contents in the TPK group was still higher than in the Normal group on the 28th day (P < 0.01, Table 2). SOD activity was decreased by nearly 75% (P < 0.01, Table 3) in the lung tissues after radiation injury, compared to the Normal group. Administration of TPK strain resulted in a significant increase of SOD activity (P < 0.01, Table 3) compared to the TP group and Saline group on the 14th day after radiation, however, SOD activity in the TPK group did still not became normalized on the 28th day (P < 0.01, Table 3). There were no significant

<table>
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<th>Gene</th>
<th>Primer sequence</th>
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<tr>
<td>KGF</td>
<td>5'-CATGGATCCATGAGCTATGATTACATGGAAG-3'</td>
</tr>
<tr>
<td></td>
<td>reverse 5'-GTCGAAATTTGATTGACCATAGGCAG-3'</td>
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<td>TGF-β</td>
<td>5'-TGCAGCTGAGAAGTACAAG-3'</td>
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<td>SP-C</td>
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<td>GADPH</td>
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<tr>
<td></td>
<td>reverse 5'-ATGTTGAGAAGACGCCAGTA-3'</td>
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Table 1. Sequences of the specific primers in Real-time PCR

Fig. 1. Histological sections of the lung on the 28th day after radiation. Inflammatory cell infiltrates and interstitial edema could be observed to decrease in the TPK group (Fig. 1A), compared to two control groups. A: A representative lung from the TPK group on the 28th day after radiation; B: A representative lung from the TP group on the 28th day after radiation; C: A representative lung from the Saline group on the 28th day after radiation. D: A representative lung from the Normal group. A, B, C, D, 200× magnification.
Cytokine mRNA expression in lung after administration

The mRNA levels of KGF, TGF-β, SP-A and SP-C in lung tissues after administration were quantified by Real-time PCR at various time points. As shown in Fig. 2A, KGF mRNA level in the TPK group increased significantly from the 3rd day to the 28th day. The maximal expression of KGF appeared on the 5th day after radiation, however, it decreased obviously on the 14th and 28th day, which was still higher than in the TP group and Saline group.

The levels of TGF-β mRNA in lung tissues continued to rise at the entire early phase of radiation induced pulmonary injury. The TGF-β mRNA level was significantly inhibited in the TPK group since the 7th day. Although TGF-β mRNA level in the TPK group increased, the increasing rate was lower than in the other groups, and the TGF-β mRNA level decreased by 40% (P < 0.01) on the 28th day (Fig. 2B), compared to the TP group and Saline group.

The changes of mRNA levels of SP-A and SP-C in the TP group and Saline group were not obvious compared to the Normal group on the first week after radiation, and the SP-A mRNA levels elevated slightly on the 5th day. And they all decreased under the normal level on the 14th day after radiation. Administration of TPK strain could increase mRNA levels of SP-A and SP-C, compared to the TP group and Saline group (Fig. 2C and 2D). SP-A mRNA level in the TPK group increased significantly on the 3rd day, and the maximal level of 1.9-fold (P < 0.01, Fig. 2C) compared to the normal level was reached on the 5th day, whereas it decreased under the normal level since the 14th day. Meanwhile, the SP-C mRNA level increased on the 5th day in the TPK group (Fig. 2D). The maximal SP-C mRNA level appeared on the 7th day, which reached to 1.48-fold (P < 0.01) of the normal level. Although the SP-C mRNA level in the TPK group declined since the 14th day, it was able to maintain at a high level until the 28th day (Fig. 2D).

Table 2. Changes in MDA contents (nmol/mg) in the lung tissues

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>3d</th>
<th>5d</th>
<th>7d</th>
<th>14d</th>
<th>28d</th>
</tr>
</thead>
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<tr>
<td></td>
<td>TPK</td>
<td>0.918 ± 0.203</td>
<td>1.305 ± 0.128</td>
<td>1.292 ± 0.171*</td>
<td>0.873 ± 0.102**</td>
<td>0.725 ± 0.088**</td>
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<td>TP</td>
<td>0.938 ± 0.154</td>
<td>1.278 ± 0.177</td>
<td>1.500 ± 0.138</td>
<td>1.339 ± 0.090</td>
<td>1.222 ± 0.112</td>
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<tr>
<td></td>
<td>Saline</td>
<td>0.878 ± 0.211</td>
<td>1.204 ± 0.199</td>
<td>1.489 ± 0.150</td>
<td>1.310 ± 0.125</td>
<td>1.251 ± 0.111</td>
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</tbody>
</table>
|      | Normal  | 0.536 ± 0.077

*p < 0.05, TPK group vs. TP group and Saline group at the same time points, by One-Way ANOVA.

