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Radiation decreases bronchial epithelial progenitor function as assessed by organoid formation

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Abstract

Objective Radiation-induced lung injury (RILI) is a serious side-effect of radiotherapy for lung cancer, in which effects on the normal lung epithelium may play a key role. Since these effects are incompletely understood, the aim of the present study was to evaluate the effect of ionizing radiation (IR) on cultured well-differentiated primary bronchial epithelial cells (PBEC) with a focus on cytotoxicity, barrier formation, inflammation and epithelial progenitor function.

Materials and methods PBEC were cultured at the Air-Liquid Interface (ALI-PBEC) to allow mucociliary differentiation. Effect of IR (1, 2, 4, 8 Gy [Gy]) on ALI-PBEC cultures was investigated by lactate dehydrogenase (LDH) release, Trans Epithelial Electrical Resistance (TEER; as a measure of barrier function), qPCR (P21/CDKNA1, MKI67, AEN, E2F1, ATF3) and immunofluorescence staining (γH2Ax-foci). The impact on epithelial progenitor function was assessed by studying organoid formation capacity of irradiated ALI-PBEC at 24 h and 7 days after IR.

Results and discussion IR increased the number of γH2Ax-foci (marker of double stranded DNA breaks) in ALI-PBEC, but did not affect markers of toxicity (LDH-release or TEER). IR did also not affect mRNA markers for inflammation or epithelial-mesenchymal transition (EMT), but did increase mRNA levels of the cell cycle inhibitor P21/CDKN1A and resulted in downregulation of the proliferation markers MKI67 and E2F1. Finally, IR of ALI-PBEC had a marked effect on organoid formation capacity, which was markedly impaired following IR in a dose-dependent manner.

Conclusion In conclusion, epithelial progenitor cell function as assessed by organoid formation capacity is strongly reduced by IR and persists for at least 7 days. Despite an effect on organoid formation capacity, DNA breaks, P21/CDKN1A expression and reduced expression of MKI67 and E2F1, this effect was not accompanied by IR-induced cytotoxicity, or an increase in markers of inflammation or EMT. This study indicates that studying the effects of IR on organoid formation is a valid and sensitive tool to study adverse effects of IR on normal lung epithelial cells and could be used as a tool to study RILI.

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Background

Radiation-induced lung Injury (RILI) is one of the main dose-limiting factors of radiotherapy for stage III non-small cell lung cancer (NSCLC). Between 5 and 25% of patients develop some form of RILI, manifesting as radiation pneumonitis within the first 6 months, or as radiation fibrosis which can occur up to one year after treatment. Both conditions can lead to shortness of breath, cough, hypoxemia, reduced quality of life or even death. With the advancements in lung cancer care resulting in longer survival, it is of utmost importance to better understand and prevent these severe side-effects.

The pathogenesis of RILI is a complex cascade that starts with DNA damage following ionizing radiation (IR) and the formation of reactive oxygen species (ROS). This results in cellular stress, cell death and inflammation, which can spiral out of control to cause radiation pneumonitis [1]. In the repair and remodelling process that starts the onset of inflammation, a pro-fibrotic environment may develop, leading to radiation-induced lung fibrosis [2]. Previous research has mainly focussed on alveolar injury as an initiating event, although recent studies in idiopathic pulmonary fibrosis (IPF) have shown that aberrant bronchial epithelial cells may also play a role in the development and continuation of lung fibrosis [3, 4] Therefore, bronchial epithelial cells are of interest in the pathogenesis of RILI. Furthermore, RILI is often accompanied by pathological changes in the airways [5], suggesting a role for effects of IR on bronchial epithelial cells. Within the airway epithelium, cells with progenitor function reside in the population of basal cells, and these cells can self-renew and act as precursors for the luminal cells [6]. Previous studies in primary bronchial epithelial cells cultured at the air-liquid interface (ALI) have shown that IR results in basal cell loss [7], and animal experiments suggest a reduction of progenitor cell function [8] and a reduction of colony formation [9]. These effects on epithelial progenitor function were confirmed in studies using irradiated mice-derived salivary gland organoids [10] and intestinal organoids [11].

