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Association of SIRT6 Expression With Risk of Pneumonitis Induced by Radiotherapy in Cancer Patients

Fengyuan Yu[1,2] | Zheng Gong[1,2] | Yuan Li[3] | Danial F. Naseem[4] | Chen Li[2] | Miaowei Wen[2] | Bingying Zhao[2] | Zhezhe Xu[2] | Shanshan Zhang[2] | Rukun Zang[2] | Ailu Wu[2] | Qingxin Han[2] | Shuhui Wu[5] | Hongwei Li[1] | Yipeng Song[1,2]

[1]Department of Radiotherapy, Qingdao University, Qingdao, China | [2]Department of Radiotherapy, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, China | [3]Department of Radiology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, PR China | [4]Department of Head and Neck Surgery, MD Anderson Cancer Center, Houston, Texas, USA | [5]Department of Otorhinolaryngology, Baoshan Hospital Affiliated with Shanghai University of Traditional Chinese Medicine, Shanghai, PR China

Correspondence: Hongwei Li (lhw176@126.com) | Yipeng Song (syp1972@sina.com)

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ABSTRACT

Thoracic tumours represent a significant proportion of malignant cancers. While radiotherapy (RT) improves prognosis, it can also lead to side effects such as radiation-induced pneumonitis (RP). Since SIRT6 is involved in DNA repair, energy metabolism and inflammation, this study aims to investigate the expression of SIRT6 in lymphocytes as a potential biomarker and therapeutic target for RP. This study included 170 patients diagnosed with thoracic tumours, all of whom underwent thoracic RT. RP was evaluated and classified as severe RP (SRP) and lower as non-severe RP (NSRP). Analyses were performed using SPSS version 26.0 and the R. Among 170 patients in this study, 124 developed NSRP, and 46 experienced SRP. The univariate analysis showed that SIRT6 expression (cOR, 0.33, 95%CI, 0.18–0.97 before RT and 0.31, 0.19–0.98 after RT), clinical factors, dosimetric parameters and haematological/serological parameters were associated with SRP before and after RT. Our multivariable logistic regression showed that SIRT6 expression was significantly associated with risk of SRP before (aOR, 0.32, 95%CI, 0.15–0.96) and after RT (aOR, 0.32, 95%CI, 0.18–0.99) after adjustment with other confounders. Moreover, the receiver operating characteristic curve analysis revealed that the combined multivariable model exhibited superior predictive capability compared to any single predictor (overall AUC, 0.93, 95%CI, 0.90–0.97 before RT and AUC, 0.91, 95%CI, 0.87–0.96 after RT). The expression of SIRT6 alone or in combination with other risk factors was associated with an increased risk of SRP, suggesting a novel approach for the prevention and treatment of radiation pneumonitis in clinical practice.

1 | Introduction

Thoracic tumours, including lung cancer, oesophageal cancer and thymoma, constitute a significant proportion of thoracic malignancies. In 2017, approximately 200,000 new cases of lung cancer were reported in the United States, with up to 80% of patients deemed ineligible for surgical intervention [1, 2]. Oesophageal cancer is the eighth most common malignancy globally and ranks as the sixth leading cause of cancer-related mortality [3, 4]. Thymoma, the most prevalent tumour of the anterior mediastinum, accounts for 50% of such cases in adults. Complete surgical resection is considered the optimal treatment

Fengyuan Yu and Zheng Gong contributed equally to this study.

for thymoma, while postoperative radiotherapy is recommended for cases of incomplete resection [5].

Radiotherapy (RT) was first recognised as a treatment for breast tumours in the early 20th century and has since become a cornerstone of both curative and palliative care for various malignancies, including lung cancer, oesophageal cancer, breast cancer and thymoma. However, damage signals from unintentionally irradiated tissues can lead to severe side effects that often limit the effectiveness of radiation therapy. One notable adverse effect is radiation-induced lung injury (RILI), which results from radiation damage to normal lung tissue during thoracic cancer radiotherapy. The most common manifestations of RILI include radiation pneumonitis (RP) and radiation pulmonary fibrosis [6]. These damage signals, which include heightened inflammatory responses, sustained free radical generation, activation of oncogenes and suppression of tumour suppressor genes, can increase the risk of developing secondary primary tumours [7]. Radiation pneumonitis results from early inflammatory responses triggered by damage to the lung parenchyma, epithelial cells, vascular endothelial cells and stroma. This inflammatory process promotes the production of pro-inflammatory cytokines and chemokines, which recruit immune cells to the lung tissue, ultimately causing pneumonitis and leading to subsequent fibrosis. Acute pneumonitis typically occurs within 2–4 months after radiation therapy, while chronic fibrosis generally manifests around 6 months after treatment [8]. The incidence of RP varies significantly across studies. Recent data indicate that lung cancer has the highest incidence of RP, ranging from 5% to 25%, followed by mediastinal lymphoma (5%–10%) and breast cancer (1%–5%) [9].

However, there are currently no effective clinical methods for the prevention or treatment of RP. It is widely recognised that the development of RP is a complex process influenced by both clinical and dosimetric factors [10]. In cases of RP with significant clinical symptoms, systemic corticosteroid therapy may offer relief after infectious causes have been ruled out [11]. Nonetheless, key biomarkers for predicting the occurrence and severity of RP in clinical practice are currently lacking. Additionally, promising therapeutic targets for RP remain elusive, highlighting the urgent need for the development of novel treatment strategies and therapeutic agents.

A primary objective in radiation oncology is to identify biomarkers that can predict radiosensitivity, enabling the customisation of treatment plans for individual patients. A substantial body of evidence suggests that genetics play a central role in radiation response, offering a promising avenue for discovering predictive markers of radiation toxicity [12–17]. Genomic studies have shown significant promise in identifying gene expression profiles that can predict late-onset radiation toxicity [13, 18, 19]. The human SIRT6 protein, part of the sirtuin family known as silent mating-type information regulation homologs (SIRT), regulates various biological processes through its ADP-ribosyl transferase and NAD+-dependent deacetylase activities. These processes include telomere DNA repair and maintenance as well as energy metabolism. Consequently, SIRT6 is implicated in a wide range of diseases, including cancer, obesity, diabetes, cardiovascular diseases and fibrosis [20]. Additionally, SIRT6 has been shown to regulate inflammation. Most evidence suggests that SIRT6 exerts its anti-inflammatory effects by inhibiting the production of inflammatory cytokines and promoting the polarisation of immune cells towards an immunosuppressive phenotype. For example, SIRT6 has been demonstrated to promote the M2 polarisation of macrophages [21].

Macrophages are key components of the innate immune system and are primarily responsible for host defence. Beyond their role in protecting the host, macrophages also maintain tissue homoeostasis and can either promote or suppress inflammatory responses [22, 23]. To fulfil these distinct functions, macrophages exhibit remarkable plasticity and can adopt a range of polarisation states, with M1 and M2 macrophages representing the two extremes [23]. M1 macrophages are induced by a combination of the Th1 cytokine interferon-gamma (IFN-γ) and toll-like receptor ligands, such as lipopolysaccharide. During M1 activation, the expression of pro-inflammatory genes, including TNF-α, IL-1β, IL-6, IL-8 and IL-12, is upregulated, along with the expression of pattern recognition receptors such as toll-like receptors and node-like receptors [24]. Macrophages can also be activated by various noninflammatory factors. Non-M1 macrophages are generally classified as M2, with Th2 cytokines IL-4 and IL-13 serving as inducers of alternatively activated macrophages [25]. Functionally, M2 macrophages suppress Th1/M1-driven inflammation, promote tissue repair and mediate Th2-driven conditions such as asthma and helminth infections [26, 27].

The involvement of lymphocytes in early radiation-induced pulmonary inflammation has been increasingly supported by a growing body of evidence [28].

Macrophages are highly plastic and heterogeneous cells with differentiation mechanisms that vary based on their response to stimuli and tissue localisation. Various T cell subsets play a key role in regulating the polarisation of macrophages into distinct phenotypes [29]. Research has also demonstrated that SIRT6 suppresses inflammation by influencing lymphocyte differentiation and function [30]. Macrophages are primarily located in tissues, whereas lymphocytes are predominantly found in the bloodstream. In terms of sample accessibility, lymphocytes are clearly more readily obtainable than macrophages.

The information presented suggests a significant relationship between SIRT6, metabolism, cancer and various inflammatory responses. However, the role of SIRT6 expression in lymphocytes within the context of RP remains unclear. Could SIRT6 serve as a biomarker alone or in combination with other risk factors to predict the risk of RP? Might it represent a potential therapeutic target for RP? To address these questions, we conducted this study.

2 | Materials and Methods

2.1 | Study Patients

This study included patients with oesophageal cancer, lung cancer and thymoma who underwent thoracic radiation therapy at Yantai Yuhuangding Hospital from October 2022 to

January 2024. The inclusion criteria were as follows: a confirmed pathological or cytological diagnosis, ineligibility for surgery, completion of thoracic radiation therapy, absence of severe complications other than radiation pneumonitis and signed informed consent before enrolment. The exclusion criteria included active concurrent cancer, a total radiation dose of less than 45 Gy, a follow-up time of less than 6 months, the presence of other severe pulmonary infectious diseases and a history of surgery or additional radiation therapy following chemoradiotherapy. A total of 191 patients were included in this study. Among them, three patients developed a second primary tumour during the follow-up period, ten patients did not undergo follow-up for various reasons (including patients' refusal to undergo genetic testing, seeking medical treatment at other institutions, etc.), and eight patients underwent surgery or radiotherapy again. Ultimately, a total of 170 patients met the inclusion criteria. The research adhered to the principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board of Yantai Yuhuangding Hospital.

2.2 | Dose-Volume Histogram (DVH) Parameters

The DVH parameters for radiation therapy primarily include the mean lung dose (MLD), the percentage of lung volume exposed to more than 20 Gy of radiation (V20), the percentage of lung volume exposed to more than 30 Gy of radiation (V30), the percentage of lung volume exposed to more than 40 Gy of radiation (V40), planning target volume (PTV) and gross tumour volume.

