Diagnostic Value of 18 F-NOTA-FAPI PET/CT in A Rat Model of Radiation-induced Lung Damage

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Research Article

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Abstract

Background: In this study, we explore the diagnostic value of a novel PET/CT imaging tracer that specifically targets fibroblast activation protein (FAP), $^{18}$F-NOTA-FAPI, in a radiation induced lung damage (RILD) rat model.

Methods: High focal radiation (40, 60, or 90 Gy) was administered to a 5-mm diameter area of the right lung in Wistar rats for evaluation of RILD induction. Lung tissues exposed to 90 Gy radiation were scanned with $^{18}$F-NOTA-FAPI PET/CT and with $^{18}$F-FDG. Dynamic $^{18}$F-NOTA-FAPI PET scanning was performed on day 42 post-irradiation. After in vivo scanning, lung cryosections were prepared for autoradiography, hematoxylin and eosin (HE) and immunohistochemical (IHC) staining.

Results: An animal model of RILD was established and validated by histopathological analysis. On $^{18}$F-NOTA-FAPI PET, RILD was first observed on days 42, 35 and 7 in the 40, 60 and 90 Gy groups, respectively. After treatment with 90 Gy, $^{18}$F-NOTA-FAPI uptake in an area of RILD emerged on day 7 (0.65±0.05 %ID/ml) and reappeared on day 28 (0.81±0.09 %ID/ml), remaining stable for 4–6 weeks. Autoradiography and HE staining IHC staining revealed that $^{18}$F-NOTA-FAPI accumulated mainly in the center of the irradiated area. IHC staining confirmed the presence of FAP+ macrophages in the RILD area, while FAP+ fibroblasts were observed in the peripheral area of irradiated lung tissue.

Conclusion: $^{18}$F-NOTA-FAPI represents a promising radiotracer for in vivo imaging of RILD in a dose- and time-dependent manner. Noninvasive imaging of FAP may potentially aiding in the clinical management of radiotherapy patients.

Introduction

Radiotherapy is a critical component in the treatment of thoracic malignancies, including esophageal cancer, lung cancer and breast cancer [1]. However, normal lung tissue inside the radiation field is vulnerable to potential injury. Radiation doses greater than 50 Gy can lead to the development of radiation-induced lung damage (RILD) in the form of acute radiation-induced pneumonitis (RIP) or late occurring radiation-induced lung fibrosis (RILF) [2, 3]. High numbers of macrophages are detected within the damaged tissue in clinical and preclinical RILD, as these cells are the first responders to organ injury and are crucial for tissue repair and re-establishment of homeostasis [4]. Once the repair process is stimulated by inflammatory cell infiltration, fibroblasts also populate the site of injury and interactions between activated macrophages and fibroblasts coordinate tissue repair after injury, with miscommunications potentially resulting in pathological healing and fibrosis [5]. Thus, close monitoring of the responses of macrophages and fibroblasts in radiated tissue can inform new strategies for preventing or delaying the progression of lung fibrosis [6].

Fibroblast activation protein (FAP) is a homodimeric membrane-bound serine protease that has intracellular and extracellular soluble truncated forms [7]. Its expression was shown to facilitate the
ability of macrophages to migrate through the collagen networks found in the dermis and in the tumor microenvironment, similar to the mechanism demonstrated for FAP-expressing (FAP+) fibroblasts [8]. FAP+ fibroblasts are selectively induced in areas of ongoing tissue remodeling, including sites of wound healing [9], fibrosis [10-12], the solid tumor microenvironment [13, 14], and rheumatoid arthritis [15]. The basal expression level of FAP in healthy human tissue is considered very low, whereas in mice, detectable levels of FAP expression were shown to be highest in the uterus, pancreas, submaxillary gland, and skin [16]. Radiolabeled FAP inhibitors (FAPIs) have been developed for noninvasive imaging of FAP expression and characterized by many groups, exhibiting rapid distribution at the target site and minimal uptake in normal organs [13, 17]. In relation to fibrosis, however, FAP remains a relatively understudied protein, and its role in the pathogenesis of this condition is unknown. In the present study, we investigated the feasibility of using a novel FAP-based positron emission tomography (PET)/computed tomography (CT) tracer, $^{18}$F-NOTA-FAPI, to monitor the injury status of lung tissue following radiation and to define the role of FAP in the development of RILD in vivo.