**p < 0.01, TPK group vs. TP group and Saline group, by One-Way ANOVA.

Table 3. Changes in SOD activity (U/mg) in the lung tissues

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>3d</th>
<th>5d</th>
<th>7d</th>
<th>14d</th>
<th>28d</th>
</tr>
</thead>
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<tr>
<td></td>
<td>TPK</td>
<td>75.40 ± 9.31</td>
<td>62.10 ± 10.12</td>
<td>75.23 ± 7.39</td>
<td>90.45 ± 9.77**</td>
<td>82.44 ± 14.92**</td>
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<tr>
<td></td>
<td>TP</td>
<td>71.18 ± 11.11</td>
<td>66.38 ± 7.94</td>
<td>65.33 ± 12.08</td>
<td>68.71 ± 7.82</td>
<td>57.11 ± 15.34</td>
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<tr>
<td></td>
<td>Saline</td>
<td>74.50 ± 13.87</td>
<td>63.50 ± 12.00</td>
<td>67.40 ± 6.59</td>
<td>69.79 ± 7.10</td>
<td>59.91 ± 7.79</td>
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</table>
|      | Normal  | 99.08 ± 12.24

**p < 0.01, TPK group vs. TP group and Saline group at the same time points, by One-Way ANOVA.

The total number of cells in BALF

The total number of cells in BALF was significantly increased on the 7th day after radiation compared to the normal level, and the maximum was attained on the 28th day (Fig. 3). Administration of TPK strain significantly inhibited the influx of total cells in BALF on the 14th day (P < 0.05, Fig. 3) and 28th day (P < 0.01, Fig. 3). There was no statistic difference in the total number of cells in BALF between the TP group and Saline group at all time points.

The effect of TPK administration on cytokine concentrations in BALF

To analyze the effect of administration of TPK strain on cytokines concentrations in BALF, we evaluated the secretion of KGF, TGF-β, SP-A and SP-C in BALF (Fig. 4). After administration, the KGF concentration in BALF elevated substantially by 93.77 ± 15.09 pg/mL (Fig. 4A) on the 3rd day. The maximal concentration was reached on the 5th day, and the KGF concentration in the TPK group remained at
higher levels than in the other groups at all time points (Fig. 4A).

The TGF-β concentrations in BALF of all the three groups elevated continuously from the 3rd day to the 28th day, and reached the maximum on the 28th day after radiation (Fig. 4B). However, the increase of TGF-β concentration in the TPK group was inhibited (P < 0.01, Fig. 4B) on the 14th and 28th day after radiation compared to the TP group and Saline group, although the TGF-β concentration in the TPK group still increased slightly.

The secretion of SP-A and SP-C in BALF of the TP group and Saline group was greatly inhibited on the 14th day and 28th day after radiation compared to the normal level (P < 0.01, Fig. 4C and 4D), which was similar to the mRNA levels (Fig. 2C and 2D). Administration of TPK strain induced an increase of SP-A concentration in BALF (P < 0.05, Fig. 4C) compared to the TP group and Saline group on the 3rd day, and the maximal level was attained by 4.72 ± 0.34 μg/mL (P < 0.01, Fig. 4C), whereas it decreased continuously from the 14th day to the 28th day (Fig. 4C). And there was a slight increase of SP-A concentration in BALF from the 3rd day to the 7th day (Fig. 4C). Meanwhile, there was not significant increase of the SP-C concentration in BALF on the 3rd day in the TPK group, compared to the TP group and Saline group (P > 0.01, Fig. 4D). A remarkable increase of SP-C concentration in the TPK group (P < 0.01, Fig. 4D) compared to the TP group and Saline group on the 5th day. The maximal SP-C concentration appeared on the 7th day, which reached by 0.81 ± 0.08 μg/mL (P < 0.01, Fig. 4D). And SP-C concentrations in BALF of the TP group and Saline group remained at a lower level than in the Normal group at all
Radiation induced pulmonary injury is a major limited factor in the treatment of thoracic tumors, which has no specific treatment and causes serious morbidity and mortality.3,24) The typical radiation-induced pulmonary injury process involves two phases: acute inflammatory pneumonia and late pulmonary fibrosis.6,9,25) In the present study, we focused on the former phase and investigated the effects of TPK strain, a strain of the attenuated Salmonella typhimurium Ty21a-recombined human KGF gene, on the X-ray induced irradiated pulmonary injury in the rats model. The results demonstrated that KGF could effectively express in lung after administration of TPK strain and radiation induced pulmonary injury was markedly ameliorated physiologically and pathologically in the TPK group, by increasing of SP-A, SP-C expression and SOD activity and decreasing of TGF-β expression and MDA contents in lung.

