原著

Regulation of the pro-α1（V）collagen gene（*Col5a1*）following ionizing radiation at the transcriptional and post-transcriptional levels.

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Abstract : Radiation-induced fibrosis（RIF）is thought to be the result of the excessive accumulation of collagen and other extracellular matrix components. Previously, we reported that the TGF-β/Smad signaling pathway plays an important role in the expression of type I collagen during RIF. Type V collagen is incorporated into fibers expressing type I collagen and has an important role in controlling the diameter of collagen fibers. It is thought that type V collagen plays a major role in the radiation-induced increase in type I collagen expression, but its role in the development of RIF remains unclear. microRNAs（miRNAs）are small, noncoding sequences approximately 20 nucleotides long that negatively regulate the gene expression at the post-transcriptional level. We previously reported that miR-29 negatively regulates the expression of type I collagen in RIF. However, the effect of miR-29 on regulating the expression of type V collagen remains unclear. In the present study, we cultured mouse fibroblasts（NIH-3T3 cells）and examined the regulation of the *Col5a1* expression at the transcriptional and post-transcriptional level by miRNAs using real-time PCR and Luciferase assay. The expression of type V collagen were increased by irradiation at the transcriptional level. Ionizing radiation increased the 3′ untranslated region activity of *Col5a1* while decreasing the expression of miR-29. This indicates that ionizing radiation cancelled the negative regulation of *Col5a1* by miR-29. In conclusion, we showed that the expression of *Col5a1* was increased by irradiation and that this increment was coordinated at the transcriptional and miR-29-mediated post-transcriptional levels.

Key words: Radiation-induced fibrosis, microRNA, collagen, TGF-β/Smad signaling pathway, extracellular matrix

1. Introduction

Radiotherapy is one of the most important non-surgical treatment methods for malignant tumors such as lung cancer. However, its efficacy is severely limited by radiation-induced fibrosis（RIF）[1].

Clinically, RIF is one of the most important factors limiting the ionizing radiation dose that can be delivered to the tumor, thus adversely affecting the patient's long-term quality of life. It is characterized by the excessive accumulation of extracellular matrix proteins, such as collagen, and is considered a serious complication because it leads to the subsequent formation of scar tissue and ultimately fatal respiratory failure[2]. Therefore, it is of great importance to clarify the mechanism underlying the gene expression in RIF and to identify appropriate treatments to prevent and attenuate the development and progression of fibrosis. However, identifying the mechanism underlying the gene expression and the intracellular signaling pathways that drive RIF has not yet been achieved.

Collagen is a major constituent of the extracellular matrix and critical for the morphology and function of organs in the body[3]. It forms fibrils within living tissues and plays an important role in maintaining the structure of connective tissues, such as skin, bone, cartilage, tendons, and teeth. In addition to connective tissue, it is also present in many organs, such as the lung, liver, and cornea, and helps maintain their function. At present, more than 45 distinct α-chains of collagen genes forming 28 types of collagen proteins have been identified[4,5]. Type I collagen is a fibrillar collagen that is the major component of the extracellular matrix. Type V collagen is a quantitatively minor fibrillar collagen and co-

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expressed in a wide range of tissues in which type I collagen is expressed *in vivo*. Furthermore, type V collagen is incorporated into fibrils of the more abundant type I collagen and acts as a regulator of the diameter of collagen fibrils[6]. The basic structure forming collagen fibers is a collagen molecule, which is constructed by gathering three polypeptide chains called α-chains to form a triple helix structure. In type V collagen, three types of α chains (α1, α2, α3) assemble to form a helical structure. The predominant molecular form of type V collagen is the heterotrimer $[\alpha 1]_2\alpha 2$ (V) in many tissues.

In the process of fibrosis, TGF-β plays an important role in a number of pathological processes, such as the promotion of fibroblast proliferation and induction of collagen production, and functions as an important mediator of fibrosis in various organ[7,8]. Ionizing radiation promotes the formation of reactive oxygen species (ROS) and induces TGF-β expression and activation[9]. Our research group previously reported that the expression of type I collagen was increased by ionizing radiation and that the TGF-β/Smad signaling pathway was involved in this increment[10]. However, the effect of ionizing radiation on the expression of type V collagen, which regulates the size of collagen fibrils, has not been fully clarified.