The aim of the present study was to study the effect of IR on cultured human primary bronchial epithelial cells (PBEC), with a focus on cytotoxicity, barrier function and markers of inflammation and epithelial to mesenchymal transition (EMT). We specifically focussed on effects of epithelial progenitor cell function by assessing organoid formation capacity, which has been previously used to study progenitor cell function and responses after IR [12]. We hypothesized that radiation-induced effects on the normal airway epithelium play a significant role in development of RILI, and that results of the present study help unravel the pathogenesis of RILI.

Materials and methods

An extensive description of all materials and methods can be found in the supplemental material. In short, PBEC were obtained from our local biobank from patients who underwent resection surgery for lung cancer at the Leiden University Medical Centre, the Netherlands. This biobank was approved by the institutional medical ethical committee (B20.042/Ab/ab and B20.042/Kb/kb). PBEC from non-COPD donors were cultured at the Air-Liquid Interface (ALI-PBEC) for 14 days to allow mucociliary differentiation. For this study, cultures from a total of 5 donors were used, and cells from an additional 12 donors were used in the preliminary and pilot experiments. After differentiation, ALI-PBEC cultures were treated with 1, 2, 4, and 8 Gy photon radiation using the YXlon X-ray generator (YXLON International GmbH, Germany). First, to assess the impact on epithelial progenitor function, PBEC were dissociated at 24 h and 7 days after IR and 90,000 cells per sample were seeded in three 30 µl hydrogel droplets and cultured to assess and count organoid formation after 8 days of culture (Supplemental Fig. 2). Additionally, the effect of IR was investigated by using qPCR to detect transcription of pre-determined genes (EMT: Vimentin; Inflammation: IL-1β, IL6, IL-8/CXCL8; Cell cycle progression: P21/CDKN1A, AEN; Proliferation: MKI67, E2F1, ATF3; primer sequences in supplemental Table 1). The Trans Epithelial Electrical Resistance (TEER) was used as a measurement of barrier function and lactate dehydrogenase (LDH) release was measured to assess direct toxic effects of IR. Finally, immunofluorescence staining of γH2Ax-foci was performed to assess DNA-damage in ALI-PBEC after IR at different dosages. Results were analysed using GraphPad Prism (GraphPad Software, CA, USA) using paired Student's t-test and one-way ANOVA.

Results

PBEC were isolated from five donors with an age range of 52–73 years and a normal lung function. There were three female and two male donors, three donors had stage I NSCLC, one donor had a typical carcinoid, and for one donor the diagnosis was unknown. ALI-PBEC cultures from these donors were exposed to 1, 2, 4 and 8 Gy photon radiation (Fig. 1A), and next subjected to various analyses at different time points after irradiation. IR caused a dose-dependent increase of γH2AX foci measured with immunofluorescence staining using confocal microscopy at 1 h after IR (Fig. 1B). Gene expression was assessed by RT-PCR at 48 h or 7 days after exposure to 2 Gy, since this is a relevant dose for lung cancer patients who undergo fractionated radiotherapy. Measurements of mRNA levels for markers associated with EMT (vimentin) and inflammation (IL-1β, IL-6, IL-8/CXCL8,) showed no change after IR (Supplemental

