

Inhibition of Smad3 Expression in Radiation-Induced Fibrosis Using a Novel Method for Topical Transcutaneous Gene Therapy

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Objective: To attempt to mitigate the effects of irradiation on murine skin after high-dose radiation using a novel transcutaneous topical delivery system to locally inhibit gene expression with small interfering RNA (siRNA) against *Smad3*.

Design: Laboratory investigation.

Setting: University laboratory.

Subjects: Twenty-five wild-type C57 mice.

Intervention: In an isolated skin irradiation model, the dorsal skin of C57 wild-type mice was irradiated (45 Gy). Just before irradiation, *Smad3* and nonsense siRNA were applied to 2 separate dorsal skin areas and then reapplied weekly. Skin was harvested after 1 and 4 weeks. *Smad3* expression were assessed by immunohistochemistry, and collagen deposition and architecture was examined using picrosirius red collagen staining.

Main Outcome Measures: Epidermal thickness was measured semiquantitatively at 4 weeks. Radiation-

induced fibrosis was measured quantitatively via tensiometry. The Young modulus, a measure of cutaneous rigidity inversely related to elasticity, was determined, with normal irradiated skin serving as a control specimen.

Results: Murine skin treated with topical *Smad3* siRNA demonstrated effective *Smad3* inhibition at 1 week and persistent suppression at 4 weeks. Collagen deposition and epidermal thickness were significantly decreased in skin treated with *Smad3* siRNA compared with control irradiated skin. Tensiometry demonstrated decreased tension in *Smad3* siRNA-treated skin, with a Young modulus of 9.29 MPa (nonirradiated normal skin, 7.78 MPa) compared with nonsense (control) siRNA-treated skin (14.68 MPa).

Conclusions: *Smad3* expression can be effectively silenced in vivo using a novel topical delivery system. Moreover, cutaneous *Smad3* inhibition mitigates radiation-induced changes in tissue elasticity, restoring a near-normal phenotype.

Arch Otolaryngol Head Neck Surg. 2010;136(7):714-719

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RADIOTHERAPY HAS BEEN widely accepted for the primary or adjuvant treatment of many head and neck cancers for nearly a century. Although radiotherapy is used to kill cancerous cells, it also damages healthy cells, leading to many acute and long-term adverse effects.^{1,2} Early damage is characterized by erythema, dry and moist desquamation, and ulceration. Long-term effects of radiotherapy include skin atrophy, decreased elasticity, soft-tissue fibrosis, microvascular damage, and impaired wound healing. Skin is particularly sensitive to radiation damage.² Damage is progressive and may continue for many years after radiotherapy. The degree of skin damage and ability of cells to recover from radiation damage mainly depend on radiation technique, dose rate,

total dose, and cellular conditions. Radiotherapy used to treat malignant neoplasms can induce a pathologic fibrotic response that can compromise patients' quality of life and may complicate later surgical intervention.^{1,3} Because of unavoidable exposure during radiotherapy, the skin is commonly subject to fibrosis yielding unpliant, discolored tissue with poor wound-healing characteristics.

In the early phase after irradiation, multiple transcriptional and signaling pathways are activated, leading to the induction of a variety of growth factors and cytokines.^{3,4} Transforming growth factor β (TGF- β) is a well-described, central regulator of physiologic and pathologic inflammation and fibrosis, including radiation-induced fibrosis.⁴⁻⁸ Transforming growth factor β acts as a potent chemotactic factor for inflammatory cells and an induction fac-



Figure 1. Mouse model of isolated skin radiation injury. Dorsal skin was clamped without compromising perfusion. The remainder of body was shielded from irradiation.

tor for extracellular matrix production by fibroblasts. Recent studies have shown that TGF- β signaling during the fibrotic response is largely dependent on Smad3, an intracellular mediator.⁸⁻¹¹ Once TGF- β binds to its receptor on fibroblasts, Smad3, a member of the Smad family of intracellular proteins, is activated and translocates to the nucleus as a transcription factor to directly activate the expression of genes involved in extracellular matrix production, including various collagens, matrix metalloproteinase 2, and tissue inhibitor of matrix metalloproteinase 1.^{12,13} Irradiated human tissue and several mammalian models of radiation injury have demonstrated increased levels of TGF- β and Smad3 in vitro and in vivo.¹⁴⁻¹⁶ Similarly, animal wounding models have shown increased Smad3 expression in areas of wound healing, especially in the epidermis and dermal hair follicles.¹⁷