Our research group has studied the regulation of *Col5a1* expression at the transcriptional level. Sakata et al. found that there is a core promoter region 231 bp upstream from the transcription start site of the gene and this core promoter region is regulated by the binding of ubiquitously expressed transcription factors Sp1 and CBF/NF-Y[11]. However, there have been no reports on the effects of ionizing radiation on the transcriptional activity in the promoter region of *Col5a1*.

MicroRNAs (miRNAs) are small, functional, highly conserved, noncoding RNAs 20-23 nucleotides in length that suppress target gene expression via sequence-specific binding to the 3′ untranslated region (3′ UTR) of target mRNAs[12]. miRNAs are suggested to play essential roles in a variety of biological processes. It has also been suggested that miRNAs play a central role in liver fibrosis[13]. Regarding the pro-fibrotic process, the miR-29 family is reported to be associated with fibrosis in various organs[14]. miR-29 mediates the repression of several collagen mRNAs under both normal and pathological conditions[15–17]. Zhang et al. found that miR-29 specifically binds to the 3′ UTR region of *Col5a1* and that miR-29 negatively regulates the expression of *Col5a1* in cultured mouse fibroblasts[18]. However, the molecular mechanism underlying the role of miR-29-mediated *Col5a1* expression in RIF remains unclear. The present study examined the molecular mechanism underlying miR-29-mediated *Col5a1* expression coordinately with the transcription level in RIF.

2. Material and methods

2.1 Cell culture and ionizing irradiation of cells

Cell culture was performed using NIH-3T3 cells. The cells were cultured at 37℃ with 5% CO_2 in DMEM containing 10% fetal bovine serum (Sanko Junyaku, Tokyo, Japan). After culturing for 24 h, the cells were irradiated with ^{137}Cs γ-rays using a Gammacell 40 (dose rate: 0.7 Gy/min). The dose rate was calculated from the half-life attenuation of ^{137}Cs and confirmed using a thermoluminescent dosimeter (TLD). The radiation doses used in this experiment was 10 Gy. We then confirmed the effect of the ionizing irradiation on type V collagen expression at 72 h after administering the irradiation.

2.2 RNA isolation and real-time polymerase chain reaction (PCR)

The procedures for the isolation of total RNA and subsequent real-time PCR were as previously described[10]. In brief, 1 μg of total RNA from NIH-3T3 cells was reverse-transcribed into cDNA using ReverTra Ace (Toyobo, Tokyo, Japan). The relative gene expression was normalized using the *GAPDH* gene as an internal control.

2.3 Plasmid constructs

Plasmid constructs used in this study were the *Col5a1*/luciferase construct containing a 1,783 bp

fragment containing the sequence from -1760 to +23 (-1760/+23 Luc), an 885 bp fragment containing the sequence from -862 to +23 (-862/+23 Luc), a 254 bp fragment containing the sequence from -231 to +23 (-231/+23 Luc) and a 24 bp fragment containing the sequence from -1 to +23 (-1/+23 Luc) of the *Col5a1* promoter. These promoter regions were cloned into the pGL3.0 Control vector. An 840 bp fragment from the 3′ UTR in *Col5a1* was cloned into the pGL3.0 Control vector (*Col5a1* 3′ UTR Luc). A 254 bp fragment of the -231/+23 promoter and an 840 bp fragment of the 3′ UTR in *Col5a1* was cloned into the pmiR-Glo vector (-231/+23 *Col5a1* 3′ UTR Luc). The mutated 3′ UTR construct was generated by side-directed mutagenesis using -231/+23 *Col5a1* 3′ UTR Luc as a template (-231/+23 *Col5a1* 3′ UTR mut Luc). These steps have been previously described[18].

2.4 The luciferase assay

Cells were seeded at 2×10^5 per 35-mm dish, cultured for 24 h and transfected with 5 μg of plasmid DNA using calcium phosphate. After culturing for another 24 h, the cells were irradiated and collected for a luciferase assay, which was performed at 72 h after irradiation. The luciferase activity was measured using a Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA).

2.5 Reagents and treatment

miR-29 mimic (Qiagen, Valencia, CA, USA) was purchased and transfected into cells using Lipofectamine 2000 (Life Technologies, GmbH, Darmstadt, Germany). For reverse transfection, miR-29 mimic (50 nM) mixed with transfection reagent was added when the cells were placed, followed by incubation for 72 h at 37 °C in 5% CO_2.