### Methods

#### Rats

Male Wistar rats, 6 weeks of age, were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. and housed in a specific pathogen-free, temperature and humidity-controlled environment with food and water in their cages. The rats were housed two per cage and allowed to acclimate for 1 week after shipping prior to treatment. All studies involving the use of rats were approved by the Shandong Cancer Hospital and Institute.

#### Rat Model

To confirm the feasibility of the proposed experiments, 12 male Wistar rats were randomly assigned to one of four radiation treatment groups (3 rats/group): sham, 40 Gy, 60 Gy, and 90 Gy. Rats were anesthetized with 2.5% pentobarbital administered intraperitoneally and secured onto a four-axis robotic positioning table of a small animal radiation research platform (SARRP; Xstrahl®, Surrey, UK). A high-resolution, treatment planning CT scan was performed, and CT images were reconstructed with an isotropic voxel size of 0.15 mm. To maximize the survival quality of rats, a circle with a diameter of 5 mm was delineated as the radiotherapy area in the outer 2/3 area of the right lung. The target area was delineated into 16–18 layers from the lower boundary of the lung according to the cone beam CT image. The isocenter for radiotherapy planning was positioned in the right lung on coronal, axial, and sagittal slices (Supplemental Fig. 1). Other parameters such as the target dose according to the dose function, the precise coordinate point and the beam exit time were calculated using MuriPlan software. An arc radiation field was irradiated by a 5 mm×5 mm square field filtered by 0.15 mm Cu in the beam tube. After treatment, the rats were examined weekly by $^{18}$F-NOTA-FAPI PET/CT scanning to confirm the presence of RILD. Almost all rats that received the 90-Gy radiation treatment developed acute RIP and late
occurring RILF based on $^{18}$F-NOTA-FAPi PET/CT and pathologic evaluations, consistent with previously published observations[18, 19].

In subsequent experiments, 15 male rats underwent CT-guided treatment simulation and delivery. During irradiation, a single dose of 90 Gy was delivered along with 220 kV X-ray energy with a 13-mA tube current. All experiments were repeated twice, and all findings were similar across all experiments (Fig. 1).

**Micro PET/CT Imaging**

Micro PET imaging was performed using a small-animal Inveon PET/CT scanner (IRIS PET/CT, Inviscan, Germany). Before $^{18}$F-fluorodeoxyglucose (FDG) PET/CT scanning, the rats were fasted for at least 6 h. The rats were anesthetized with 1.5%–2% isoflurane in a 0.4 L/min flow of air. Static PET/CT images (10 min) were acquired 1 h after injection of $^{18}$F-NOTA-FAPi (7–9 MBq; days 7, 14, 21, 28, 36, 38, 42 after radiation treatment) or $^{18}$F-FDG (8–10 MBq; day 22 after radiation treatment). Dynamic PET scanning was performed with $^{18}$F-NOTA-FAPi (day 42 after radiation treatment) for 90 min.

Images were reconstructed by software that uses a 3-dimensional ordered-subsets expectation maximum algorithm with attenuation correction. The acquired data were then Fourier-rebinned in 46-time frames (6 · 5 s, 21 · 10 s, 8 · 120 s, 8 · 300 s, and 3 · 600 s) and reconstructed using the same 3-dimensional ordered-subsets expectation maximum algorithm. The obtained images were reconstructed using 3Dimensional Ordered Subsets Implementations Monte Carlo (3D-OSEM-MC) without attenuation correction and then processed using the OsiriX MD 7.0 (Pixmeo, Switzerland). Regions of interest were drawn over areas of RILD and main organs, and average signal levels in the regions were measured. The analyzed results were corrected with a decay curve, and signal intensities were recorded as percentage injected dose per milliliter of tissue (%ID/ml).

**Autoradiography**

To assess the specificity of $^{18}$F-NOTA-FAPi accumulation and to confirm that uptake of $^{18}$F-NOTA-FAPi in the area of RILD was due to saturable binding to FAP, a group of treated rats (n=3, day 42 after radiation treatment) were injected with $^{18}$F-NOTA-FAPi and killed 1 h later. Serial short-axis cryosections 50-µm thick were prepared from the harvested lungs, and consecutive sections were used for autoradiography.