Studies on *Smad3* knockout mice suggest that Smad3 has an important relationship to fibrosis in vivo.¹⁴⁻¹⁶ The predominant postradiation damage in skin is increased thickness of the epithelial spinous layer (acanthosis) and pronounced dermal fibrosis, characterized by increased deposition and disorganized architecture of collagens. *Smad3* knockout mice are protected against radiation-induced cutaneous injury, with decreased epidermal acanthosis and dermal inflammation, and show improved wound healing in previously irradiated skin compared with wild-type mice.¹⁶ The objective of our study was to inhibit Smad3 expression in vivo at the skin level. Using a novel transcutaneous topical delivery system to locally inhibit gene expression with small interfering RNA (siRNA) previously described by this laboratory,¹⁸ we attempted to mitigate the effects of irradiation on murine skin after high-dose radiotherapy by inhibiting cutaneous Smad3 expression. We report herein the changes in histopathologic phenotype and biomechanical properties of irradiated murine skin after treatment with *Smad3* siRNA.

METHODS

ANIMAL IRRADIATION

Dorsal skin of wild-type C57 mice (n=25) was irradiated with a single dose of 45 Gy, delivered by a standard linear accelerator (Varian Medical Systems, Inc, Palo Alto, California), as described by an isolated skin injury model from this laboratory¹⁹ that delivers clinically relevant radiation doses, causing reproducible skin fibrosis without systemic radiation exposure (**Figure 1**). By distracting the dorsal skin while shielding the mouse's body, the skin can be specifically targeted by high-dose radiation without compromising skin perfusion. Dorsal hair was removed using a commercial chemical exfoliator (Nair; Church & Dwight Co, Inc, Lakewood, New Jersey) before all



Figure 2. Topical small interfering RNA (siRNA) gel matrix application on intact skin. Preformed wells were constructed from thick hydrocolloid dressing (DuoDERM; ConvaTec, Skillman, New Jersey). The agarose-siRNA gel complex was reapplied weekly and secured in place with plastic wrap.

treatments. Before irradiation, the dorsal skin was pretreated with a detergent (1% Triton X-100; Fisher Scientific, Pittsburgh, Pennsylvania) to exfoliate the outer stratum corneum and to allow better penetration of the siRNA treatment.

TOPICAL siRNA TRANSFECTION

An agarose matrix-based system to topically deliver siRNA precisely to skin was described previously by this laboratory.¹⁷ Briefly, a 1.1% agarose gel (UltraPure; Invitrogen, Carlsbad, California) is complexed with 500 pmol of *Smad3* siRNA (Silencer select siRNA; Applied Biosciences, Foster City, California) or nonsense (control) siRNA (Applied Biosciences) and a 65% volume of reduced-serum, antibiotic-free media (OptiMEM; Invitrogen), for a total volume of 200 μ L of agarose-siRNA gel per application site. The application area was demarcated using a 1.5-cm circular frame constructed from 1-mm-thick hydrocolloid dressing (DuoDERM; ConvaTec, Skillman, New Jersey) (**Figure 2**). The gel was applied directly onto the pretreated dorsal skin and secured in place with plastic wrap before irradiation. This matrix was reapplied weekly after irradiation, based on previous optimization studies. The delivery system targets specific RNA production at the skin level locally without off-target effects. A total of 10 animals were included in each treatment group (*Smad3* vs nonsense siRNA). A separate cohort of 5 animals undergoing irradiation did not receive any treatment.

ANIMAL HARVEST AND ANALYSIS BY IMMUNOHISTOCHEMISTRY

After irradiation, the animals were observed daily and 5 animals from each treatment group were humanely killed after 1 and 4 weeks. Time points were chosen to exemplify early and progressive stages of fibrosis after radiation exposure, based on previous findings in an isolated radiation skin injury animal model from this laboratory.¹⁹ The dorsal skin was removed and divided into sections for histopathologic examination and tissue tensiometry. The histopathologic sections were transferred to 10% neutral buffered formalin for 24 hours, then transferred to 70% ethanol in preparation for paraffin embedding. Formalin-fixed, paraffin-embedded tissue samples were cut into