In the previous reports, Keratinocyte growth factor (KGF) can prevent lung from multiple injuries,15–18) however, this mechanism is still unclear. It has been reported that KGF may protect lung tissues by stimulating proliferation and differentiation of alveolar type II cells in vitro and in vivo.1,4,20) KGF also inhibits inflammatory response in the lung injury process.27) Moreover, KGF may enhance alveolar fluid clearance from the air spaces to maintain the gas exchange by stimulating Na-K-ATPase expression,28,29) and improve pulmonary edema after pulmonary injury.30) KGF is relatively safe for the cancer patients because KGF is not able to stimulate majority of cancer cells proliferation.31) KGF is widely used to treat various of lung injury in recent studies, however, KGF protein agent is limited to clinical treatment because of its rapid degradation, highly cost, and difficulties in achieving adequate delivery to the distal lung.22,29) Thus, the gene therapy of KGF attracts much attention, and the most important point of gene therapy is to develop the suitable vector to transfer the exogenous gene into the cells effectively. Liposome is a common non-viral vector for KGF gene therapy, however, its transfection is transient and low efficient in tissues.22,32) Although adenovirus can mediate the exogenous gene to various tissues and express effectively, it is limited by the short duration of transgene expression.33,34) The attenuated Salmonella typhimurium is a notable vector for gene therapy, which has been proved to be safe for human35) and is able to synthesize recombinant cytokines at high levels.36) And it is widely used in gene therapy and DNA vaccine,37–39) so we chose the attenuated Salmonella typhimurium as the gene therapy vector in this study.

In this study, KGF gene could be effectively expressed in lung tissues after administration of TPK strain. Interestingly, the KGF expression level markedly decreased on the 14th day in both mRNA level and protein level (Fig. 2A and Fig. 4A). So we administered TPK strain twice to maintain the...
high expression level of KGF in lung of the rats. And administration of TPK strain attenuated the pulmonary injury on the histology (Fig. 1) and reduced the total number of cells in BALF (Fig. 3), compared to the TP group and Saline group, however, there was no significant difference of the total protein in BALF among the three groups at all time points (data not shown).

Radiotherapy may induce the generation of reactive oxygen species (ROS) in lung tissues, and result in direct damage to the alveolar epithelium. Superoxide dismutase (SOD) is the direct product of ROS induced lipid peroxidation, analysis of the MDA contents may measure the lipid peroxidation levels. Sener et al. reported that MDA contents in lung tissues elevate after exposed to radiation. Superoxide dismutases (SOD) is able to protect lung tissues against ROS by catalyzing the dismutation of superoxide radical to hydrogen peroxide and oxygen. Although the mechanism is still not completely clear, many therapies based on SOD are developed to ameliorate the radiation induced pulmonary injury, such as polyethylene glycol-modified (PEGylated) superoxide dismutase (PEG-SOD), mimics of SOD and the gene therapy of SOD. In this study, results demonstrated that SOD contents decreased significantly on the 7th day in the TPK group, compared to the TP group and Saline group (Table 2). As known, inflammatory cells are the main sources of ROS, then this decrease of MDA contents in the TPK group could be attributed to the inhibition of the inflammatory response by KGF, as shown in Histological sections (Fig. 1). Compared to the TP group and Saline group, SOD activity in the TPK group remarkably increased on the 14th day (Table 3), which also could result in reducing of MDA contents. And the increase of SOD activity may be due to the promotion of alveolar type II cells proliferation induced by KGF, which secretes SOD in lung. These results showed that KGF could prevent lung from radiative injury in rats model via interference with the damaging reactive species cascades initiated by radiation.