**Histological Analysis**

Hematoxylin and eosin (HE) staining (days 7, 14, 21, 28, 42 after radiation treatment) was used to determine the location and extent of areas of RILD, while Masson's trichrome staining was used to assess overall collagen deposition. The expression levels of FAP, as a marker of activated macrophages and fibroblasts, and glucose transporter1 (GLUT1), as a glucose metabolism marker, were evaluated by immunohistochemical (IHC) staining[20]. For IHC analysis, paraffin-embedded sections of lung tissue were deparaffinized, and goat serum (Boster Biological Technology, Pleasanton, CA) was used to block nonspecific binding sites. The sections were incubated with primary antibodies targeting FAP-alpha.
(1:100 dilution, ab53066; Abcam) and GLUT1 (1:200 dilution, ba128033; Abcam). Full-specimen images were captured using Axio Scan.Z1 (Zeiss).

Statistical Analyses

Statistical comparisons were performed using the two-tailed Mann-Whitney U test (GraphPad Software, Inc., San Diego, CA). Differences for which the P value was 0.05 or less were considered to be significant.

Results

Responses of Rat Normal Lung to Different Doses of Irradiation

To obtain an initial estimated dose–response curve for lung injury produced by radiation of different doses, areas of normal rat lung were irradiated with three different doses using a 5-mm collimator. In this feasibility study, no RIP was observed in the 40 Gy and 60 Gy groups. However, the rats in the 40 Gy and 60 Gy groups did show RILF on micro-PET/CT as well as $^{18}$F-NOTA-FAP1 uptake in week 6 ($0.76 \pm 0.02 \% \text{ID/ml}$) and week 5 ($0.92 \pm 0.06 \% \text{ID/ml}$) after radiation treatment (Fig. 2a). In rats of the 90 Gy group, $^{18}$F-NOTA-FAP1 uptake in areas of RIP emerged on day 7 ($0.65 \pm 0.05 \% \text{ID/ml}$) and reappeared in week 4 after radiation treatment ($0.81 \pm 0.09 \% \text{ID/ml}$). Additionally, in these rats, obvious RILD lesions were observed on micro-PET/CT, and histologic evaluation confirmed the presence of pathological changes associated with RILF in the irradiated lung tissue (Fig. 2b).

Rapid Biodistribution and Accumulation of $^{18}$F-NOTA-FAP1 in RILD

A series of dynamic images (axial and coronal sections) collected from 5–90 min after injection of $^{18}$F-FAP1 in the irradiated lung are presented in Fig. 3a (day 42 after radiation treatment with 90 Gy). Dynamic measurements over the course of the 90-min post-injection period revealed fast biodistribution and specific tracer uptake in the site of lung injury in vivo (Fig. 3b), and FAPI demonstrated the highest uptake level in the injured lung tissue at 60 min after injection ($0.93 \pm 0.09 \% \text{ID/ml}$), followed by a marker decrease within 90 min ($0.53 \pm 0.01 \% \text{ID/ml}$).

Dynamic Uptake of $^{18}$F-NOTA-FAP1 in RILD

Based on our initial results showing the feasibility of observing differences in RILD severity by $^{18}$F-FAP1-PET/CT imaging in the rat model, we next investigated the potential value of $^{18}$F-FAP1-PET scanning for identifying severe cases of RILD. In vivo longitudinal PET/CT images (cross-sections) of a representative rat subjected to damaging lung irradiation are shown in Fig.4a. Differential $^{18}$F-NOTA-FAP1 uptake in the area of RILD compared with the normal lung emerged on day 7 ($0.26 \pm 0.01 \% \text{ID/ml}$), and the uptake was significantly elevated in the irradiated area ($0.65 \pm 0.05 \% \text{ID/ml}$, $P<0.01$), suggesting an early inflammatory response in the lung. Interestingly, the uptake decreased to the background level at 2–3 weeks after irradiation (2 weeks: $0.35 \pm 0.09 \% \text{ID/ml}$, 3 weeks: $0.27 \pm 0.06 \% \text{ID/ml}$) before a second increase was observed at 4 week and a stable period from 4–6 weeks (4 weeks: $0.81 \pm 0.09 \% \text{ID/ml}$, 5 weeks: $0.90 \pm 0.07$
%ID/ml, 6 weeks: 0.93±0.09 %ID/ml; Fig. 4c), suggesting the development of RILF. The exact location of 18F-NOTA-FAPI uptake within the area of RILD was further verified by autoradiography. In autoradiographic images, increased 18F-NOTA-FAPI uptake was observed predominantly in the injured lung area, while no significant tracer uptake was observed in contralateral normal lung tissue (P<0.05; Fig. 5). Intense 18F-NOTA-FAPI uptake observed in the same irradiated area that was identified on 18F-FDG PET/CT scans (1.93±0.17 %ID/ml; day 22 after radiation treatment; Fig. 4b).