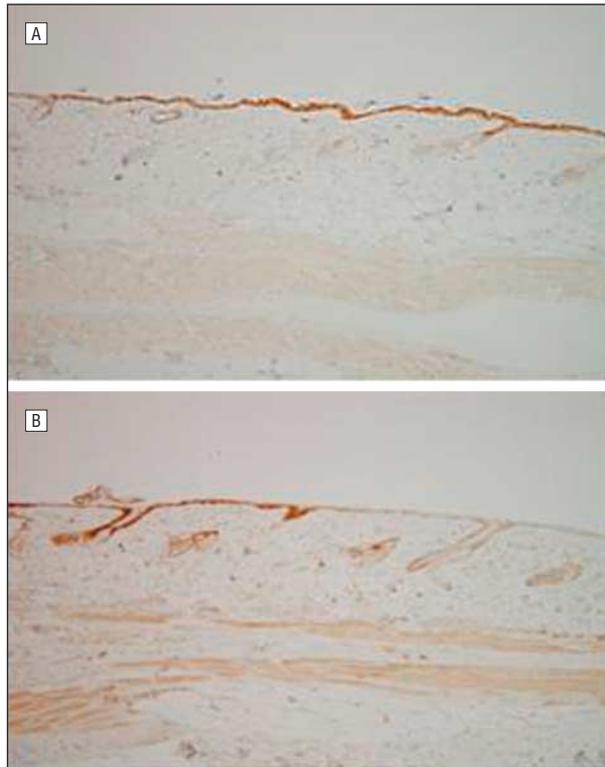


Figure 3. Results of immunohistochemistry studies at week 1. A, Diffuse epidermal Smad3 (brown) staining on nonsense (control) small interfering RNA (siRNA)-treated skin (original magnification $\times 20$). B, Near-complete inhibition of Smad3 expression in area treated with *Smad3* siRNA (original magnification $\times 20$). Treatment area is on the right half of the slide; normal skin, the left half.

5- μ m sections and stained with hematoxylin-eosin. For immunolocalization of Smad3, deparaffinized sections were blocked with 10% rabbit serum and incubated overnight with Smad3 primary rabbit antibody (14 μ g/mL) (R&D Systems, Minneapolis, Minnesota) in blocking buffer. Sections were washed, incubated with biotinylated antirabbit IgG secondary antibody, washed again, then incubated with a preformed avidin-biotinylated enzyme complex, as directed by the kit manufacturer (Vectastain Elite ABC Kit; Vector Laboratories, Inc, Burlingame, California). Normal wild-type mice undergoing irradiation (without any siRNA treatment) were used as positive controls for Smad3 expression. For negative controls, we stained normal, wild-type irradiated murine skin with primary antibody alone.

To analyze the collagen content and architecture of irradiated skin, deparaffinized sections were stained for 1 hour with 0.1% picosirius red solution (Sirius Red; Sigma-Aldrich Corp, St Louis, Missouri). The intensity of red staining correlated with the degree of collagen deposition. Epidermal thickness was measured using commercially available image-editing software (Adobe Photoshop CS3 Image Editor; Adobe Systems Inc, San Jose, California). Results are expressed as mean (SEM) in pixels. Statistical significance was determined by the 2-tailed *t* test.

TISSUE TENSIOMETRY

Fresh tissue sections stored in normal saline solution were cut into thin, uniform (4 \times 20-mm) sections immediately after harvest for tissue tensiometry to quantitatively measure radiation-induced fibrosis. These sections were distracted to measure cutaneous rigidity using a tensiometer (Chatillon TCD-200; Commercial Scale Co, Inc, Agawam, Massachusetts), and stress-

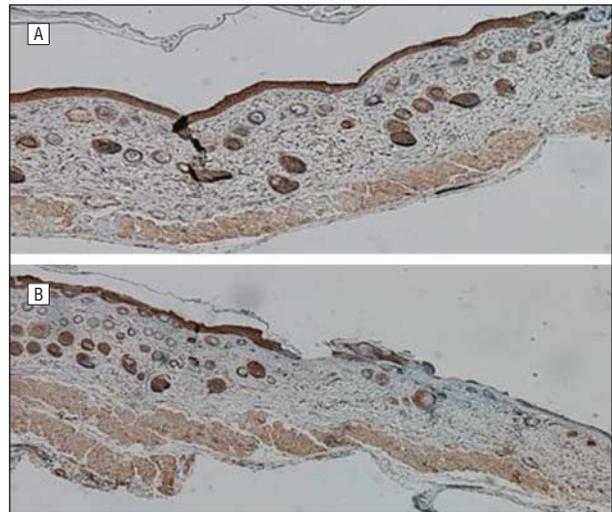


Figure 4. Results of immunohistochemistry studies at week 4. A, Significant epidermal Smad3 (dark brown) staining in nonsense small interfering RNA (siRNA)-treated skin (original magnification $\times 20$). B, Absence of Smad3 expression in *Smad3* siRNA treatment area (right half of slide) (original magnification $\times 20$).

strain curves were generated to measure the Young modulus, a coefficient of rigidity inversely related to elasticity. The Young modulus was calculated for all 3 of the following groups: normal (nonirradiated), *Smad3* siRNA-treated (irradiated), and nonsense siRNA-treated (irradiated) skin. Results are presented as mean (SEM) Young modulus, and statistical significance was calculated by the *t* test.