2.6 Statistical analyses

All data are expressed as the mean ± standard deviation. Each experiment was repeated at least three times in triplicate. Microsoft Excel (Redmond, WA, USA) was used to perform all statistical analyses. Student's *t*-test was used for comparisons between groups. P values of < 0.05 were considered to indicate statistical significance.

3. Results

3.1 Changes in the expression of type V collagen by irradiation

To evaluate the effect of irradiation on the expression of type V collagen, we measured the collagen V expression in NIH-3T3 cells using real-time PCR. As shown in Figs.1A and 1B, the expression of *Col5a1* and *Col5a2* were increased by irradiation. In contrast, the expression of *Col5a3* was not affected by irradiation (Fig. 1C).

Next, we performed a luciferase assay to examine the effect of irradiation on the transcriptional activity of *Col5a1*. Fig.1D shows a schematic illustration of the luciferase construct used in this study. The luciferase activity in the basal promoter region of *Col5a1* (-231/+23) was increased by irradiation (Fig. 1E). These results indicate that *Col5a1* expression is increased at the transcriptional level by irradiation.

3.2 Changes in the *Col5a1* 3′ UTR activity by irradiation

To assess the effect of irradiation on the 3′ UTR of *Col5a1*, a luciferase assay was performed. As shown in Fig. 2A, we used a luciferase construct in which the 3′ UTR region of *Col5a1* had been inserted downstream of the luciferase gene. The pGL3 Control vector used in this study contained an SV40 promoter and was a vector often used in general luciferase assays. The luciferase activity of the construct containing the 3′ UTR of *Col5a1* (Col5a1 3′ UTR Luc) was markedly reduced compared to the empty control vector (SV40 3′ UTR Luc) (Fig. 2B). In contrast, the luciferase activity in Col5a1 3′ UTR Luc was increased by irradiation.

The relationship between the *Col5a1* promoter and the 3′ UTR was then investigated using a luciferase construct containing the basic promoter region (-231/+23) of *Col5a1* (Fig. 2C). As shown in Fig.2D, the luciferase activity of the construct containing the 3′ UTR of *Col5a1* (-231/+23 Col5a1 3′ UTR Luc)