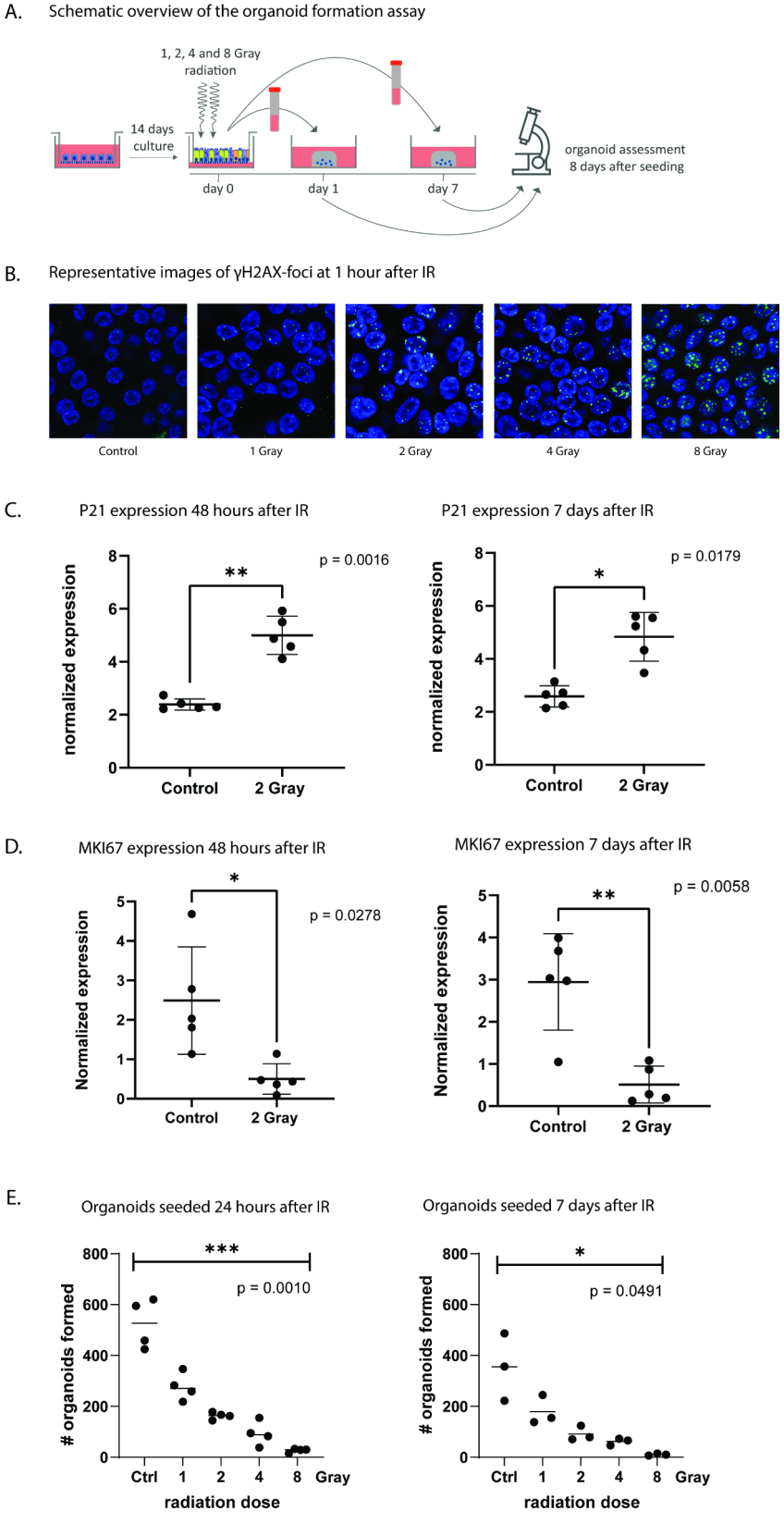

Fig. 1 Effects of IR on ALI-PBEC. (**A**) general experimental design with specific details for organoid formation (see materials and methods for details) (**B**) Immunofluorescence staining of γH2AX-foci (green) in DAPI-stained nuclei (blue) 1 h after radiation. Expression of P21/CDKN1A (**C**) and MKI67 (**D**) analysed by qPCR at 48 h and 7 days post-IR ($n=5$) E. organoid formation capacity: number of organoids based on 15 brightfield images at 10x magnification per organoid culture initiated at 24 h ($n=4$) and 7 days ($n=3$) following radiation (one-way ANOVA, 24 h: $p=0.0010$, 7 days: $p=0.0491$)

Fig. 1A). Preliminary experiments evaluating the same markers at other time-points and dosages also showed no change after IR (data not shown). However, expression of the cell cycle inhibitory marker P21/CDKN1A was significantly upregulated at 48 h and remained upregulated up to 7 days (Fig. 1C), and a similar pattern was seen for DNA damage response (DDR)-associated marker AEN (Supplemental Fig. 1B). The proliferation marker MKI67 was shown to be decreased significantly in the irradiated samples at 48 h and remained decreased at 7 days after IR (Fig. 1D). The same pattern could be observed for the cycle regulator E2F1 [13], but not for another proliferation-associated marker, ATF3 (Supplemental Fig. 1B). Furthermore, there were no changes in morphology, barrier function (TEER) or LDH-release, indicating that IR to ALI-PBEC did not cause cytotoxicity (Supplemental Fig. 1C, D). Importantly, IR caused a dose-dependent decrease in organoid formation capacity when assessed at 1 or 7 days after IR (Fig. 1E).