All animal experiments were repeated in triplicate for validity.

RESULTS

SMAD3 EXPRESSION AT THE SKIN LEVEL WITH TOPICAL siRNA DELIVERY

As early as 1 week after irradiation, immunohistologic examination of irradiated dorsal skin sections topically treated with *Smad3* siRNA revealed near-complete inhibition of Smad3 expression in the epidermis (**Figure 3**). The extent of inhibition was limited precisely to the topical treatment area. The skin outside the treatment range exhibited strong Smad3 staining, as would be expected in the early fibrotic phase after irradiation. Four weeks after irradiation, the effects were more pronounced, with effective silencing of Smad3 expression in the epidermis and dermis in the area treated weekly with topical *Smad3* siRNA (**Figure 4**). This effect did not penetrate beyond the subdermal muscular layer.

EPIDERMAL THICKNESS AND DENSITY OF DERMAL COLLAGEN DEPOSITION

Four weeks after irradiation, the epidermal layer in the dorsal skin treated with *Smad3* siRNA was markedly thinner than that of nonsense siRNA-treated skin (**Figure 5**). The mean epidermal height of the *Smad3* siRNA-treated skin was 27.6 (3.3) pixels compared with 77.5 (20.2) pixels when measured using the image-editing software ($P = .06$) (**Figure 6**). Dermal fibrosis is related not only to the quantity of collagen produced but to its quality of

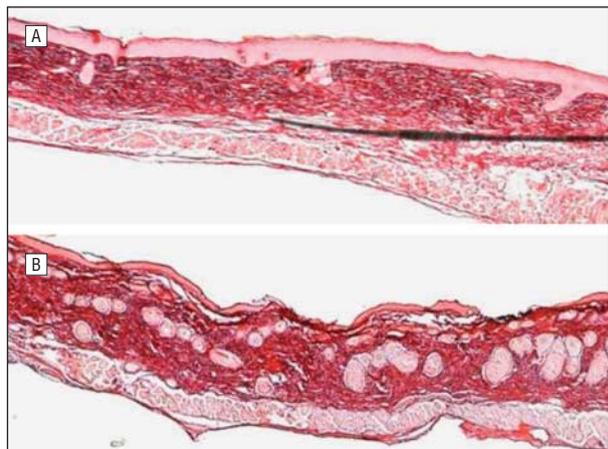


Figure 5. Epidermal growth at week 4 (picrosirius red collagen stain). A, Nonsense small interfering RNA (siRNA)-treated skin with thicker, more stratified epidermal layer (light pink) (original magnification $\times 10$). B, *Smad3* siRNA-treated skin with thin epidermal layer (original magnification $\times 10$).

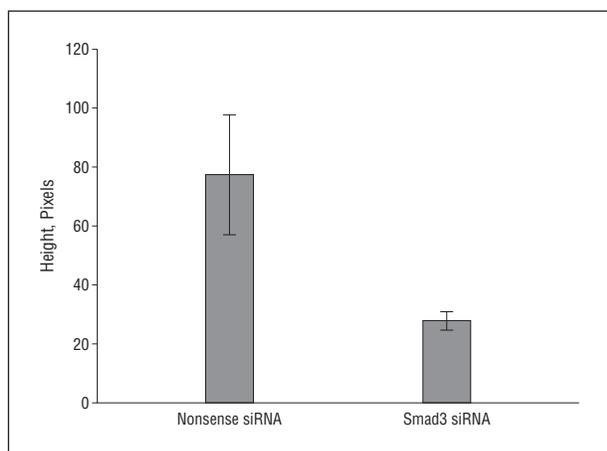


Figure 6. Epidermal height at week 4. Mean (SEM) epidermal height of small interfering RNA (siRNA)-treated skin was 27.6 (3.3) pixels vs 77.5 (20.2) pixels in the nonsense *Smad3* siRNA-treated group ($P = .06$).

organization in dermal architecture. Picrosirius red staining for collagen was markedly diminished qualitatively in the *Smad3* siRNA group compared with nonsense siRNA (**Figure 7**). *Smad3* siRNA-treated skin displayed a loose, basket-weave-type pattern, whereas there was a denser, darker collagen pattern in the nonsense siRNA-treated skin 4 weeks after irradiation. These phenotypic effects were limited to the treatment areas and did not affect the untreated skin or subdermal structures.