**Involvement of FAP in the Pathogenesis of RILD**

On HE-stained sections of the irradiated lung tissue, the radiation damage was confined to a small circular area (Fig. 6). On day 7 after irradiation, significant cell injury was observed in the irradiated area of the rat lungs, with foamy macrophages present both within and around the scarred area. Many foamy macrophages and fibroblasts were present by day 28 and remained until day 42. IHC staining showed abundant FAP expression in activated macrophages in the injured lung area. Masson's trichrome staining revealed slight collagen deposition in the injured lung area on day 7 with the amount of collagen deposition gradually increasing thereafter.

On day 42 after radiation, we observed a very interesting “delamination” phenomenon in the injured rat lung tissue. At the center of the irradiated area, we observed a large number of infiltrating FAP+ macrophages and collagen deposition. However, at the border of the irradiated area, we observed thickening of the alveolar walls and a decrease in alveolar air space, leading to the disappearance of the alveolar structure. At the remote area distant from the irradiated area, a large number of FAP+ fibroblasts had gathered and a small amount of collagen had been deposited.

**Discussion**

Lung tissue is highly sensitive to radiation, and accordingly, the lung is classified as a major dose-limiting organ in radiotherapy. Both acute and delayed radiation-induced pulmonary toxicity have been demonstrated at the pathophysiological and cellular levels [21]. Recent studies have shown that FAP expression on activated fibroblasts defines a distinct subset of fibroblasts associated with matrix remodeling in the context of tissue (lung, liver, cardiac, skin, etc.) fibrosis [22]. Both human and murine RILD is associated with a high level of macrophage infiltration, and pulmonary macrophages are able to trigger the onset and maintenance of RILD [4, 23]. Previous studies have shown that FAP may facilitate the macrophage's ability to migrate through the collagen networks found in the dermis and in the tumor microenvironment, similar to the role of FAP demonstrated in fibroblast migration [8, 24]. FAP expression is one of several candidate biologic processes that have been targeted for molecular imaging [25, 26]. As a small-molecule enzyme inhibitor with high affinity to FAP [27], FAPI has shown promising pharmacokinetic properties regarding target accumulation and retention time [28]. Different from previous studies of FAPI-based tracers using 68Ga, we label FAPI with 18F, which has been shown to be more effective [29]. In this comprehensive evaluation of 18F-NOTA-FAPI PET for assessing RILD, micro-PET scans showed increases in FAPI uptake in irradiated lung tissue, and the specificity of the PET signal
was confirmed by autoradiography and IHC staining, corroborating the high expression of FAP by activated macrophages and fibroblasts. The present study is the first to explore the role of 18F-NOTA-FAPI PET/CT in the evaluation and diagnosis of RILD, with pathological examinations used as the gold standard and excluding confounding factors in an animal model. The pathogenesis of RILD in patients remains incompletely understood, and greater insight is needed into the events that govern the conversion of what begins as a normal healing process after lung injury into an uncontrolled fibroproliferative response resulting in irreversible scarring, tissue distortion, and progressive decline in lung function. The experiments in this study showed the feasibility of imaging the time course of macrophage and fibroblast activation in irradiated tissue. Previous studies have confirmed that irradiated lung fibroblasts themselves can contribute to macrophage activation through the secretion of cytokines. Thus, the signals seem to be bidirectional with functional crosstalk occurring between fibroblast and macrophages in RILD, as described during prostate carcinoma progression [30]. Our findings identified a novel fibrogenic process that involves and sustains macrophage and fibroblast activation. Tracer uptake increased at day 7 after radiation and then decreased to the background level by 2–3 weeks before increasing again by 4 weeks and remaining stable thereafter. In clinical settings, RILD can be classified as early (<6 months, i.e., acute RIP) and late (>6 months, i.e., RILF) [31]. Therefore, we evaluated the histopathologic changes that occurred at several time points to provide a better understanding of the progression of RILD and its consequences. Interestingly, histopathologic analysis revealed steps in the inflammatory process associated with the acute phase in people receiving radiotherapy [32]. In our rat model, these steps were seen within 1 week