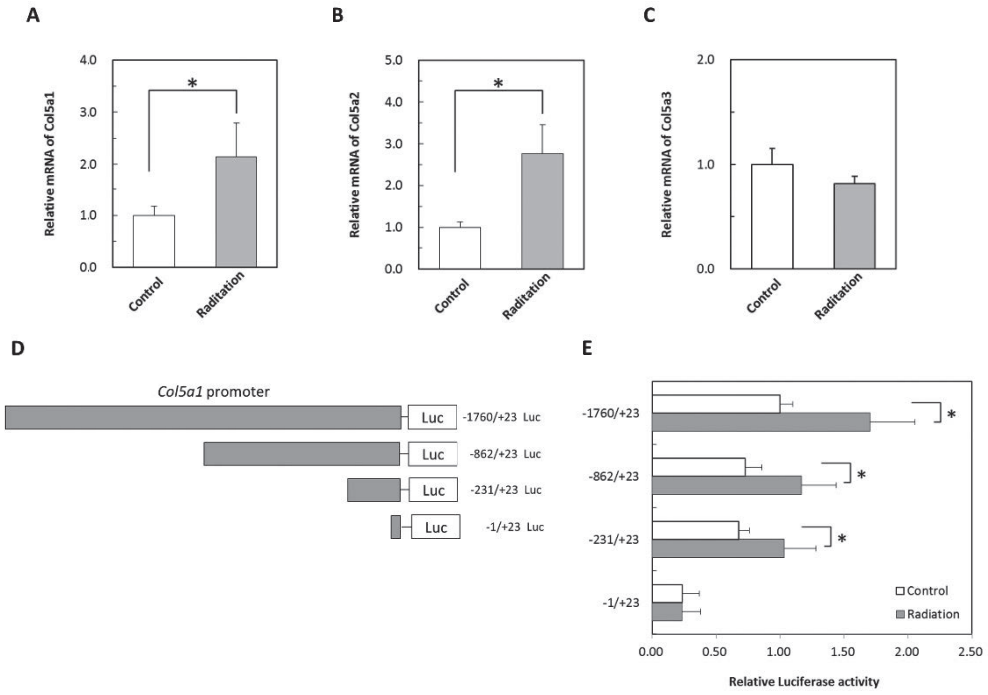

Figure 1 – The effect of irradiation on the expression of type V collagen
The expression of type V collagen in normal and irradiated NIH-3T3 cells (A) pro-α1 collagen gene (*Col5a1*), (B) pro-α2 collagen gene (*Col5a2*) and (C) pro-α3 collagen gene (*Col5a3*). as assessed by real-time PCR. (D) A schematic illustration of the luciferase reporter construct containing the Col5a1 promoter. (E) The promoter activity of Col5a1 gene in normal and irradiated NIH-3T3 cells, as assessed by a luciferase assay. Each value is normalized to the corresponding value non-irradiation. The results are shown as the mean ± S.D. (n=3). Statistical comparisons between groups were performed using Student's *t*-test (*$p < 0.05$).

decreased overall, but the increase induced by irradiation was large. These results indicate that radiation-induced changes in the *Col5a1* expression, along with changes in the transcriptional activity, also affect the 3′ UTR activity.

3.3 Effect of miR-29 on the *Col5a1* 3′ UTR activity

First of all, we examined the effect of miR-29 on the *Col5a1* 3′ UTR during exposure to ionizing radiation using a luciferase assay. As shown in Fig. 3A, we generated a wild-type construct (-231/+23 *Col5a1* 3′ UTR WT Luc) and a mutant construct (-231/+23 *Col5a1* 3′ UTR mut Luc). The luciferase activities were generally higher in -231/+23 *Col5a1* 3′ UTR mut Luc than in -231/+23 *Col5a1* 3′ UTR WT Luc (Fig. 3B). In addition, both -231/+23 *Col5a1* 3′ UTR WT Luc and -231/+23 *Col5a1* 3′ UTR mut Luc showed increased luciferase activity by irradiation.
To assess the effect of miR-29 on the expression of *Col5a1*, NIH-3T3 cells were then transfected with miR-29 mimics. As shown in Fig. 3C, it was confirmed that miR-29 mimic transfection increased the expression of miR-29. However, miR-29 repressed the *Col5a1* mRNA expression (Fig. 3D). Furthermore, miR-29 mimics decreased the luciferase activity of -231/+23 *Col5a1* 3′ UTR WT Luc but hardly altered the luciferase activity of -231/+23 *Col5a1* 3′ UTR mut Luc (Fig. 3E). These results indicate that miR-29 specifically binds to the 3′ UTR of *Col5a1* and negatively regulates the *Col5a1* mRNA expression.

3.4 Effect of miR-29 on radiation-induced *Col5a1* expression changes

We confirmed that the expression of *Col5a1* was increased by ionizing radiation at the transcriptional activity level and at the 3′ UTR activity level. We therefore investigated how miR-29 affected the radiation-induced increment in the expression of *Col5a1*. Initially, we measured the expression of miR-29 due to irradiation using real-time PCR. As shown in