Discussion

These results show that exposure of differentiated human bronchial epithelial cells to IR results in a loss of epithelial progenitor cell function as assessed by an organoid formation assay, which persists for at least 7 days. Whereas IR did cause DNA breaks, caused an increase in gene expression of the cell cycle inhibitor P21/CDKN1A and a decrease in the proliferation markers MKI67 and ETF1, it did not affect viability, barrier function or production of a set of markers for inflammation and EMT, even at high IR dose. These findings extend previous findings on effects of IR on mouse airway epithelial progenitor function [8], effects on progenitor function in mouse salivary glands [10] and intestinal organoids [11]. They are in line with previous findings in a similar ALI-PBEC culture model as used in the present study, that showed that IR decreases the number of basal cells in such cultures [8]. Since basal cells constitute a heterogeneous population of cells displaying various functions, the present study adds by showing that IR decreases progenitor cell function specifically.

This finding is relevant for a better understanding of the pathogenesis of RILI, in view of the essential role of airway epithelial progenitor cells in repair and inflammation. Whereas its capacity to self-renew is essential for repair following injury, recent studies in chronic obstructive pulmonary disease (COPD) demonstrate that alterations in the composition of progenitor subpopulations may also impact on inflammation and remodelling of tissue [14]. These results suggest that IR may also have long-term effects on inflammation and tissue remodelling through alterations in airway epithelial progenitor populations. It is possible that this remodelling process also plays a role, together with the supporting stromal cells, in the airway pathology and development of bronchial stenosis, a known side effect of radiotherapy to the lungs with major impact on quality of life [15].

In line with previous studies on bronchial [8] and alveolar [16] epithelial cells, we found no effect of radiation on inflammation or markers of viability in our bronchial epithelial model. These studies similarly exposed PBEC [8] and alveolar epithelial cells [15] to different dosages of IR and did not find any effect on inflammation or EMT. The marked effect on the organoid formation capacity described in the current study could thus not be readily explained by changes in these markers of viability studied, but most likely results from the inhibitory effect of radiation on the cell cycle through the DDR which activates P53. The activation of the DDR is further supported by the occurrence of γH2AX foci and upregulation of AEN and P21. After activation of P53, it transcriptionally activates P21, which is a key factor in cell cycle arrest [17], and remained increased in our samples for at least 7 days. This cell cycle arrest allows the cells time for DNA repair, but here we show it also leads to a strong reduction of self-renewal in the progenitor cell population. Another related possibility is that IR induces senescence in the progenitor cell population. Senescence has been linked to the development of other pulmonary fibrotic diseases as well as COPD [18]. Others have also shown the occurrence of senescent progenitor cells in irradiated salivary gland organoids in mice [10], accompanied by decreased organoid formation which was rescued by senolytic drug ABT-263. The cell cycle arrest marker P21/CDKN1A that we found to be increased following radiation, is also associated with senescence [19], and therefore it is tempting to speculate that the observed effect of radiation on organoid formation is linked to the induction of senescence. However, we did not observe an increase in other markers of senescence or a senescence-associated secretory phenotype (SASP). It is possible that increases in these markers of senescence, or even EMT and inflammation, were not detected because the progenitor cell population is only a small proportion of the total cell population, and differences may therefore not have been detected in the current experimental setup.

This study has several strengths. Firstly, we used bronchial epithelial cells isolated from macroscopical normal tissue from several patients with lung cancer, who could be eligible for radiotherapy. These cells are therefore relevant to the patient category and disease development. Secondly, we used mucociliary differentiated cultures of primary cells reflecting the composition of the bronchial epithelium in vivo. Thirdly, there was very limited interdonor variability in the organoid formation capacity. Finally, the doses we used correspond to doses that are administered in the clinical setting for fractionated radiotherapy for lung cancer and some cases of stereotactic