2.3 | Follow-Up and Evaluation of Radiation Pneumonitis

The diagnosis of radiation pneumonitis was based on the Common Terminology Criteria for Adverse Events version 5.0 from the National Cancer Institute [31], with consensus reached by two radiation oncologists. Radiation pneumonitis was diagnosed when consolidation or ground-glass opacities were observed on chest X-ray or CT images. These radiographic findings were confined to the radiation field with relatively well-defined borders, and other potential causes of lung changes due to radiation were excluded. Patients were typically monitored with contrast-enhanced CT imaging every 2–3 months. A comprehensive evaluation was conducted by combining imaging results with clinical symptoms, and the symptom with the highest severity during follow-up was selected as the outcome. Patients were classified into the severe radiation pneumonitis (SRP) group if they had grade 3 or higher RP, while those with grade 2 or lower were categorised as the non-SRP (NSRP) group.

2.4 | Haematological and Serological Parameter Testing

Haematological and serological examinations were conducted on the enroled patients before radiotherapy and at each follow-up. These tests included assessments of the white blood cell count, C-reactive protein (CRP) and various cytokines, such as IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN-α, IFN-γ and TNF-α.

2.5 | RNA Extraction and Quantitative PCR Analysis

Haematological tests were performed on the enroled patients before radiation therapy and at each follow-up visit. Additionally, extra blood samples were collected from the patients for laboratory analysis. Lymphocytes were first isolated from the patients' blood samples using a lymphocyte separation reagent. Total RNA was then extracted using TRIzol reagent. First-strand cDNA was synthesised from the total RNA using TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (TransGen). Radiation therapy quantitative PCR was performed using PerfectStart Green qPCR SuperMix on a CFX Maestro system (Bio-Rad Laboratories) for SIRT6 expression, with GAPDH serving as the internal loading control. The Bio-Rad CFX Maestro real-time monitoring system was used according to the manufacturer's instructions. Relative expression levels were calculated using the 2^(−ΔΔCT) method for relative quantification.

2.6 | Statistical Analysis

Descriptive statistics were used to summarise baseline patient and treatment characteristics. Univariate and multivariate analyses were performed using binary logistic regression to assess the relationship between risk factors and RP. To avoid instability and imprecision in the coefficient estimates, multicollinearity was tested, and highly correlated dosimetric and biological variables were excluded from the final multivariate logistic regression model. Based on the independent variables, curve estimation was used to assess the risk of SRP, providing recommended cutoff values. The predictive ability of the identified covariates for SRP was evaluated using the area under the curve (AUC) from the receiver operating characteristic (ROC) analysis. Statistical analyses were performed using SPSS 26.0, with a p-value < 0.05 (two-sided) considered statistically significant. The R programming language was utilised for the visualisation of the chart, ROC curve and calibration curve. Decision curve analysis was employed to evaluate the differentiation, accuracy and practicality of the mode.

3 | Results

3.1 | Patient Clinical and Dose-Volume Characteristics

Clinical characteristics included 11 factors: sex, age, primary tumour location, pathology, clinical stage, respiratory system history (particularly COPD), diabetes, smoking history, synchronous chemotherapy, synchronous immunotherapy and RP classification. Details of patient demographics and characteristics are presented in Table 1. Dosimetric parameters included

TABLE 1 | Clinical characteristics and DVH parameters of 170 patients.

Characteristics	No. of patients (%) or mean ± SD
Sex	
Male	136 (80)
Female	34 (20)
Age	62.9 ± 10.0
Primary tumour location	
Oesophagus	33 (19.4)
Lung	121 (71.2)
Thymus	16 (9.4)
Pathology	
Squamous carcinoma	75 (44.1)
Others	95 (55.9)
Clinical stage	
I/II	55 (32.3)
III/IV	115 (67.7)
History in respiratory system	
Yes	68 (40)
No	102 (60)
Diabetes	
Yes	88 (51.8)
No	82 (48.2)
Smoking history	
Yes	88 (51.8)
No	82 (48.2)
Synchronous chemotherapy	
Yes	96 (56.5)
No	74 (43.5)
Synchronous immunotherapy	
Yes	50 (29.4)
No	120 (70.6)
RP classification	
1–2	124 (73.0)
3–5	46 (27.0)
RT Technique	
IMRT	109 (64.1)
TOMO	61 (35.9)
MLD (Gy)	1004.2 ± 283.3
PTV Volume (cm^3)	347.4 ± 130.0
GTV Volume (cm^3)	84.8 ± 68.0
Whole lung V20 (%)	18.4 ± 6.3
Whole lung V30 (%)	11.2 ± 4.5
Whole lung V40 (%)	6.3 ± 3.3

Abbreviation: DVH, dose-volume histogram; SD, standard deviation.

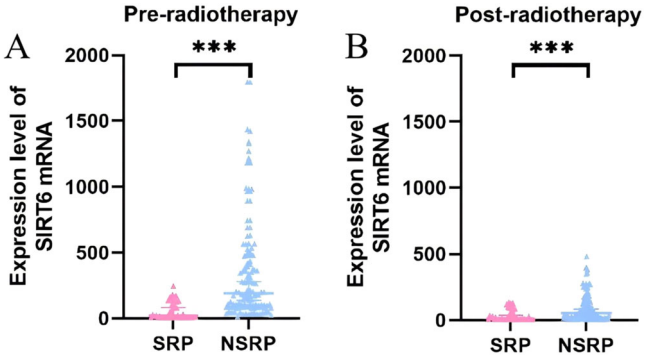

FIGURE 1 | The SIRT6 expression levels in the SRP and NSRP groups before and after radiotherapy. A: SIRT6 expression levels in the SRP and NSRP groups before radiotherapy. B: SIRT6 expression levels in the SRP and NSRP groups after radiotherapy (***$p < 0.001$). NSRP, non-severe RP; SRP, severe radiation-induced pneumonitis.

seven factors: radiotherapy technique, MLD, PTV volume, GTV volume, whole lung V20, whole lung V30 and whole lung V40.

3.2 | Expression of SIRT6 mRNA in Blood Lymphocytes Detected by qPCR

Blood samples were collected from 170 tumour patients who underwent chest RT both before treatment and within 6 months posttreatment. Lymphocytes were isolated from these samples, and total RNA was extracted from the lymphocytes. The expression of SIRT6 mRNA was then measured using a one-step qPCR method. When normalised to GAPDH, the mean SIRT6 mRNA expression in blood lymphocytes was 280.92 ± 366.28 before radiotherapy and 81.17 ± 94.65 within 6 months postradiotherapy. Before radiotherapy, the mean SIRT6 mRNA expression in blood lymphocytes was 61.61 ± 66.16 in the SRP group and 362.28 ± 397.58 in the NSRP group. Within 6 months postradiotherapy, the mean SIRT6 mRNA expression was 38.37 ± 43.53 in the SRP group and 97.05 ± 103.33 in the NSRP group (Figure 1).

3.3 | Haematological and Serological Indicators

Inflammatory cytokines, white blood cells and CRP levels were measured in blood samples collected from patients both pre- and postradiotherapy. Notably, the pre-radiotherapy levels of TNF-α, IL-1β, IL-2 and IL-6 were significantly higher in the SRP group than in the NSRP group, while IL-10 and IL-4 expression were markedly higher in the NSRP group than in the SRP group. However, no significant differences were observed between the two groups in terms of white blood cell count, CRP, IL-5, IFN-α, IFN-γ, IL-8, IL-12p70 or IL-17A (Figure 2).

Postradiotherapy, the patients in the SRP group exhibited significantly higher levels of CRP, IL-17A, TNF-α, IL-1β, IFN-γ and IL-6 than those in the NSRP group. In contrast, IL-4 and IL-10 levels were significantly higher in the NSRP group than in the SRP group. No notable differences were observed between the two groups in terms of white blood cell count, IL-5, IFN-α, IL-2, IL-12p70 or IL-8 (Figure 2).

3.4 | Univariate Analysis of Radiation Pneumonia

In this study, 46 out of 170 patients (27.06%) developed grade 3 or higher RP postradiotherapy, including 36 cases of grade 3 RP, 9 cases of grade 4 RP and 1 case of grade 5 RP. The results of the univariate logistic regression analysis for SRP before radiotherapy are shown in Table 2. Among the 32 variables used, 13 were significantly associated with the occurrence of SRP. Similarly, the results of the univariate logistic regression analysis for SRP postradiotherapy are presented (Table 2), where 17 variables were found to be significantly associated with the development of SRP.

3.5 | Multivariate Analysis of Radiation Pneumonia

Based on the above results, a multivariate logistic regression analysis was conducted for SRP. For the pre-radiotherapy SRP model, the final variables included age, concurrent immunotherapy, concurrent chemotherapy, diabetes, respiratory system history and other relevant clinical factors. Additionally, dosimetric parameters, such as MLD, V20 and PTV, were incorporated, along with serological parameters, including TNF-α, IL-1β, IL-6 and IL-10, as well as the relative expression level of SIRT6. For the postradiotherapy SRP model, the final variables included age, concurrent immunotherapy, concurrent chemotherapy, diabetes, respiratory system history, and other relevant clinical factors. Additionally, dosimetric parameters, such as MLD, V20, V30, and PTV, were included, along with serological parameters, including TNF-α, IFN-γ, IL-1β, IL-6, IL-10 and CRP, as well as the relative expression level of SIRT6. Regression coefficients were obtained using the maximum likelihood method, and the corresponding odds ratios were calculated. Detailed results are presented in Table 3.

The multivariate analysis before radiotherapy revealed that respiratory system history, V20 and MLD were independent risk factors for SRP, while SIRT6 was identified as an independent protective factor. The postradiotherapy multivariate analysis identified respiratory system history, V20, MLD and IL-6 as independent risk factors for SRP, while IL-10 and SIRT6 were identified as independent protective factors.