after irradiation. The fibrosis process in humans began at a very early time point, and we observed fibroblast hyperplasia on day 7 post-irradiation that resulted in collagen deposition in parenchymal tissue at the same time point. Therefore, FAPI PET/CT in the rat model appears to be a good translational model for clinical RILD, allowing for dynamic monitoring of fibroblast activation. This model may be useful for identifying a time window during which fibrosis can still be prevented and the disease course altered. FAP has been identified in a wide range of cancer types and shows minimal expression in normal tissues [13, 17]. Accordingly, several groups have successfully provided proof-of-concept that alpha therapy targeting FAP in the cancer stroma is effective [33, 34]. In light of our present findings, however, somewhat heightened caution is needed for such endeavors. For treatment with FAPIs, side effects associated with tracer accumulation in normal tissues or benign lesions may present a major issue, as benign lesions including pulmonary fibrosis, which showed increased FAPI uptake, are common [35, 36]. Additionally, many patients with primary lung cancers or other intrathoracic malignancies undergo adjuvant radiation therapy. If future treatment regimens include a pharmacologic FAPI, we need to ensure that patients will not be at increased risk of developing RILD, and a better understanding of the role of FAP in human fibrosing conditions is required. In this study, 18F-FDG PET/CT also contributed to the confirmation of RILD diagnosis. Several groups have investigated FDG-PET imaging as a method for assessing RIP. Guo et al. concluded that 18F-FDG imaging may not be able to diagnose aseptic radiation pneumonia in a murine model [37], whereas Abdulla et al. proposed that global lung parenchymal glycolysis and the mean standard uptake value in lung parenchyma may serve as useful biomarkers to quantify lung inflammation on FDG PET/CT following thoracic radiation therapy [38]. The imaging targets for the 18F-FDG and 18F-NOTA-FAPI tracers are different, with 18F-FDG
revealing glucose metabolism imaging and 18F-NOTA-FAPI showing FAP expression. Because 18F-NOTA-FAPI provides protease imaging, this method can help guide research into the related mechanisms of RILD that involve FAP. Although we were able to demonstrate that the process of RILD can be accurately assessed by FAPI PET, this study has several limitations. First, we investigated RILD induced by a single high dose of radiation in order to ensure the consistency of the target area. It is possible that RILD caused by radiation delivered in multiple fractions might differ from that produced by a single dose. Although fractionation was not applied in our study, we still assume that our findings provide a valuable reference for understanding ablative dose focal radiation-induced damage to the normal lung. In clinical settings, local control rates of 85–90% are now expected with SBRT when biologically equivalent doses of >100Gy are delivered in several fractionations [39]. In addition, based on our study of the time–dose response relationship in radiosensitive rats and previous studies on RILD, we chose 90Gy as a time-effective and well-tolerated ablative dose. A strength of our study was that we used radiotherapy simulation, planning, and delivery to induce targeted RILD, which resembles the clinical situation, and damage was observed in and limited to a local area of the right lung, which represents better accuracy than the previous use of ordinary X-ray irradiation to simulate radiotherapy [40]. Moreover, we obtained pathologic confirmation that all irradiated rat lung tissue developed RILD, and the dynamic changes that we observed clearly showed that macrophages and fibroblasts play important roles in the resulting lung fibrosis. More research should be carried out to elucidate the cellular and molecular mechanisms involved.

**Conclusion**

RIP and RILF remain dose-limiting forms of radiation-induced lung toxicity with relevant impacts on the success of thoracic radiotherapy. In this study, 18F-FAPI PET/CT imaging specifically detected inflamed lung tissue containing macrophages expressing FAP in a rat model of RILD, and the findings obtained using this novel tracer can increase our understanding of the dynamic sequential events that occur after radiation of normal lung tissues. Moreover, imaging of FAP expression will be helpful in future attempts to mediate these reactions to reduce the side effects of radiation therapy.

**Abbreviations**

RILD: Radiation-induced Lung Damage; RILF: Radiation-induced Lung Fibrosis; FAP: Fibroblast Activation Protein; FAPIs: FAP Inhibitors; HE: Hematoxylin and Eosin; GLUT1: Glucose Transporter1; IHC: Immunohistochemical.