body radiation therapy (SRBT). However, this study also has some limitations. Firstly, this in vitro model lacks stromal and immune cells and the interplay with and between these cell types. This might be a relevant feature in the development of RILI, as was recently shown by Dasgupta et al. who developed an alveolus-on-a-chip combined with primary endothelial cells and immune cells to model radiation-induced lung injury in vitro [16]. To study inflammation and senescence, the addition of immune cells and endothelial cells to our culture might be essential, since addition of peripheral blood mononuclear cells in increased responses to radiation in the alveolus-on-chip model [15], and endothelial cells have been shown to be susceptible for senescence and EMT after IR [20]. However, an advantage is that our model is simple and easy to use compared to the more technically challenging alveolus-on-chip or other co-culture models, and it is relevant for addressing questions regarding the basal epithelial cell population. A second limitation is that the organoid medium used in the culture is a rich medium containing several growth factors and the ROCK-inhibitor Y-27,632, which enhances cell survival. This medium could therefore influence the survival of the basal cells and may have caused an underestimation of the effect of radiation. A third limitation is that we used cells from donors that were diagnosed with lung cancer. Whereas this can be a considered a relevant population because these are the type of patients that undergo radiotherapy, cells from such patients may respond different compared to those from healthy donors. However, the cell donors had a lung function within the normal range; only a low tumour stage; and one donor had a typical carcinoid that has a different origin than NSCLC. Despite the heterogeneity in their baseline characteristics, the responses are similar in all donors, supporting the relevance of our findings. Nevertheless, it would be interesting to compare responses to those in cells derived from normal healthy donors. Finally, although we did observe a sustained increase in senescent marker P21/CDKN1A, we were unable to find a radiation-induced increase in other markers of senescence such as SASP or activity of β-galactosidase, and we can therefore cannot formally conclude that senescence plays a role in the diminished proliferation. Future studies are required using other senescence-associated markers to demonstrate a role of senescence in the decreased organoid formation observed following radiation.

In conclusion, epithelial progenitor cell function as assessed by organoid formation capacity is strongly reduced by IR and persists for at least 7 days. Despite an effect on DNA breaks and P21/CDKN1A expression, this effect was not accompanied by IR-induced cytotoxicity, changes in barrier function, or markers of inflammation and EMT. This study indicates that studying the effects of IR on organoid formation is a valid and sensitive tool to study adverse effects of IR on normal lung tissue, that could be used to study radiomitigators or other radiation modalities.

Abbreviations

AEN	Apoptosis Enhancing Nuclease
ATF3	Activating Transcription Factor 3
ALI-PBEC	Air-Liquid Interface-Primary Bronchial Epithelial Cells
ANOVA	Analysis of Variance
CDKN1A	Cyclin-Dependent Kinase Inhibitor 1 A
COPD	Chronic Obstructive Pulmonary Disease
CXCL8	Chemokine (C-X-C motif) ligand 8 (also known as IL-8)
EMT	Epithelial-Mesenchymal Transition
E2F1	E2F Transcription Factor 1 Gy: Gray
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8 (also known as CXCL8)
IR	Ionizing Radiation
LDH	Lactate Dehydrogenase
LUMC	Leiden University Medical Centre
MKI67	Marker Of Proliferation Ki-67
NSCLC	Non-Small Cell Lung Cancer
PBEC	Primary Bronchial Epithelial Cells
qPCR	Quantitative Polymerase Chain Reaction
RILI	Radiation-Induced Lung Injury
ROS	Reactive Oxygen Species
SASP	Senescence-Associated Secretory Phenotype
SRBT	Stereotactic Body Radiation Therapy
TEER	Trans Epithelial Electrical Resistance

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03105-z.