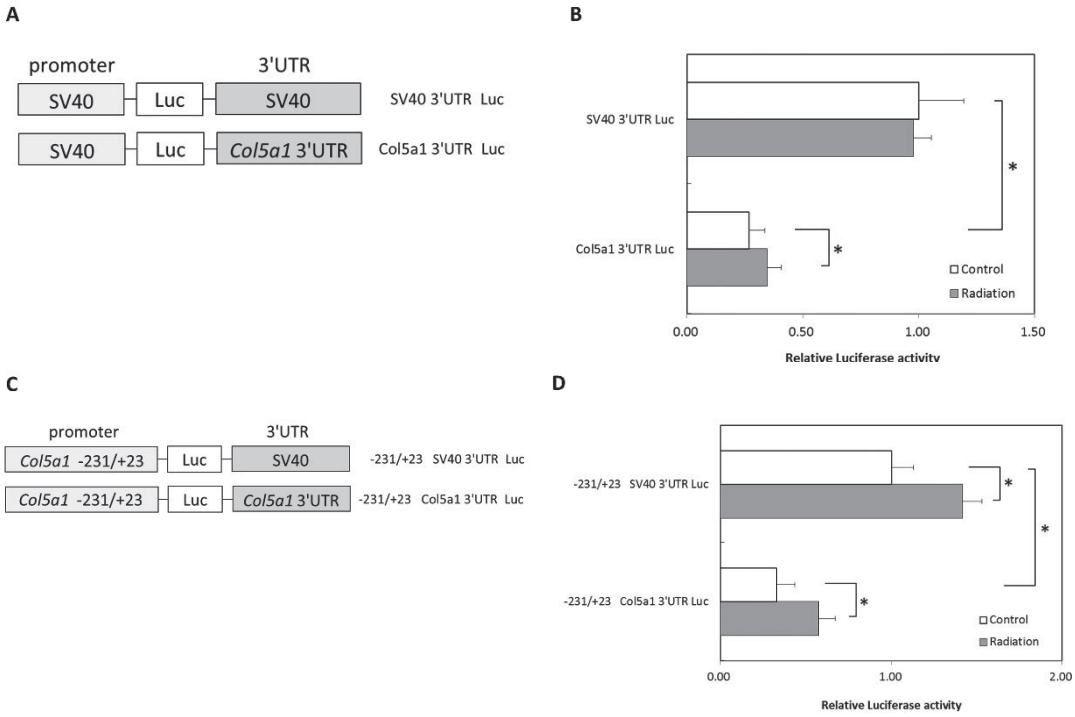

Figure 2 −The luciferase activity of the Col5a1 3′ UTR using the SV40 and *Col5a1* promoter.
(A) A schematic illustration of the luciferase reporter construct containing the *Col5a1* 3′ UTR. The luciferase construct contains the *Col5a1* 3′ UTR downstream of the luciferase gene. (B) The luciferase activity of *Col5a1* 3′ UTR in normal and irradiated NIH-3T3 cells, as assessed by a luciferase assay. The histograms indicated the activity normalized to the luciferase construct containing SV40 3′ UTR. (C) A schematic illustration of the luciferase reporter construct containing two domains *Col5a1* promoter and 3′ UTR upstream and downstream of the luciferase gene. (D) The luciferase activity of *Col5a1* promoter and 3′ UTR in normal and irradiated NIH-3T3 cells, as assessed by a luciferase assay. The histograms indicated the activity normalized to the luciferase construct containing two domain Col5a1 promoter and SV40 3′ UTR upstream and downstream of the luciferase gene. The results are shown as the mean ± S.D. (n=3). Statistical comparisons between groups were performed using Student's t-test (*$p < 0.05$).

Fig. 4A, the expression of miR-29 was reduced by irradiation. Furthermore, miR-29 mimics significantly reduced the expression of *Col5a1* and attenuated the increase in *Col5a1* expression by irradiation (Fig. 4B). These results indicate that miR-29 suppresses radiation-induced increase in the *Col5a1* expression by reducing the 3′ UTR activity of *Col5a1*.

4. Discussion

Our research group previously conducted a promoter analysis in the transcribed region of *Col5a1*[11] and assessed the post-transcriptional regulation of expression by miRNAs in the 3′ UTR region[18]. In the present study, we investigated the effects of ionizing radiation on *Col5a1* transcriptional and 3′ UTR activities in relation to RIF. In irradiated NIH-3T3 cells, we found that the expression of *Col5a1* was increased at the transcript level using a luciferase assay and real-time PCR. As a result of measuring the 3′ UTR activity of *Col5a1* using a luciferase assay, we found that miR-29 repressed the expression of *Col5a1*. In contrast, the expression of miR-29 was decreased by irradiation, thus cancelling the negative regulation of miR-29 expression. These results indicate that the radiation-induced increment in *Col5a1* expression is coordinated at the transcriptional and post-transcriptional levels (Fig. 5).

Type V collagen is incorporated into abundant type I collagen to control fibrogenesis of collagen fibers. Type V collagen is also present within the matrix of most connective tissues and has multiple physiological and pathological functions. Sakata et al. reported that the binding of transcription factors CBF/NF-Y and SP1 in the proximal promoter region of the *Col5a1*