3.6 | The ROC Curve and Prediction Nomogram

The ROC curve directly represents the sensitivity and specificity of a diagnostic test through the AUC. The higher the ROC, the more that the curve is positioned towards the upper-left corner and the larger the AUC, the higher the clinical diagnostic value of the test. Therefore, to conduct further ROC curve analysis, we used the occurrence of SRP as the test variable. For the pre-radiotherapy prediction model, the ROC curve was established using pre-radiotherapy SIRT6 expression, V20, MLD and

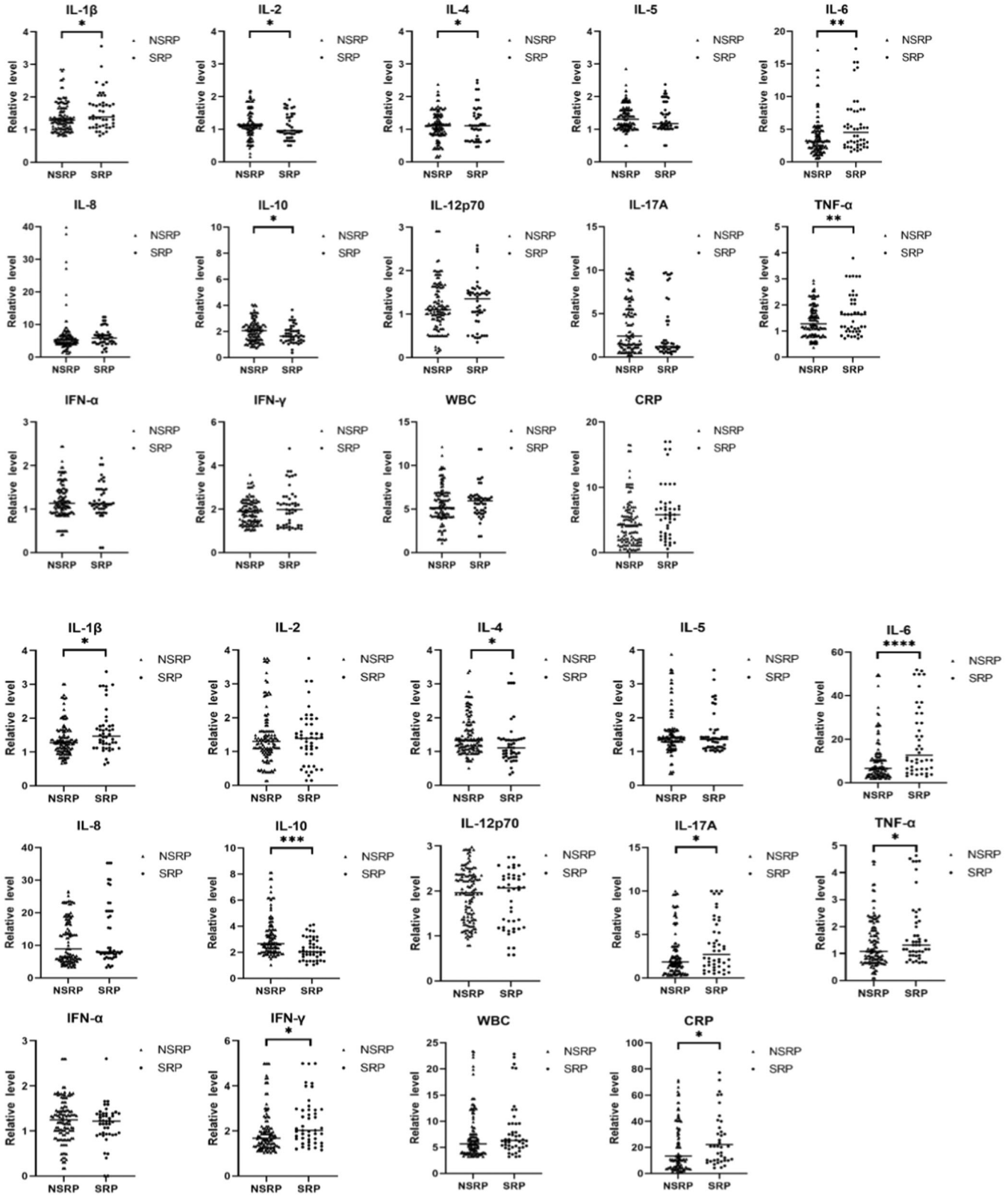

FIGURE 2 | Differences in haematological and serological markers before radiotherapy and after radiotherapy (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

respiratory system history as state variables. For the post-radiotherapy prediction model, the ROC curve was established using postradiotherapy SIRT6 expression, V20, MLD, IL-6, IL-10 and respiratory system history as state variables. From Table 4, it is evident that the ROC curves of the prediction models outperformed any individual variables. The ROC AUC for before the RT prediction model was 0.93 (95% CI: 0.90–0.97) for after the RT prediction model, it was 0.91 (95% CI: 0.87–0.96). The predictive model was visualised in nomogram shown in Figures 3 and 4. The calibration curve showed that the

TABLE 2 | Univariate logistic regression analysis for SRP before and after radiotherapy.

Variable	Before radiotherapy		After radiotherapy	
	p-value	Crude OR (95% CI)	*p*-value	Crude OR (95% CI)
Sex	0.931		0.931	
Male		1.00 (ref.)		1.00 (ref.)
Female		0.96 (0.42–2.25)		0.96 (0.42–2.25)
Age	0.010*	1.05 (1.01–1.09)	0.010*	1.05 (1.01–1.09)
Primary tumour location	0.080		0.080	
Oesophagus		1.00 (ref.)		1.00 (ref.)
Left lung		1.62 (0.51–5.19)		1.62 (0.51–5.19)
Right lung		3.08 (1.04–9.11)		3.08 (1.04–9.11)
Both lungs		2.40 (0.46–12.5)		2.40 (0.46–12.5)
Thymus		2.55 (0.61–10.6)		2.55 (0.61–10.6)
Pathology	0.780		0.780	
Squamous carcinoma		1.00 (ref.)		1.00 (ref.)
Adenocarcinoma		0.78 (0.33–1.85)		0.78 (0.33–1.85)
Small cell carcinoma		0.75 (0.31–1.84)		0.75 (0.31–1.84)
Thymoma		1.10 (0.34–3.52)		1.10 (0.34–3.52)
Clinical stage	0.257		0.257	
I		1.00 (ref.)		1.00 (ref.)
II		0.35 (0.07–1.83)		0.35 (0.07–1.83)
III		0.42 (0.08–2.05)		0.42 (0.09–2.05)
IV		0.91 (0.17–4.77)		0.91 (0.17–4.77)
History in respiratory system	< 0.001*		< 0.001*	
Yes		4.86 (2.35–10.1)		4.86 (2.35–10.1)
No		1.00 (ref.)		1.00 (ref.)
Diabetes	0.014*		0.014*	
Yes		2.43 (1.19–4.94)		2.43 (1.19–4.94)
No		1.00 (ref.)		1.00 (ref.)
Smoking history	0.779		0.779	
Yes		0.91 (0.46–1.79		0.91 (0.46–1.80)
No		1.00 (ref.)		1.00 (ref.)
Synchronous chemotherapy	0.016*		0.016*	
Yes		2.46 (1.18–5.11)		2.46 (1.18–5.11)
No		1.00 (ref.)		1.00 (ref.)
Synchronous immunotherapy	0.040*		0.04*	
Yes		2.11 (1.03–4.31)		2.11 (1.03–4.31)
No		1.00 (ref.)		1.00 (ref.)
RT Technique	0.859		0.859	
IMRT		1.00 (ref.)		1.00 (ref.)
TOMO		1.07 (0.53–2.15)		1.07 (0.53–2.15)
MLD (Gy)	0.004*	1.00 (1.00–1.01)	0.004*	1.00 (1.00–1.01)
PTV Volume (cm^3)	0.027*	1.00 (1.00–1.01)	0.027*	1.00 (1.00–1.01)
GTV Volume (cm^3)	0.308	1.00 (1.00–1.01)	0.308	1.01 (1.00–1.01)
Whole lung V20 (%)	< 0.001*	1.22 (1.12–1.32)	< 0.001*	1.22 (1.12–1.32)
Whole lung V30 (%)	0.562	1.02 (0.95–1.10)	0.562	1.02 (0.95–1.10)
Whole lung V40 (%)	0.919	1.01 (0.91–1.11)	0.919	1.01 (0.91–1.11)

(Continues)

TABLE 2 | (Continued)

Variable	Before radiotherapy		After radiotherapy	
	p-value	Crude OR (95% CI)	*p*-value	Crude OR (95% CI)
WBC (10^9/L)	0.371	1.08 (0.92–1.26)	0.279	1.04 (0.97–1.11)
CRP (mg/L)	0.893	1.00 (0.96–1.05)	0.048*	1.01 (1.00–1.01)
IL-1β (pg/mL)	0.049*	1.96 (1.00–3.81)	0.046*	1.70 (1.01–2.87)
IL-2 (pg/mL)	0.059	1.43 (0.99–2.07)	0.550	0.88 (0.59–1.33)
IL-4 (pg/mL)	0.054	1.75 (0.99–3.11)	0.049*	0.51 (0.26–0.99)
IL-5 (pg/mL)	0.982	0.99 (0.42–2.33)	0.792	0.94 (0.54–1.60)
IL-6 (pg/mL)	0.004*	1.16 (1.05–1.28)	< 0.001*	1.06 (1.03–1.08)
IL-8 (pg/mL)	0.497	0.98 (0.92–1.04)	0.156	1.03 (0.99–1.08)
IL-10 (pg/mL)	0.043*	0.61 (0.38–0.98)	< 0.001*	0.45 (0.29–0.70)
IL-12p70 (pg/mL)	0.528	1.21 (0.67–2.22)	0.542	0.84 (0.48–1.47)
IL-17A (pg/mL)	0.110	0.91 (0.81–1.02)	0.037*	1.13 (1.01–1.27)
IFN-α	0.096	1.55 (0.93–2.55)	0.505	0.81 (0.43–1.52)
IFN-γ	0.101	1.50 (0.92–2.42)	0.019*	1.47 (1.07–2.01)
TNF-α	0.006*	1.86 (1.20–2.88)	0.028*	1.43 (1.04–1.98)
SIRT6 expression (high vs. low)	< 0.001*	0.33 (0.18–0.97)	0.001*	0.31 (0.19–0.98)

Abbreviation: SRP, severe radiation-induced pneumonitis.
*$p < 0.05$.