Supplementary Material 1

Supplementary Material 2

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Author contributions
MK, PH, AS drafted the experiments, MK and DN performed the experiments, MK and PH wrote the manuscript, AS and KvDW reviewed and corrected the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Human material that was used in this study was obtained from the local biobank, approved by the institutional medical ethical committee of the LUMC (B20.042/Ab/ab and B20.042/Kb/kb). Patients were enrolled via a no-objection system for coded anonymous use of such tissue (www.coreon.org) and consent was waived by the local METC. However, as of 01-09-2021 active informed consent is used in this biobank.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Giuranno L, Ient J, De Ruysscher D, Vooijs MA. Radiation-Induced Lung Injury (RILI). Front Oncol. 2019;9.
2. Kuipers ME, van Doorn-Wink KCJ, Hiemstra PS, Slats AM. Predicting Radiation-Induced Lung Injury in patients with Lung Cancer: challenges and opportunities. Int J Radiat Oncol Biol Phys. 2024;118(3):639–49.
3. Asghar S, Monkley S, Smith DJF, Hewitt RJ, Grime K, Murray LA, et al. Epithelial senescence in idiopathic pulmonary fibrosis is propagated by small extracellular vesicles. Respir Res. 2023;24(1):51.
4. Chakraborty A, Mastalerz M, Ansari M, Schiller HB, Staab-Weijnitz CA. Emerging roles of Airway Epithelial Cells in idiopathic pulmonary fibrosis. Cells. 2022;11(6).
5. Bucknell NW, Belderbos J, Palma DA, Iyengar P, Samson P, Chua K, et al. Avoiding toxicity with Lung Radiation Therapy: an IASLC Perspective. J Thorac Oncol. 2022;17(8):961–73.
6. Watson JK, Rulands S, Wilkinson AC, Wuidart A, Ousset M, Van Keymeulen A, et al. Clonal Dynamics Reveal two distinct populations of basal cells in slow-turnover Airway Epithelium. Cell Rep. 2015;12(1):90–101.
7. Giuranno L, Roig EM, Wansleeben C, van den Berg A, Groot AJ, Dubois L, et al. NOTCH inhibition promotes bronchial stem cell renewal and epithelial barrier integrity after irradiation. Stem Cells Transl Med. 2020;9(7):799–812.
8. Fouillade C, Curras-Alonso S, Giuranno L, Quelennec E, Heinrich S, Bonnet-Boissinot S, et al. FLASH irradiation spares lung progenitor cells and limits the incidence of radio-induced senescence. Clin Cancer Res. 2020;26(6):1497–506.
9. Farin AM, Manzo ND, Kirsch DG, Stripp BR. Low- and high-LET radiation drives clonal expansion of lung progenitor cells in vivo. Radiat Res. 2015;183(1):124–32.
10. Peng X, Wu Y, Brouwer U, van Vliet T, Wang B, Demaria M, et al. Cellular senescence contributes to radiation-induced hyposalivation by affecting the stem/progenitor cell niche. Cell Death Dis. 2020;11(10):854.
11. Fujimichi Y, Otsuka K, Tomita M, Iwasaki T. Ionizing radiation alters organoid forming potential and replenishment rate in a dose/dose-rate dependent manner. J Radiat Res. 2022;63(2):166–73.
12. Serrano Martinez P, Giuranno L, Vooijs M, Coppes RP. The Radiation-Induced Regenerative response of adult tissue-specific stem cells: models and signaling pathways. Cancers (Basel). 2021;13(4).
13. Wu L, Timmers C, Maiti B, Saavedra HI, Sang L, Chong GT, et al. The E2F1-3 transcription factors are essential for cellular proliferation. Nature. 2001;414(6862):457–62.
14. Rao W, Wang S, Duleba M, Niroula S, Goller K, Xie J, et al. Regenerative metaplastic clones in COPD Lung Drive inflammation and fibrosis. Cell. 2020;181(4):848–e6418.
15. Miller KL, Shafman TD, Anscher MS, Zhou SM, Clough RW, Garst JL, et al. Bronchial stenosis: an underreported complication of high-dose external beam radiotherapy for lung cancer? Int J Radiat Oncol Biol Phys. 2005;61(1):64–9.
16. Dasgupta Q, Jiang A, Wen AM, Mannix RJ, Man Y, Hall S, et al. A human lung alveolus-on-a-chip model of acute radiation-induced lung injury. Nat Commun. 2023;14(1):6506.
17. Abuetabh Y, Wu HH, Chai C, Al Yousef H, Persad S, Sergi CM, et al. DNA damage response revisited: the p53 family and its regulators provide endless cancer therapy opportunities. Exp Mol Med. 2022;54(10):1658–69.
18. Aghali A, Koloko Ngassie ML, Pabelick CM, Prakash YS. Cellular Senescence in Aging lungs and diseases. Cells. 2022;11(11).
19. Di Micco R, Krizhanovsky V, Baker D. d'Adda Di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol. 2021;22(2):75–95.
20. Bouten RM, Dalgard CL, Soltis AR, Slaven JE, Day RM. Transcriptomic profiling and pathway analysis of cultured human lung microvascular endothelial cells following ionizing radiation exposure. Sci Rep. 2021;11(1):24214.

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