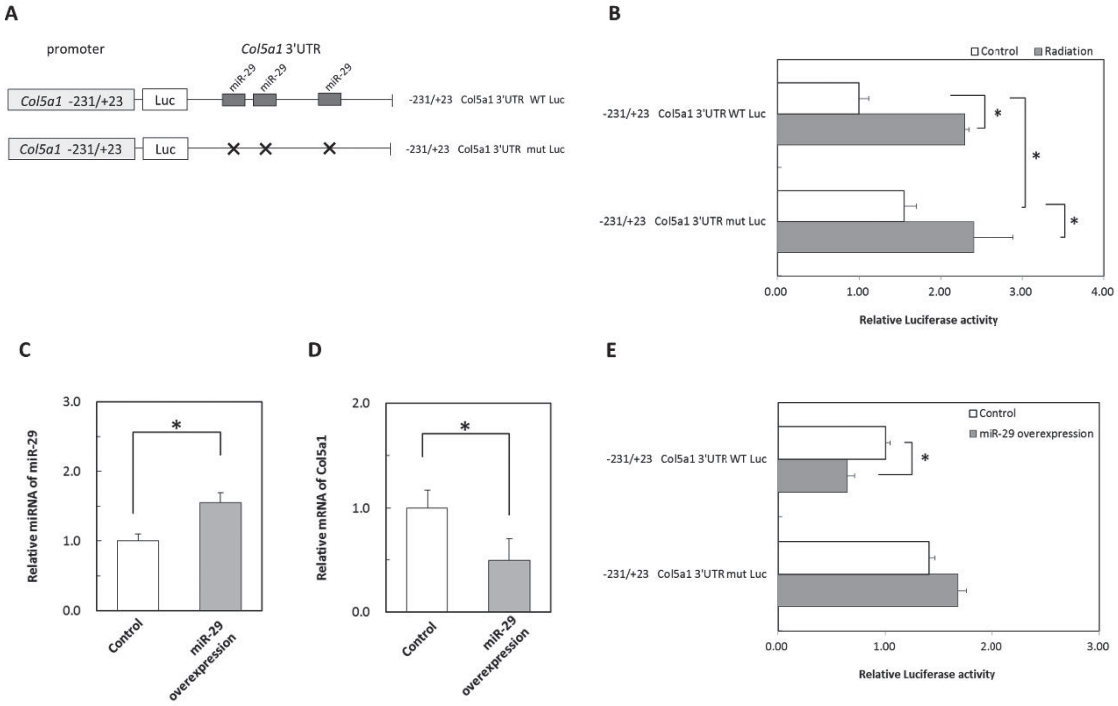

Figure 3 – The effect of miR-29 on the expression of Col5a1.

（A）A schematic illustration of the luciferase wild-type and mutant reporter construct. The miR-29 binding sites in the 3′ UTR of *Col5a1* mRNA were obtained using TargetScan（http://targetscan.org/）.（B）The luciferase activity of Col5a1 3′ UTR in wild-type and mutant reporter construct. The histograms indicated the activity normalized to the luciferase construct in the wild-type reporter construct.（C）The expression of mature miR-29 after the transfection with the miR-29 mimic using a real-time PCR. The effect of the overexpression of miR-29 on the expression of *Col5a1*（D）and the luciferase activity of *Col5a1* 3′ UTR（E）. The results are shown as the mean ± S.D.（n=3）. Statistical comparisons between groups were performed using Student's t-test（$*p < 0.05$）.

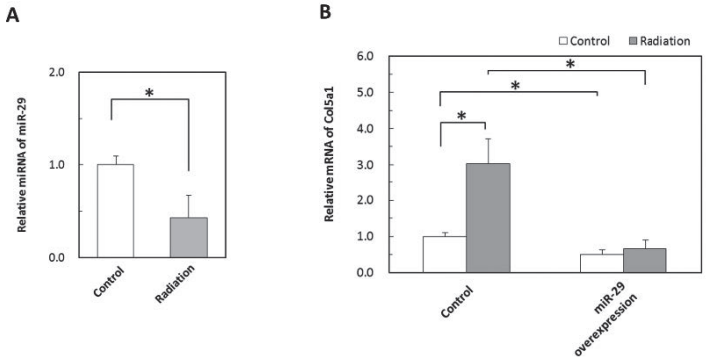

Figure 4 – The effect of miR-29 on radiation-induced *Col5a1* expression changes.

（A）The expression of mature miR-29 in normal and irradiated NIH-3T3 cells, as assessed by a real-time PCR.（B）The effect of the overexpression of miR-29 on the mRNA expression levels of *Col5a1* in normal and irradiated NIH-3T3 cells. The results are shown as the mean ± S.D.（n=3）. Statistical comparisons between groups were performed using Student's t-test（$*p < 0.05$）. .

regulates the promoter activity of *Col5a1*[11]. TGF-β is a potential pro-fibrotic factor and induces the expression of collagens. The expression of *Col5a1* has been reported to be activated by TGF-β, resulting in the increased deposition of collagen V as well as collagen types I and III fibrils[19]. However, there has been no report on the site to which TGF-β responds in this proximal promoter region. In the present study, ionizing radiation increased the transcriptional activity of the proximal promoter region of the *Col5a1*, suggesting that the TGF-β response site exists within this proximal promoter region.