TABLE 3 | Multivariate logistic regression analysis for SRP before and after radiotherapy.

Variable	Before radiotherapy		After radiotherapy	
	p-value	Adjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)
Age	0.262	1.05 (0.97–1.13)	0.094	1.07 (0.99–1.14)
History in respiratory system	0.028*	3.86 (1.16–12.9)	0.019*	4.72 (1.29–17.3)
Diabetes	0.252	2.31 (0.55–9.62)	0.585	1.48 (0.36–5.99)
Synchronous chemotherapy	0.194	2.51 (0.63–10.1)	0.150	2.56 (0.71–9.21)
Synchronous immunotherapy	0.759	1.24 (0.32–4.79)	0.070	3.56 (0.90–13.9)
MLD (Gy)	0.008*	1.00 (1.00–1.01)	0.032*	1.00 (1.00–1.01)
PTV volume (cm^3)	0.411	1.00 (0.99–1.10)	0.131	1.00 (0.99–1.01)
Whole lung V20 (%)	0.002*	1.27 (1.09–1.49)	0.002*	1.24 (1.08–1.41)
CRP (mg/L)	0.877	1.01 (0.97–1.11)	0.706	1.00 (0.99–1.01)
IL-1β (pg/mL)	0.061	4.77 (0.93–24.4)	0.086	2.25 (0.89–5.70)
IL-4 (pg/mL)	0.061	1.73 (0.96–3.01)	0.526	0.71 (0.25–2.04)
IL-6 (pg/mL)	0.124	1.16 (0.96–1.41)	0.015*	1.05 (1.01–1.10)
IL-10 (pg/mL)	0.941	0.97 (0.39–2.42)	0.037*	0.46 (0.22–0.96)
IL-17A (pg/mL)	0.131	0.93 (0.84–1.08)	0.099	1.21 (0.97–1.51)
IFN-γ	0.110	1.51 (0.91–2.54)	0.272	1.35 (0.79–2.31)
TNF-α	0.113	2.39 (0.81–7.01)	0.401	1.33 (0.68–2.60)
SIRT6 relative expression	< 0.001*	0.32 (0.15–0.96)	0.002*	0.32 (0.18–0.99)

*$p < 0.05$.

diagnostic efficiency of the model is in alignment with the actual diagnosis. Finally, the decision curve analysis result showed that the model has a satisfactory positive net benefit, suggesting that the model can provide clinically relevant predictions of SRP (Figures 3 and 4).

4 | Discussion

Generally, RP occurs about 4 weeks after conventional fractionated radiotherapy. Signs of pulmonary infection include unilateral or bilateral lung opacities, tree-in-bud opacities and

TABLE 4 | The SRP prediction model before and after RT.

Variable	Before RT		After RT	
	AUC	95% CI	AUC	95% CI
History in respiratory system	0.69	0.60–0.78	0.69	0.60–0.78
MLD (Gy)	0.63	0.53–0.72	0.63	0.53–0.72
Whole lung V20 (%)	0.74	0.66–0.82	0.74	0.66–0.82
IL-6 (pg/mL)	0.62	0.40–1.21	0.70	0.61–0.79
IL-10 (pg/mL)	0.57	0.34–1.32	0.70	0.61–0.79
SIRT6 relative expression	0.85	0.79–0.91	0.71	0.63–0.80
Prediction model	0.93	0.90–0.97	0.91	0.87–0.96

Abbreviations: RT, radiotherapy; SRP, severe radiation-induced pneumonitis.

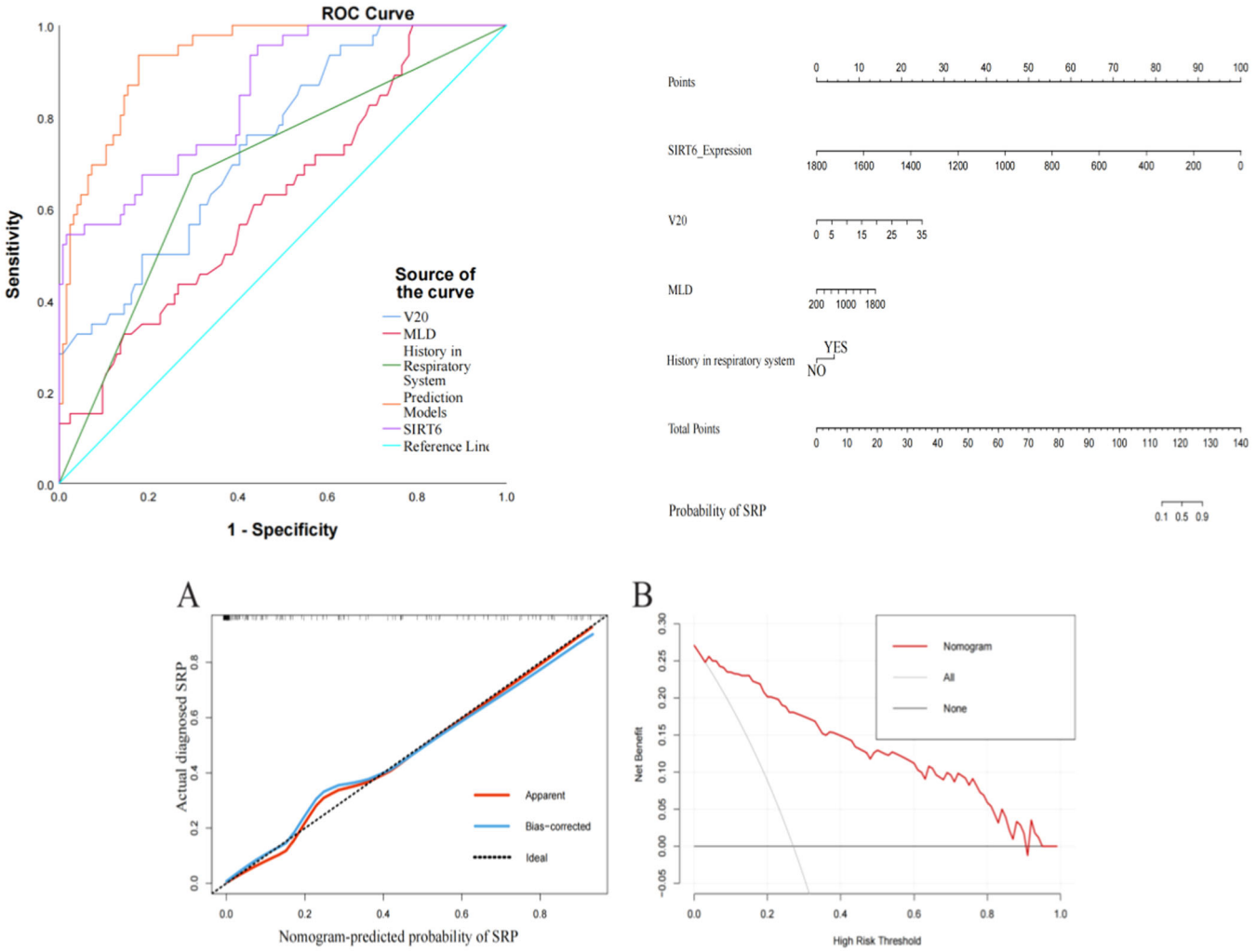

FIGURE 3 | The ROC, prediction nomogram of SRP, and calibration curve and decision curve (A: calibration curve. B: decision curve) before RT. ROC, receiver operating characteristic; RT, radiotherapy; SRP, severe radiation-induced pneumonitis.

cavitation appearing before the completion of radiation. Radiation-induced necrosis and local recurrence can also present with cavitation; however, these typically develop some time after the end of treatment [32].

There are no standard laboratory or imaging tests that can definitively identify RP, as it is primarily a clinical diagnosis. Some patients may exhibit elevated white blood cell counts, erythrocyte sedimentation rates, or CRP levels; however, these findings are nonspecific. Differential complete blood cell counts are commonly used to assess most patients, while additional laboratory tests may be conducted as needed to evaluate other potential causes [9]. This is consistent with our findings, which indicate that while differences in complete blood cell counts are