**Declarations**

**Ethics approval and consent to participate**

Approval from the Shandong Cancer Hospital and Institute animal care committee was obtained.

**Consent for publication**
Not applicable

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

There is no conflict of interests to declare.

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**Authors’ contributions**

SHY and YCW conceived of the study and participated in its designed; XTQ participated in the experiments and drafted the manuscript; SJW is responsible for the preparation of $^{18}$F-NOTA-FAPI-04 and $^{18}$F-FDG; XLL carried out the radiation in rats. JHD and are responsible for collecting PET/CT images; KC evaluated PET/CT images; ZSM carried out the pathology; JJ carried out the nuclear medicine. All authors have read and approved the final manuscript.

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Figures
Figure 1

**Experimental timeline.** R = radiation, $^{18}$F-FAPI-PET/CT = $^{18}$F-fibroblast activation protein inhibitor-emission tomography (PET)/computed tomography (CT), $^{18}$F-FDG = $^{18}$F-fluorodeoxyglucose.
**Figure 2**

**18**F-NOTA-FAPI PET/CT and pathologic evaluations of rat lung tissues with different radiation doses. **a** Micro-CT imaging of FAPI uptake in irradiated rat lung tissue. Rats in the 40 Gy and 60 Gy groups showed no RIP and began to show RILF on days 42 and 35 after radiation, respectively. In contrast, rats in 90 Gy group showed 18F-NOTA-FAPI uptake in areas of RIP on day 7, with reappearance of tracer uptake in the irradiated area on day 28 after radiation. **b** Representative micrographs of HE-stained lung sections from the 40 Gy, 60 Gy, and 90 Gy groups at days 42, 35, and 28, respectively, showed that the radiation damage was confined to a small area.
Figure 3

In vivo dynamic imaging of $^{18}$F-FAPI uptake in irradiated rat lung tissue. a Serial PET/CT images (axial and coronal sections) from 90 min of dynamic scanning. White arrows indicate area of pulmonary fibrosis. b Corresponding time–activity curves for fibrosis in lung tissue (average and SD, n=3). p.i. = post-injection.
Figure 4

In vivo imaging of $^{18}$F-FAPI uptake in longitudinal study. a Static PET/CT matched axial slices from the same rat subjected to radiation and scanned 1 h after injection of $^{18}$F-FAPI on days 7, 15, 21, 28, 36, 38 and 42 after radiation treatment. Dashed lines separate tracer uptake in lung from uptake in heart, and white circles show representative regions of interest (2-dimensional) drawn over area of RILD. b Static PET/CT matched axial slices in the same rat scanned 1 h after injection of $^{18}$F-FDG on day 22 after radiation treatment. c Corresponding time–activity curves for RILD from day 7 to day 42 after radiation (average and SD, n=3).

Figure 5

Binding specificity test. a Serial coronal sections of PET/CT image from 60-min static scan on day 42 after radiation treatment (white arrow). b Autoradiographs of irradiated lungs show increased $^{18}$F-FAPI
uptake in area of RIP at day 42 after radiation treatment (red arrow). c HE-stained parallel section showed acute RIP (black arrow). d Comparison of average intensities on autoradiographic imaging between areas of RILF and normal lung (n=3).

Figure 6

Correlation between $^{18}$F-NOTA-FAPI uptake and FAP expression. On day 7 after irradiation, foamy macrophages appeared in the damaged lung area, and large numbers of foamy macrophages and
fibroblasts reappeared at day 28, remaining until day 42. IHC staining showed abundant FAP expression in the activated macrophages. Slight collagen deposition was detected on day 7, and the amount of collagen gradually increased thereafter based on Masson's trichrome staining. On day 42 after radiation, infiltration of a large number of FAP+ macrophages were observed (black arrows) along with collagen deposition at the center of the irradiated area. At the border of the irradiated area, thickening of the alveolar walls and a decrease in alveolar air space was observed, leading to disappearance of the alveolar structure (white arrows). At the remote area distant from the irradiated lung tissue, a large number of FAP+ fibroblasts gathered. From the center of the irradiated area to the remote area distant from the irradiated lung tissue, the number of collagens gradually decreased (yellow arrows).

**Supplementary Files**

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