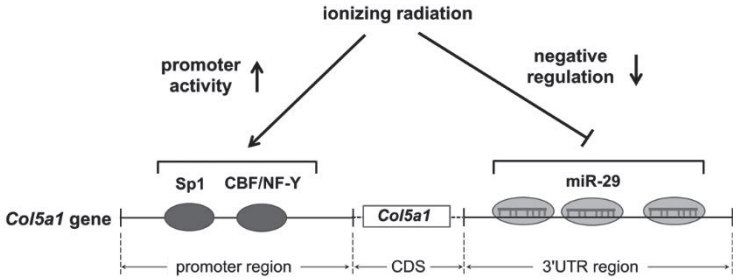

Figure 5 – Schematic illustration of the regulation of the *Col5a1* expression following ionizing radiation at the transcriptional and miR-29-mediated post-transcriptional levels.

In the present study, we obtained evidence that, in addition to regulating the expression of *Col5a1* at the transcriptional level, miR-29 acts on the post-transcriptional regulation of the expression as well. Ionizing radiation increased the *Col5a1* 3′ UTR activity, indicating that specific binding of miR-29 to the *Col5a1* 3′ UTR is attenuated, i.e. the expression of the miR-29 expression is reduced by irradiation. Gene expression is controlled by complex interactions of many different factors. It is reported that miR-29 mediates TGF-β-induced ECM synthesis through the PI3K-AKT pathway in human pulmonary fibrosis[20]. Furthermore, in renal fibrosis, the TGF-β/Smad signaling pathway was shown to be involved in regulating miR-29 expression[21]. Our group previously identified the proximal promoter region of miR-29 and showed that ionizing radiation reduces the transcriptional activity of this region[22]. The decrease in miR-29 expression caused by ionizing radiation in the present study is also thought to be due to a decrease in the transcriptional activity of this proximal promoter region.

Since miR-29 regulates ECM metabolism in various organ fibrosis, it has attracted attention as an anti-fibrotic factor. In the activation of human stellate cells in liver fibrosis, miR-29 acts as a negative regulator of type I collagen expression by interacting with the 3′ UTR of *COL1A1* and SP1[23]. In lung cancer, miR-29 also plays an important role in regulating cell proliferation, differentiation, and apoptosis of cancer cells[24]. Our previous studies have also shown that miR-29 is involved in the co-expression of type I and V collagen, indicating that it is essential for the formation of fibril under pathological conditions such as organ fibrosis and various cancers.

This study still has some limitations. First, we used only one type of cell, fibroblasts. However, the cellular mechanism involved in RIF have been seen as multicellular processes involving various types of cells, such as myofibroblasts. Second, the upstream regulation mechanisms of miR-29 is still uncertain in the present study and we cannot say how ionizing radiation induced a decrease of the expression of miR-29 through TGF-β/Smad signaling pathway. Further studies are needed to develop the clinical application of miR-29 in RIF.

In conclusion, we demonstrated that, in association with RIF, the expression of *Col5a1* is increased in irradiated mouse fibroblasts at the level of transcription and miR-29-mediated post-transcriptional regulation of expression. Like the profibrotic factor TGF-β, miR-29 plays an important role as an antifibrotic factor in RIF. These results indicate the potential utility of miR-29 as a therapeutic target in RIF.

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References

1) Wang B, Wei J, Meng L, Wang H, Qu C, Chen X, Xin Y Jiang X, Advances in pathogenic mechanisms and