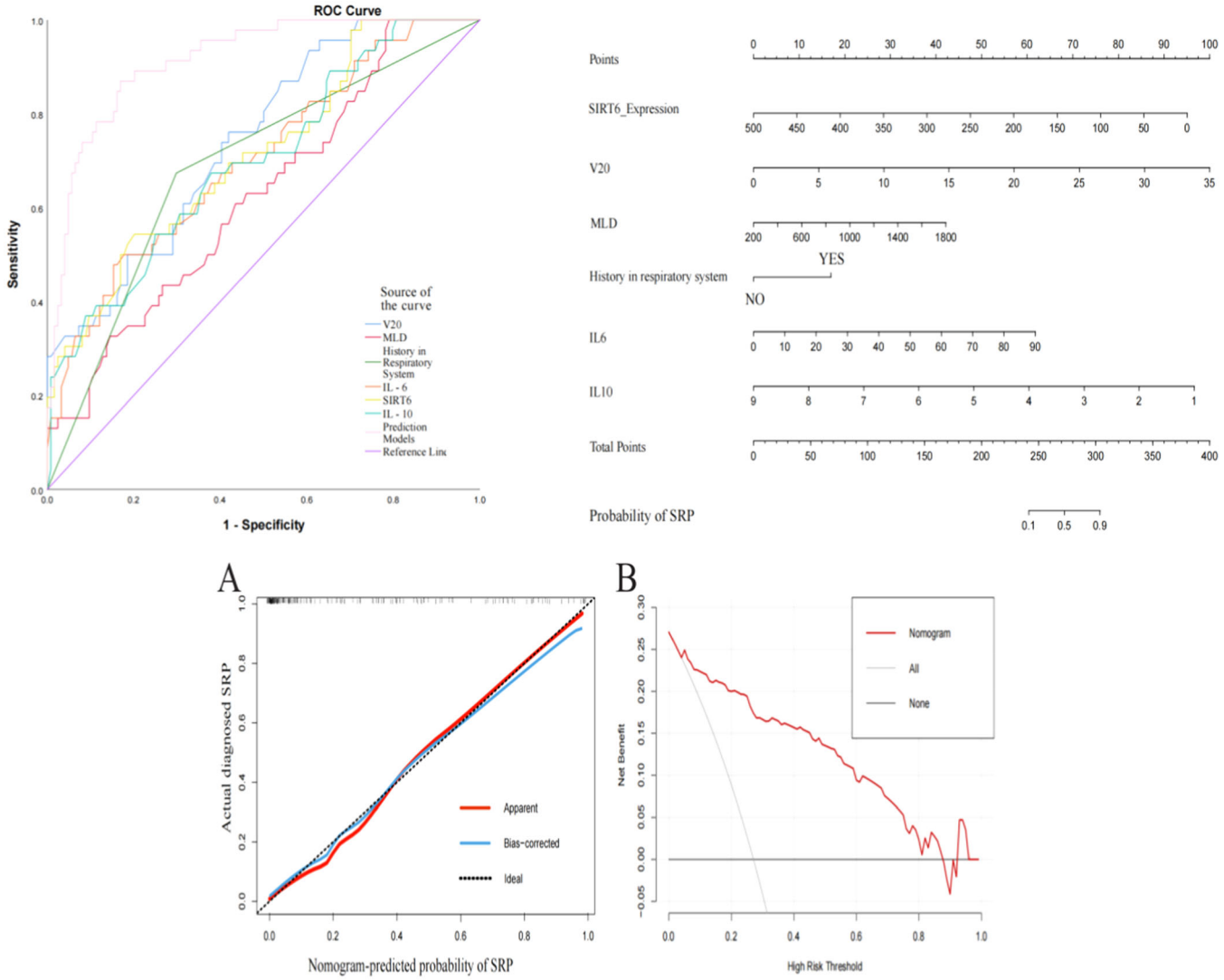

FIGURE 4 | The ROC, prediction nomogram of SRP, and calibration curve and decision curve (A: calibration curve. B: decision curve) after RT. ROC, receiver operating characteristic; RT, radiotherapy; SRP, severe radiation-induced pneumonitis.

useful for assessing the patient's current physical condition, they hold no predictive value for RP.

The goal of chest radiotherapy is to optimise tumour control while preventing SRP. Identifying patients at risk for SRP may allow for individualised treatment modifications, such as reducing the radiation dose to critical normal tissues or adding radioprotective agents, thereby decreasing the incidence of SRP and expanding the therapeutic window [33, 34]. Therefore, identifying the risk factors for SRP is of critical importance.

Previous studies have indicated that radiation dose is a key risk factor for the development of radiation pneumonitis, including parameters such as MLD and DVH metrics like lung V20 and V30 [32]. Generally, particularly in lung cancer patients, radiation oncologists aim to limit radiation pneumonitis by minimising the lung volume exposed to 20 or 30 Gy (represented as V20 and V30, respectively). Higher V20 values have been demonstrated to be predictive of radiation pneumonitis [9]. The simultaneous study by Tsujino, Leprieur, Pinnix and Farr confirmed that the incidence of RP increases with higher V20 values [35]. Therefore, strict limitations are imposed on V20 in concurrent chemoradiotherapy protocols. Tsujino's multivariate analysis during concurrent chemoradiotherapy indicated that $V20 \geq 26\%$ is a significant risk factor for developing Grade 3 or higher RP [36]. Lastly, a meta-analysis by Zhang et al. demonstrated that RP frequently occurs in patients receiving radiotherapy with $V20 > 25\%$ [9]. Our findings further validate the predictive value of MLD and V20 in the occurrence of RP. Although there is no standardised definition for normal lung dose calculation, and the cutoff values for dosimetric parameters remain debated, both clinical trials and the National Comprehensive Cancer Network practice guidelines have identified V20 and MLD as critical constraints in lung dose assessment [37].

In addition to dosimetric factors, the occurrence of RP following radiotherapy is also closely associated with patient health status, disease condition and treatment modalities. Factors such as age, respiratory system history, interstitial lung disease, diabetes

and concurrent systemic chemotherapy or immunotherapy are all significant contributors to this condition [37].

The SIRT protein family consists of NAD+-dependent deacetylases that have been strongly conserved during evolution in eubacteria, archaea and eukaryotes, especially mammals. Due to their reliance on NAD+ as a primary substrate, sirtuins are also closely linked to cellular energy sensing [38]. Beyond energy sensing, these proteins also regulate DNA repair, mitochondrial structure, mitochondrial metabolism, inflammation, redox homoeostasis and even cell death. Altered sirtuin activity is associated with various pathologies, including neurodegenerative diseases, obesity, diabetes, cancer, cardiovascular diseases, in particular, fibrosis and age-related degenerative conditions. Due to their essential roles in cellular longevity, telomere maintenance and DNA repair, sirtuins are often referred to as anti-ageing proteins [20]. Sirtuin 6 (SIRT6) is a crucial chromatin-regulating protein within the Sirtuin (SIRT) family. SIRT6 catalyses the deacetylation of histone H3 lysines K9, K18 and K56 [39].

SIRT6 has been a target of investigation in research on mammalian longevity [40]. In a recent study, Roichman et al. found that overexpression of SIRT6 under the CAG promoter increased the median lifespan of male mice by 27% and that of female mice by 15% compared to wild-type controls [41]. SIRT6 exhibits diverse roles in cancer, acting to either suppress or promote tumours depending on the specific context [42]. Abbotto et al. have demonstrated that SIRT6 plays a promoting role in skin cancer development, suggesting that targeting SIRT6 with inhibitors could be an effective strategy for treating cutaneous squamous cell carcinoma [43].

In this study, we observed that patients with low SIRT6 expression in lymphocytes postradiotherapy had a significantly higher likelihood of developing SRP than those with high expression. This finding uncovers a previously unknown link between SIRT6 and RP, suggesting that SIRT6 may have the potential to modulate inflammatory responses.

Evidence indicates that overexpression of SIRT6 can suppress various inflammatory responses, such as collagen-induced arthritis and inflammation induced by hypoxia in human osteoblasts [44]. A study of bronchial asthma revealed that SIRT6 is involved in airway remodelling, finding that SIRT6 provides protection by reducing the recruitment of inflammatory cells, blocking the accumulation of bronchial mucus and preventing allergen-induced inflammation in bronchial asthma [45]. TNF-α and NF-κB are two of the most critical pro-inflammatory factors involved in inflammation. The anti-inflammatory effects of SIRT6 are primarily attributed to its interaction with pro-inflammatory gene expressions related to TNF-α, NF-κB and c-Jun, thereby modulating these factors in a specific manner [20]. Research indicates that SIRT6 can exert anti-inflammatory effects by inhibiting the function of downstream TNF-α, which is known to activate nuclear factor NF-κB (a potent pro-inflammatory cytokine) [45]. Research by Kawahara et al. demonstrated that SIRT6 exerts its anti-inflammatory effects by physically interacting with the NF-kB subunit RELA (v-rel avian reticuloendotheliosis viral oncogene homolog A). This interaction recruits SIRT6 to the promoters of NF-kB target genes, where it functions as a corepressor of NF-kB. By deacetylating H3K9 on the target gene promoters, SIRT6 silences NF-kB target genes, thereby exerting its anti-inflammatory effects [46].

Furthermore, SIRT6-deficient mice exhibited increased chronic inflammation in the liver and adipose tissue. Mechanistically, SIRT6 knockout in human umbilical vein endothelial cells (HUVECs) leads to increased expression of NF-κB and pro-inflammatory cytokines, such as IL-1β, IL-6 and IL-8. Conversely, overexpression of SIRT6 is associated with reduced NF-κB transcriptional activity [44].

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by the proliferation of fibroblast-like synoviocytes and cartilage destruction. Research indicates that SIRT6 protects chondrocytes from RA by uniquely inhibiting the infiltration and polarisation of synovial macrophages [47]. Mechanistically, the deficiency of macrophage SIRT6 leads to inflammation by stabilising the FOXO1 protein through the regulation of its acetylation. In contrast, overexpression of SIRT6 in synovial macrophages of RA patients reduces associated inflammatory responses [48]. Additionally, a study investigating the correlation between cartilage-specific SIRT6 deficiency and osteoarthritis in mice indicates that the absence of SIRT6 exacerbates the severity of posttraumatic and age-related osteoarthritis [49]. This suggests that SIRT6 may play a role in suppressing the inflammatory processes associated with arthritis.

Thoracic aortic aneurysm (TAA) progresses asymptomatically and is characterised by aortic dilation. SIRT6 expression was significantly reduced in the culture medium of sporadic human TAA tissues. In mice, SIRT6 gene knockout in vascular smooth muscle cells accelerates the formation and rupture of TAA, reduces survival rates and increases vascular inflammation and ageing following angiotensin II infusion. Transcriptomic analysis identifies IL-1β as a key target of SIRT6. Elevated levels of IL-1β are associated with vascular inflammation and ageing in both human and mouse TAA samples. Chromatin immunoprecipitation assays reveal that SIRT6 binds to the II1b promoter, partially suppressing its expression by reducing the acetylation of H3K9 and H3K56. Pharmacological inhibition of IL-1β signalling using gene knockout of IL-1β or the receptor antagonist anakinra can ameliorate the exacerbated vascular inflammation, ageing, TAA formation and reduced survival observed in SIRT6-deficient mice [50]. This is consistent with our findings, where patients in the NSRP group exhibited high SIRT6 expression and low IL-1β levels.