TGF-β is a profibrogenic cytokine, which plays a critical role in radiation induced pulmonary injury and fibrosis. TGF-β plays roles in this process from multiple aspects, including cell apoptosis and differentiation, inflammatory response, collagen formation and regulation of extracellular matrix function. The radiation induced TGF-β increase may promote tumor metastases, and enhance radiotherapy risk. TGF-β levels elevate in serum and lung tissues rapidly after radiation, and inhibition of the TGF-β expression could attenuate the radiation induced pulmonary injury. In this study, the results demonstrated that TGF-β expression increased since the 3rd day after radiation and administration of TPK strain could markedly inhibit the increase of TGF-β induced by radiation in both mRNA level and protein level on the 14th day and 28th day (Fig. 2B and Fig. 4B). SOD is able to decrease ROS levels and TGF-β expression after radiation, therefore, the increase of SOD induced by TPK strain may help to explain the decrease of TGF-β level in TPK group.

The lung-specific surfactant proteins (SPs), involving SP-A, SP-B, SP-C and SP-D, stabilize the alveoli and prevent them from collapse by reducing alveolar surface tension. SP-A is an important component of the innate immune system, which can prevent virus and bacteria infection by mediating the innate host defense mechanisms and attenuate inflammatory response in pulmonary injury process. At present, serum SP-A assays has been used as a diagnostic method for detection of radiation pneumonitis. To test the impact of TPK strain on lung-specific surfactant proteins in the irradiated rats model, we detected the mRNA levels and concentrations in BALF of SP-A and SP-C. The mRNA level and concentration in BALF of SP-A elevated in the early phase after radiation and then declined on the 14th day and 28th day, which may be relevant to the self-regulation mechanism of radiation. Administration of TPK strain could promote the secretion of SP-A and inhibit the decrease induced by radiation. In previous study, KGF pretreatment is reported to prevent lung from P. aeruginosa infection induced pneumonia, and we observed less inflammatory response in the histological sections of the TPK group (Fig. 1), so we assumed that administration of TPK strain may inhibit inflammatory response and decrease the risk of bacteria infection via enhancing the SP-A expression, which could improve host immunity.

SP-C may improve gas exchange and stabilize the surface film, and the recombinant SP-C protein (Venticute) has been encountered in clinical researches. And SP-C only secreted by alveolar type II cells, is a surface marker characteristic of alveolar type II cells. Compared to SP-A, SP-C may be more sensitive to irradiation injury, and the impact of TPK strain on SP-C was delayed. The Real-time PCR and ELISA results demonstrated that SP-C decreased immediately after radiation in the TP group and Saline group, and SP-C in the TPK group could maintain at a higher level than the Normal group until the 7th day. Although the SP-C level in the TPK group decreased on the 14th day and 28th day compared to the Normal group, it remained higher than in the TP group and Saline group (Fig. 2C and Fig. 4C). This probably supplied an indirect evidence that the high level of KGF in the TPK group could inhibit apoptosis and promote proliferation of alveolar type II cells, as progenitors for restoration of alveolar epithelium, are able to differentiate into alveolar type II cells and secret pulmonary surface-active material to maintain gas exchange, so it is necessary in pulmonary injury repairing process. Hence, administration of TPK strain may improve radiation induced pulmonary injury via protecting alveolar type II cells.

In summary, the gene therapy of attenuated Salmonella typhimurium Ty21a-recombined KGF gene (TPK strain)
ameliorated radiation-induced pulmonary injury in an experimental rats model at acute inflammatory pneumonitis phase. Administration of TPK strain could decrease radiation-induced ROS and TGF-β expression in lung tissues, and increase SP-A and SP-C levels against radiation damage. These suggested that this gene therapy is a promising potential treatment for radiation-induced pulmonary injury. And further studies are needed to assess the effects of TPK strain on the pulmonary fibrosis phase.

ACKNOWLEDGEMENTS
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REFERENCES