management of radiation-induced fibrosis, Biomed. Pharmacother., 121, 109560, 2020.
2) Rodemann HP, Bamberg M,Cellular basis of radiation-induced fibrosis. Radiother. Oncol. 32, 83-90, 1995.
3) Gordon MK, Hahn RA, Collagens, Cell Tissue. Res., 339, 247-257, 2010.
4) van der Rest M, Garrone R, Collagen family of proteins, FASEB J., 5, 2814-2823, 1991.
5) Shoulders MD and Raines RT, Collagen Structure and Stability, Annu. Rev. Biochem., 78, 929-958,2009.
6) Birk DE, Type V collagen: heterotypic type I/V collagen interactions in the regulation of fibril assembly, Micron, 32, 223-237, 2001.
7) Border WA, Noble NA, Transforming growth factor b in tissue fibrosis, N. Engl. J. Med., 331, 1286-1292, 1994.
8) Pohlers D, Brenmoehl J, Löffler I, Müller CK, Leipner C, Schultze-Mosgau S, TGF-β and fibrosis in different organs −molecular pathway imprints. Biochim. Biophys. Acta., 1792, 746-756, 2009.
9) Martin M, Lefaix J, Delanian S, TGF−β1 and radiation fibrosis, a master switch and a specific therapeutic target? Int. J. Radiat. Oncol. Biol. Phys., 47 277-290, 2000.
10) Yano H, Hamanaka R, Nakamura M, Sumiyoshi H, Matsuo N, Yoshioka H, Smad, but not MAPK, pathway mediates the expression of type I collagen in radiation induced fibrosis, Biochem. Biophys. Res. Commun., 418, 457-463, 2012.
11) Sakata-Takatani K, Matsuo N, Sumiyoshi H, Tsuda T, Yoshioka H, Identification of a functional CBF-binding CCAAT-like motif in the core promoter of the mouse pro-α1 (V) collagen gene (Col5a1), Matrix Biol., 23, 87-99, 2004.
12) Bartel DP, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell, 116, 281-297, 2014.
13) Jiang XP, Ai WB, Wan LY, Zhang YQ, Wu JF, The roles of microRNA families in hepatic fibrosis, Cell Biosci., 7, 2017.
14) Noetel A, Kwiecinski M, Elfimova N, Huang J, Odenthal M, microRNA are central players in anti- and profibrotic gene regulation during liver fibrosis, Front Physiol., 19, 1-6, 2012.
15) Friedman SL, Liver fibrosis: from mechanisms to treatment, Gastroenterol Clin. Biol., 31, 812-814, 2007.
16) Maher TM, Wells AU, Laurent GJ, Idiopathic pulmonary fibrosis: multiple causes and multiple mechanisms? Eur. Respir. J., 30, 835-839, 2007.
17) Elnakish MT, Kuppusamy P, Khan M, Stem cell transplantation as a therapy for cardiac fibrosis, J. Pathol., 229, 347-354, 2013.
18) Zhang JJ, Yano H, Sasaki T, Matsuo N, Yoshioka H, The pro-α1 (V) collagen gene (Col5a1) is coordinately regulated by miR-29b with core promoter in cultured cells, Connect. Tissue Res., 59,263-273, 2018.
19) Vittal R, Mickler EA, Fisher AJ, Zhang C, Rothhaar K, Gu H, Brown KM, Emtiazjoo A, Lott JM, Frye SB, Smith GN, Sandusky GE, Cummings OW, Wilkes DS, Correction: Type V Collagen Induced Tolerance Suppresses Collagen Deposition, TGF-β and Associated Transcripts in Pulmonary Fibrosis, PLoS One, 13, e0209107, 2018.
20) Yang T, Liang Y, Lin Q, Liu J, Luo F, Li X, Zhou H, Zhuang S, Zhang H, miR-29 mediates TGFβ1-induced extracellular matrix synthesis through activation of PI3K-AKT pathway in human lung fibroblasts, J. Cell. Biochem., 114, 1336-1342, 2013.
21) Qin W, Chung AC, Huang XR, Meng XM, Hui DS, Yu CM, TGF-β/Smad3 signaling promotes renal fibrosis by inhibiting miR-29, J. Am. Soc. Nephrol., 22, 1462-1474, 2011.
22) Yano H, Hamanaka R, Nakamura-Ota M, Zhang JJ, Matsuo N, Yoshioka H, Regulation of type I collagen expression by microRNA-29 following ionizing radiation, Radiat. Environ. Biophys., 57, 41-54, 2018.
23) Ogawa T, Iizuka M, Sekiya Y, Yoshizato K, Ikeda K, Kawada D, Suppression of type I collagen production by microRNA-29b in cultured human stellate cells, Biochem. Biophys. Res. Commun., 391, 316-321, 2010.
24) Liu X, Lv X, Yang Q, Jin H, Zhou W, Fan Q, MicroRNA-29a Functions as a Tumor Suppressor and Increases Cisplatin Sensitivity by Targeting NRAS in Lung Cancer, Technol. Cancer Res. Treat., 17, 1-8, 2018.