It has been reported that SIRT6 is involved in vascular inflammation, as its downregulation has been shown to increase the production of pro-inflammatory cytokines in HUVECs [51]. Meanwhile, previous studies have demonstrated that overexpression of SIRT6 alleviates cisplatin-induced acute kidney injury, including inflammation and apoptosis, by inhibiting the ERK1/2-signalling pathway [52]. Liu reported that gut epithelial cell-specific knockout of SIRT6 increases susceptibility to colitis induced by dextran sulphate sodium (DSS) in mice and that SIRT6 expression in the colon is reduced in both mouse models with DSS-induced colitis and patients with ulcerative colitis [53]. In a study of diabetes-associated periodontitis

patients, it was observed that macrophage SIRT6 deficiency led to impaired cytoplasmic function and severe periodontal damage. This suggests that SIRT6 plays a suppressive role in diabetes-related inflammatory responses [54]. Another study investigating the protective effects of SIRT6 in vascular smooth muscle cells found that overexpression of SIRT6 or SIRT6 in ApoE (apolipoprotein E) knockout mice reduced atherosclerosis, markers of ageing and inflammation compared to control mice of the same litter [55].

To our knowledge, SIRT6 is primarily defined as an anti-inflammatory protein that suppresses the expression of NF-κB target genes and other pro-inflammatory cytokines, with limited reports on its pro-inflammatory effects. However, some studies indicate that SIRT6 can catalyse the hydrolysis of TNF-α at lysines 19 and 20, facilitating its secretion from cells and thereby promoting inflammatory responses [56].

Post-RT, the initial responders are resident immune cells that produce pro-inflammatory cytokines and growth factors. Within the irradiated cells, damage is first detected by sensors, and then a signal cascade is transmitted through effectors. Typically, these early responses are propagated through post-translational modifications such as phosphorylation, leading to the relatively rapid activation of effectors, including the production and release of cytokines [57]. The early immune response to radiation includes the secretion of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α, into the extracellular space. These cytokines activate resident immune cells, including lymphocytes and macrophages [58]. Following irradiation, some cytokines induced, such as TNF-α, IL-1α, IL-1β and IL-17, exhibit pro-inflammatory effects. Conversely, other cytokines, including IL-4, IL-10 and TGFβ, possess anti-inflammatory properties. TGF-β, IL-4 and IL-10 are key cytokines driving competitive anti-inflammatory responses, including the activation of M2 macrophages and the production of extracellular matrix necessary for fibrosis and tissue contraction during wound repair [59]. Radiation-induced inflammatory cytokines stimulate the expression of subsequent fibrotic cytokines, such as the TGF-β family and VEGF. These cytokines, in turn, promote the progression from pneumonitis to pulmonary fibrosis [60]. To balance this process, both IL-22 and IL-10 can downregulate the pneumonitis response by inhibiting pro-inflammatory cytokines and the function of antigen-presenting cells [61, 62]. IL-4 is primarily expressed by Th2 cells and drives the production of macrophages, fibroblasts and epithelial cells. IL-4 is involved in the differentiation of Th2 cells and inhibits the activity of Th1 cells. IL-4 stimulates arginase activity in macrophages and converts arginine into ornithine. Ornithine is a precursor for polyamines and collagen, ultimately contributing to the deposition of the extracellular matrix (ECM) [63]. Additionally, animal studies have shown that IL-17A plays a crucial role in RILI due to its expression varying at different stages of radiation-induced lung damage [64]. The expression of IL-17A notably increased 1 week after irradiation, peaked at 4 weeks, and then declined by 8 weeks postirradiation. In another study, IL-17A antibody treatment alleviated RP and subsequent fibrosis and improved survival rates following irradiation [65]. In our study, TNF-α, IL-6, IL-1β and IFN-γ were identified as independent risk factors for RP. A high expression of IL-17A was associated with SRP, while IL-4 and IL-10 were protective factors for RP, demonstrating anti-inflammatory effects. These findings are consistent with the existing evidence.

Prior research suggests that lymphocytes play a crucial role in the early-stage lung inflammation induced by radiation [66]. Studies have indicated that activated T lymphocytes may contribute to the production of pro-inflammatory cytokines such as IFN-γ and IL-17 [67–69]. Increasing evidence indicates that the recruitment and polarisation of macrophages play a crucial role in the development of RP, with macrophages contributing to the establishment of a pro-inflammatory and profibrotic environment [70]. Macrophages are highly plastic and heterogeneous cells that differentiate into various phenotypes due to differences in their response to stimuli and tissue localisation. In this context, different T cell subsets play a pivotal role in regulating macrophage differentiation into distinct phenotypes. IFN-γ induces M1 or classical activation of macrophages, while IL-4, IL-13 and IL-10 promote alternative activation or M2 macrophages [71]. Th1 cells secrete IFN-γ, which significantly contributes to antifibrotic and immunoregulatory activities. IFN-γ can induce M1 macrophages to express high levels of inflammatory cytokines, such as IL-2, IL-23 and nitric oxide (NO), thereby promoting the inflammatory process. Th2 cells primarily secrete IL-4 and IL-13, which, along with TGF-β1, stimulate collagen synthesis [72]. The imbalance between Th1 and Th2 cells promotes the development of RP. Studies have also shown that following irradiation, the levels of M1 macrophage-associated pro-inflammatory factors such as TNF-α, IL-1 and IL-6 are elevated in plasma [73]. Studies have also indicated that SIRT6 suppresses inflammation by affecting lymphocyte differentiation and function. In S6KO mice, observations include superficial epithelial erosion in the colon leading to spinal hypertrophy, colitis, acute subcutaneous fat loss and severe lymphopenia associated with increased lymphocyte apoptosis. Flow cytometry analysis revealed a 50-fold reduction in CD4+-CD8+ double-positive cells in the thymus and a 10-fold decrease in splenic lymphocytes and progenitor B cells in the bone marrow [30]. This suggests that SIRT6 may exert its role in inflammation regulation by influencing lymphocyte differentiation and function.

The evidence suggests that macrophage polarisation is involved in the development of RP and that this polarisation is regulated by cytokines secreted by lymphocytes. SIRT6 has demonstrated significant value in modulating inflammation across various cell types and shows regulatory effects on the secretion of multiple cytokines. However, the relationship between SIRT6 expression in lymphocytes and the development of RP remains inconclusive. Our study demonstrates that, following radiotherapy, lymphocyte overexpression of SIRT6 effectively reduces the incidence of SRP among cancer patients undergoing chest irradiation, while patients with low SIRT6 expression have a significantly higher probability of developing SRP than those with high SIRT6 expression. This suggests that SIRT6 may be a potential therapeutic target for RP.

Since the discovery of sirtuins as promising therapeutic targets and their increasing role in mitigating complications related to ageing and other degenerative conditions, significant momentum has been gained in the development of sirtuin modulators. Research efforts

driven by ethnopharmacological and pharmaceutical studies have focused extensively on exploring both natural and synthetic sirtuin modulators [74]. Additionally, several well-known sirtuin modulators have been reported to provide benefits in various experimental disease models. It has been shown to play a positive role in fibrosis by addressing lung inflammation and oxidative stress-related pathologies. This knowledge has the potential to assist researchers and clinicians in developing new, nontoxic therapies for treating RP [75]. When evaluating their effects, it is crucial to consider the sirtuin specificity/selectivity of the modulators, as many target multiple sirtuins. The overall effects may be pleiotropic and may sometimes mediate through indirect stimulation of sirtuins rather than directly inducing specific enzymatic activity. These factors are important to consider, as they can potentially impact biological effects. Finally, the bioavailability, systemic tolerance and toxicity of these modulators should also be carefully assessed.

Our research findings indicate that SIRT6 not only is a potential therapeutic target for RP but also holds promise as a predictor of RP severity. We incorporated pre-radiotherapy patient lymphocyte SIRT6 expression levels, serological parameters, clinical characteristics and dosimetric parameters into a predictive model. Through multivariate logistic regression analysis, we found that pre-radiotherapy lymphocyte SIRT6 expression levels were negatively correlated with the severity of RP postradiotherapy.

In our study, the production of the pro-inflammatory factor IL-17A was found to be closely associated with the level of SIRT6 expression. A study involving 46 patients with non-small cell lung cancer assessed pre-radiotherapy levels of Ape-1, ICAe-1, ICAM-1 and IL-17A using enzyme-linked immunosorbent assay. The study found that high levels of Ape-1, ICAM-1 and IL-17A were associated with an increased risk of RP [76]. This further suggests that SIRT6 may hold predictive value for the occurrence and progression of RP. Additionally, a study assessing the prognostic value of serum SIRT6 levels in patients with acute ischaemic stroke (AIS) found that SIRT6 levels were negatively correlated with mortality. This suggests that SIRT6 may be a potential prognostic predictor and therapeutic target for AIS [77].

Our study elucidates the potential role of SIRT6 in the development and progression of RP. However, further research is required to validate the potential value of SIRT6 as a predictive biomarker in an independent, larger validation cohort. Due to the small sample size of this study, we were unable to conduct a comprehensive analysis of potential confounding factors. Additionally, our findings require validation through animal and cellular experiments.

In conclusion, our study demonstrates that low lymphocyte SIRT6 levels before radiotherapy and within 6 months of post-treatment are associated with a high risk for SRP. These findings offer a novel therapeutic strategy for the prevention and treatment of RP in clinical practice.

Author Contributions

Yipeng Song and Hongwei Li conceived and designed the study; Fengyuan Yu and Zheng Gong performed the experiments; Fengyuan Yu, Chen Li, Miaowei Wen, Zhezhe Xu, Shanshan Zhang and Bingying Zhao performed patient collection and clinical data interpretation; Fengyuan Yu, Rukun Zang, Qingxin Han and Ailu Wu performed the statistical analysis; Fengyuan Yu drafted the paper; Shuhui Wu, Yuan Li and Danial F. Naseem revised the manuscript; Yipeng Song and Hongwei Li supervised the study. Fengyuan Yu and Zheng Gong contributed equally to this study, and they are co-first authors. All authors read and approved the final paper.

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Ethics Statement

All procedures performed involving human participants were in accordance with the ethical standards of the Institutional Review Board of the Ethics Committee of Yantai Yuhuangding Hospital (2021-399).

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

The original contributions presented in the study are included in the article/Supporting Information. Further inquiries can be directed to the corresponding author. The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer Statistics, 2017," *CA: A Cancer Journal for Clinicians* 67, no. 1 (2017): 7–30.

2. Y. Tian, J. Ma, X. Jing, et al., "Radiation Therapy for Extensive-Stage Small-Cell Lung Cancer in the Era of Immunotherapy," *Cancer Letters* 541 (2022): 215719.

3. A. Pennathur, M. K. Gibson, B. A. Jobe, and J. D. Luketich, "Oesophageal Carcinoma," *Lancet* 381, no. 9864 (2013): 400–412.

4. J. Li, J. Xu, Y. Zheng, et al., "Esophageal Cancer: Epidemiology, Risk Factors and Screening," *Chinese Journal of Cancer Research* 33, no. 5 (2021): 535–547.

5. A. Giannopoulou, I. Gkiozos, K. J. Harrington, and K. N. Syrigos, "Thymoma and Radiation Therapy: A Systematic Review of Medical Treatment," *Expert Review of Anticancer Therapy* 13, no. 6 (2013): 759–766.

6. Z. Zhang, J. Zhou, V. Verma, et al., "Crossed Pathways for Radiation-Induced and Immunotherapy-Related Lung Injury," *Frontiers in Immunology* 12 (2021): 774807.

7. R. Yahyapour, E. Motevaseli, A. Rezaeyan, et al., "Reduction-Oxidation (Redox) System in Radiation-Induced Normal Tissue Injury: Molecular Mechanisms and Implications in Radiation Therapeutics," *Clinical and Translational Oncology* 20, no. 8 (2018): 975–988.

8. Q. Dasgupta, A. Jiang, A. M. Wen, et al., "A Human Lung Alveolus-on-a-Chip Model of Acute Radiation-Induced Lung Injury," *Nature Communications* 14, no. 1 (2023): 6506.

9. A. N. Hanania, W. Mainwaring, Y. T. Ghebre, N. A. Hanania, and M. Ludwig, "Radiation-Induced Lung Injury," *Chest* 156, no. 1 (2019): 150–162.

10. Y. Yan, J. Fu, R. O. Kowalchuk, et al., "Exploration of Radiation-Induced Lung Injury, From Mechanism to Treatment: A Narrative Review," *Translational Lung Cancer Research* 11, no. 2 (2022): 307–322.

11. T. J. Bledsoe, S. K. Nath, and R. H. Decker, "Radiation Pneumonitis," *Clinics in Chest Medicine* 38, no. 2 (2017): 201–208.

12. G. C. Barnett, C. M. L. West, A. M. Dunning, et al., "Normal Tissue Reactions to Radiotherapy: Towards Tailoring Treatment Dose by Genotype," *Nature Reviews Cancer* 9, no. 2 (2009): 134–142.

13. H. B. Forrester, J. Li, T. Leong, M. J. McKay, and C. N. Sprung, "Identification of a Radiation Sensitivity Gene Expression Profile in Primary Fibroblasts Derived From Patients who Developed Radiotherapy-Induced Fibrosis," *Radiotherapy and Oncology* 111, no. 2 (2014): 186–193.

14. W. L. Chang, C. H. Hsieh, I. Y. Kuo, C. H. Lin, Y. L. Huang, and Y. C. Wang, "Nutlin-3 Acts as a DNA Methyltransferase Inhibitor to Sensitize Esophageal Cancer to Chemoradiation," *Molecular Carcinogenesis* 62, no. 2 (2023): 277–287.

15. F. Qin, Z. Bian, L. Jiang, et al., "A Novel High-Risk Model Identified by Epithelial-Mesenchymal Transition Predicts Prognosis and Radioresistance in Rectal Cancer," *Molecular Carcinogenesis* 63, no. 11 (2024): 2119–2132.

16. X. Zhou, Y. Tong, C. Yu, et al., "FAP Positive Cancer-Associated Fibroblasts Promote Tumor Progression and Radioresistance in Esophageal Squamous Cell Carcinoma by Transferring Exosomal lncRNA AFAP1-AS1," *Molecular Carcinogenesis* 63, no. 10 (2024): 1922–1937.

17. Y. Shu, J. Lan, H. Luo, H. Fu, X. Xiao, and L. Yang, "FOS-Mediated PLCB1 Induces Radioresistance and Weakens the Antitumor Effects of CD8(+) T Cells in Triple-Negative Breast Cancer," *Molecular Carcinogenesis* 64, no. 1 (2025): 162–175.

18. G. C. Barnett, D. Thompson, L. Fachal, et al., "A Genome Wide Association Study (GWAS) Providing Evidence of an Association Between Common Genetic Variants and Late Radiotherapy Toxicity," *Radiotherapy and Oncology* 111, no. 2 (2014): 178–185.

19. L. Fachal, A. Gómez-Caamaño, G. C. Barnett, et al., "A Three-Stage Genome-Wide Association Study Identifies a Susceptibility Locus for Late Radiotherapy Toxicity at 2q24.1," *Nature Genetics* 46, no. 8 (2014): 891–894.

20. S. Kugel and R. Mostoslavsky, "Chromatin and Beyond: The Multitasking Roles for SIRT6," *Trends in Biochemical Sciences* 39, no. 2 (2014): 72–81.

21. Y. Li, J. Jin, and Y. Wang, "SIRT6 Widely Regulates Aging, Immunity, and Cancer," *Frontiers in Oncology* 12 (2022): 861334.

22. Y. Okabe and R. Medzhitov, "Tissue Biology Perspective on Macrophages," *Nature Immunology* 17, no. 1 (2016): 9–17.

23. T. A. Wynn, A. Chawla, and J. W. Pollard, "Macrophage Biology in Development, Homeostasis and Disease," *Nature* 496, no. 7446 (2013): 445–455.

24. P. J. Murray, J. E. Allen, S. K. Biswas, et al., "Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines," *Immunity* 41, no. 1 (2014): 14–20.

25. Y. Zhang, J. Zhang, S. Zhao, et al., "Single-Cell Rna Sequencing Highlights the Immunosuppression of IDO1(+) Macrophages in the Malignant Transformation of Oral Leukoplakia," *Theranostics* 14, no. 12 (2024): 4787–4805.

26. F. O. Martinez, L. Helming, R. Milde, et al., "Genetic Programs Expressed in Resting and IL-4 Alternatively Activated Mouse and Human Macrophages: Similarities and Differences," *Blood* 121, no. 9 (2013): e57–e69.

27. J. Van den Bossche, B. Malissen, A. Mantovani, P. De Baetselier, and J. A. Van Ginderachter, "Regulation and Function of the E-Cadherin/Catenin Complex in Cells of the Monocyte-Macrophage Lineage and DCs," *Blood* 119, no. 7 (2012): 1623–1633.

28. A. Paun, A. Kunwar, and C. K. Haston, "Acute Adaptive Immune Response Correlates With Late Radiation-Induced Pulmonary Fibrosis in Mice," *Radiation Oncology* 10 (2015): 45.

29. H. Aegerter, B. N. Lambrecht, and C. V. Jakubzick, "Biology of Lung Macrophages in Health and Disease," *Immunity* 55, no. 9 (2022): 1564–1580.

30. R. Mostoslavsky, K. F. Chua, D. B. Lombard, et al., "Genomic Instability and Aging-Like Phenotype in the Absence of Mammalian Sirt6," *Cell* 124, no. 2 (2006): 315–329.

31. E. Basch, B. B. Reeve, S. A. Mitchell, et al., "Development of the National Cancer Institute's Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE)," *Journal of the National Cancer Institute* 106, no. 9 (2014): dju244.

32. F. Chen, J. Niu, M. Wang, H. Zhu, and Z. Guo, "Re-Evaluating the Risk Factors for Radiation Pneumonitis in the Era of Immunotherapy," *Journal of Translational Medicine* 21, no. 1 (2023): 368.

33. L. Giuranno, J. Ient, D. De Ruysscher, and M. A. Vooijs, "Radiation-Induced Lung Injury (RILI)," *Frontiers in Oncology* 9 (2019): 877.

34. G. Hou, J. Li, W. Liu, J. Wei, Y. Xin, and X. Jiang, "Mesenchymal Stem Cells in Radiation-Induced Lung Injury: From Mechanisms to Therapeutic Potential," *Frontiers in Cell and Developmental Biology* 10 (2022): 1100305.

35. C. C. Pinnix, G. L. Smith, S. Milgrom, et al., "Predictors of Radiation Pneumonitis in Patients Receiving Intensity Modulated Radiation Therapy for Hodgkin and Non-Hodgkin Lymphoma," *International Journal of Radiation Oncology*Biology*Physics* 92, no. 1 (2015): 175–182.

36. K. Tsujino, T. Hashimoto, T. Shimada, et al., "Combined Analysis of V20, VS5, Pulmonary Fibrosis Score on Baseline Computed Tomography, and Patient Age Improves Prediction of Severe Radiation Pneumonitis After Concurrent Chemoradiotherapy for Locally Advanced Non-Small-Cell Lung Cancer," *Journal of Thoracic Oncology* 9, no. 7 (2014): 983–990.

37. R. Bensenane, S. Helfre, K. Cao, et al., "Optimizing Lung Cancer Radiation Therapy: A Systematic Review of Multifactorial Risk Assessment for Radiation-Induced Lung Toxicity," *Cancer Treatment Reviews* 124 (2024): 102684.

38. J. Brenmoehl and A. Hoeflich, "Dual Control of Mitochondrial Biogenesis by Sirtuin 1 and Sirtuin 3," *Mitochondrion* 13, no. 6 (2013): 755–761.

39. M. Ji, H. Jiang, Z. Li, et al., "Sirt6 Attenuates Chondrocyte Senescence and Osteoarthritis Progression," *Nature Communications* 13, no. 1 (2022): 7658.

40. Z. Guo, P. Li, J. Ge, and H. Li, "SIRT6 in Aging, Metabolism, Inflammation and Cardiovascular Diseases," *Aging and Disease* 13, no. 6 (2022): 1787–1822.

41. A. Roichman, S. Elhanati, M. A. Aon, et al., "Restoration of Energy Homeostasis by SIRT6 Extends Healthy Lifespan," *Nature Communications* 12, no. 1 (2021): 3208.

42. F. Fiorentino, A. Mai, and D. Rotili, "Emerging Therapeutic Potential of SIRT6 Modulators," *Journal of Medicinal Chemistry* 64, no. 14 (2021): 9732–9758.

43. E. Abbotto, C. Miro, F. Piacente, et al., "SIRT6 Pharmacological Inhibition Delays Skin Cancer Progression in the Squamous Cell Carcinoma," *Biomedicine & Pharmacotherapy* 166 (2023): 115326.

44. G. Liu, H. Chen, H. Liu, W. Zhang, and J. Zhou, "Emerging Roles of SIRT6 in Human Diseases and Its Modulators," *Medicinal Research Reviews* 41, no. 2 (2021): 1089–1137.

45. K. Ma, N. Lu, F. Zou, and F. Z. Meng, "Sirtuins as Novel Targets in the Pathogenesis of Airway Inflammation in Bronchial Asthma," *European Journal of Pharmacology* 865 (2019): 172670.

46. T. L. A. Kawahara, E. Michishita, A. S. Adler, et al., "SIRT6 Links Histone H3 Lysine 9 Deacetylation to NF-κB-Dependent Gene Expression and Organismal Life Span," *Cell* 136, no. 1 (2009): 62–74.

47. S. J. Woo, H. S. Noh, N. Y. Lee, et al., "Myeloid Sirtuin 6 Deficiency Accelerates Experimental Rheumatoid Arthritis by Enhancing Macrophage

Activation and Infiltration Into Synovium," *EBioMedicine* 38 (2018): 228–237.

48. C. W. Zhang, X. Wu, D. Liu, et al., "Long Non-Coding RNA PVT1 Knockdown Suppresses Fibroblast-Like Synoviocyte Inflammation and Induces Apoptosis in Rheumatoid Arthritis Through Demethylation of Sirt6," *Journal of Biological Engineering* 13 (2019): 60.

49. J. A. Collins, C. J. Kim, A. Coleman, et al., "Cartilage-Specific Sirt6 Deficiency Represses IGF-1 and Enhances Osteoarthritis Severity in Mice," *Annals of the Rheumatic Diseases* 82, no. 11 (2023): 1464–1473.

50. Y. N. Ding, T. T. Wang, S. J. Lv, et al., "SIRT6 Is an Epigenetic Repressor of Thoracic Aortic Aneurysms via Inhibiting Inflammation and Senescence," *Signal Transduction and Targeted Therapy* 8, no. 1 (2023): 255.

51. M. Lappas, "Anti-Inflammatory Properties of Sirtuin 6 in Human Umbilical Vein Endothelial Cells," *Mediators of Inflammation* 2012 (2012): 1–11.

52. Z. Li, K. Xu, N. Zhang, et al., "Overexpressed SIRT6 Attenuates Cisplatin-Induced Acute Kidney Injury by Inhibiting ERK1/2 Signaling," *Kidney International* 93, no. 4 (2018): 881–892.

53. F. Liu, H. F. Bu, H. Geng, et al., "Sirtuin-6 Preserves R-Spondin-1 Expression and Increases Resistance of Intestinal Epithelium to Injury in Mice," *Molecular Medicine* 23 (2017): 272–284.

54. B. Li, Z. Xin, S. Gao, et al., "SIRT6-regulated Macrophage Efferocytosis Epigenetically Controls Inflammation Resolution of Diabetic Periodontitis," *Theranostics* 13, no. 1 (2023): 231–249.

55. M. O. J. Grootaert, A. Finigan, N. L. Figg, A. K. Uryga, and M. R. Bennett, "SIRT6 Protects Smooth Muscle Cells From Senescence and Reduces Atherosclerosis," *Circulation Research* 128, no. 4 (2021): 474–491.

56. H. Jiang, S. Khan, Y. Wang, et al., "SIRT6 Regulates TNF-α Secretion through Hydrolysis of Long-Chain Fatty Acyl Lysine," *Nature* 496, no. 7443 (2013): 110–113.

57. L. Ma, A. Gonzalez-Junca, Y. Zheng, et al., "Inflammation Mediates the Development of Aggressive Breast Cancer Following Radiotherapy," *Clinical Cancer Research* 27, no. 6 (2021): 1778–1791.

58. D. Schaue, E. L. Kachikwu, and W. H. McBride, "Cytokines in Radiobiological Responses: A Review," *Radiation Research* 178, no. 6 (2012): 505–523.

59. T. A. Wynn and T. R. Ramalingam, "Mechanisms of Fibrosis: Therapeutic Translation for Fibrotic Disease," *Nature Medicine* 18, no. 7 (2012): 1028–1040.

60. H. Malekzadeh, Y. Surucu, S. Chinnapaka, et al., "Metformin and Adipose-Derived Stem Cell Combination Therapy Alleviates Radiation-Induced Skin Fibrosis in Mice," *Stem Cell Research & Therapy* 15, no. 1 (2024): 13.

61. M. N. Abdelnabi, G. S. Hassan, and N. H. Shoukry, "Role of the Type 3 Cytokines IL-17 and IL-22 in Modulating Metabolic Dysfunction-Associated Steatotic Liver Disease," *Frontiers in immunology* 15 (2024): 1437046.

62. H. Wang, F. Zhou, C. Zhao, et al., "Interleukin-10 Is a Promising Marker for Immune-Related Adverse Events in Patients With Non-Small Cell Lung Cancer Receiving Immunotherapy," *Frontiers in Immunology* 13 (2022): 840313.

63. D. M. Mosser and J. P. Edwards, "Exploring the Full Spectrum of Macrophage Activation," *Nature Reviews Immunology* 8, no. 12 (2008): 958–969.

64. Y. Sheng, K. Chen, W. Jiang, et al., "PD-1 Restrains IL-17A Production From γδ T Cells to Modulate Acute Radiation-Induced Lung Injury," *Translational Lung Cancer Research* 10, no. 2 (2021): 685–698.

65. B. Z. Wang, L. P. Wang, H. Han, et al., "Interleukin-17A Antagonist Attenuates Radiation-Induced Lung Injuries in Mice," *Experimental Lung Research* 40, no. 2 (2014): 77–85.

66. F. Liu, B. Qiu, Y. Xi, et al., "Efficacy of Thymosin α1 in Management of Radiation Pneumonitis in Patients With Locally Advanced Non-Small Cell Lung Cancer Treated With Concurrent Chemoradiotherapy: A Phase 2 Clinical Trial (GASTO-1043)," *International Journal of Radiation Oncology*Biology*Physics* 114, no. 3 (2022): 433–443.

67. G. Manson, J. Norwood, A. Marabelle, H. Kohrt, and R. Houot, "Biomarkers Associated With Checkpoint Inhibitors," *Annals of Oncology* 27, no. 7 (2016): 1199–1206.

68. A. A. Tarhini, H. Zahoor, Y. Lin, et al., "Baseline Circulating IL-17 Predicts Toxicity While TGF-β1 and IL-10 Are Prognostic of Relapse in Ipilimumab Neoadjuvant Therapy of Melanoma," *Journal for Immunotherapy of Cancer* 3 (2015): 39.

69. K. Esfahani and W. H. Miller, Jr., "Reversal of Autoimmune Toxicity and Loss of Tumor Response by Interleukin-17 Blockade," *New England Journal of Medicine* 376, no. 20 (2017): 1989–1991.

70. S. K. Wculek, I. Heras-Murillo, A. Mastrangelo, et al., "Oxidative Phosphorylation Selectively Orchestrates Tissue Macrophage Homeostasis," *Immunity* 56, no. 3 (2023): 516–530.e9.

71. C. Yunna, H. Mengru, W. Lei, and C. Weidong, "Macrophage M1/M2 Polarization," *European Journal of Pharmacology* 877 (2020): 173090.

72. T. A. Wynn, "Fibrotic Disease and the T(H)1/T(H)2 Paradigm," *Nature Reviews Immunology* 4, no. 8 (2004): 583–594.

73. C. N. Sprung, H. B. Forrester, S. Siva, and O. A. Martin, "Immunological Markers That Predict Radiation Toxicity," *Cancer Letters* 368, no. 2 (2015): 191–197.

74. H. Dai, D. A. Sinclair, J. L. Ellis, and C. Steegborn, "Sirtuin Activators and Inhibitors: Promises, Achievements, and Challenges," *Pharmacology & Therapeutics* 188 (2018): 140–154.

75. S. Mazumder, M. Barman, U. Bandyopadhyay, and S. Bindu, "Sirtuins as Endogenous Regulators of Lung Fibrosis: A Current Perspective," *Life Sciences* 258 (2020): 118201.

76. L. Guo, G. Ding, W. Xu, et al., "Prognostic Biological Factors of Radiation Pneumonitis After Stereotactic Body Radiation Therapy Combined With Pulmonary Perfusion Imaging," *Experimental and Therapeutic Medicine* 17, no. 1 (2019): 244–250.

77. L. Liberale, S. Ministrini, M. Arnold, et al., "Serum Circulating Sirtuin 6 as a Novel Predictor of Mortality After Acute Ischemic Stroke," *Scientific Reports* 12, no. 1 (2022): 20513.