TISSUE ELASTICITY IN IRRADIATED SKIN

The Young modulus measures the stiffness of an elastic material and is defined as the ratio of the amount of applied force (stress) over the amount of change in length of a material under mechanical compression (strain). Skin elasticity decreased in irradiated skin as measured by an increase in the Young modulus (**Figure 8**). However, this was reversed in irradiated skin treated with *Smad3* siRNA, with a reduction in Young modulus (4 weeks after irradiation). The Young modulus of *Smad3* siRNA-treated skin (9.29 [0.02] MPa) was not only signifi-

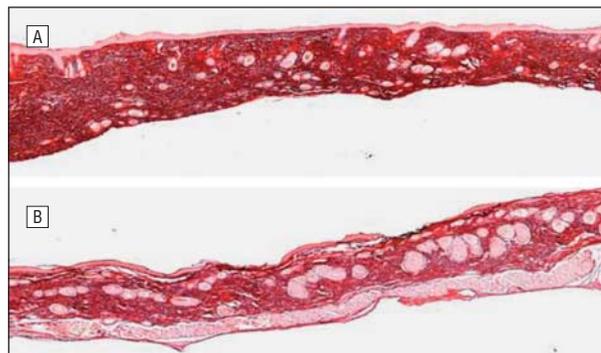


Figure 7. Picrosirius red collagen staining. A, Nonsense small interfering RNA (siRNA)-treated skin with dense, dark collagen staining (original magnification $\times 10$). B, *Smad3* siRNA skin with lighter, loose staining pattern (original magnification $\times 10$).

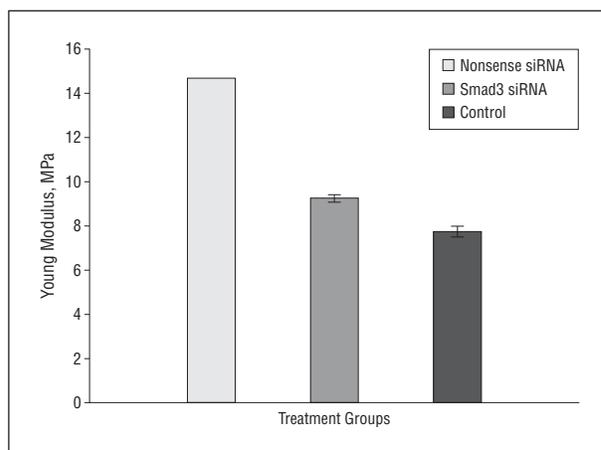


Figure 8. Young modulus (mean [SEM]) for measuring cutaneous rigidity. Irradiated *Smad3* small interfering RNA (siRNA)-treated skin (9.29 [0.02] MPa) was more elastic than irradiated nonsense siRNA-treated skin (14.68 [0.10] MPa; $P = .002$). *Smad3* siRNA-treated skin more closely resembled normal, nonirradiated skin (7.78 [0.21] MPa; $P = .007$).

cantly decreased compared with the nonsense siRNA group (14.68 [0.10] MPa; $P = .002$), but approached the Young modulus of normal, nonirradiated skin (7.78 [0.21] MPa; $P = .007$).

COMMENT

Although there are multiple signaling pathways involved in the TGF- β -dependent fibrosis pathway, *Smad3* is a specific signaling mediator that is essential for the synthesis of collagens 1, 3, 6, and 7; metalloproteinase 2; tissue inhibitor of metalloproteinase 1; and various other proteins involved in extracellular matrix deposition.^{9,13} Other complex processes of TGF- β -dependent chemotaxis and inhibition of epithelial migration also appear to be *Smad3* dependent, implicating this pathway in wound healing and fibrosis, particularly after irradiation.¹⁴ The recruitment of fibroblasts in irradiated skin is largely dependent on TGF- β autoinduction by activated *Smad3*.⁹

In this study, we show that inhibition of *Smad3* gene expression after irradiation can be effectively performed at the cutaneous level using a topical transcuta-

neous delivery system through intact, otherwise normal skin. Immunohistochemical staining shows a dramatic decrease in Smad3 expression in the epidermal and dermal areas treated with *Smad3* siRNA. Given the growing evidence linking Smad3 expression to TGF- β -dependent fibrosis pathways, including radiation-induced fibrosis, this observed decrease in Smad3 expression after irradiation suggests that skin treated topically with *Smad3* siRNA may be less susceptible to fibrosis. This may be analogous to the observation that *Smad3* knockout mice show significantly less acanthosis, ulceration, hyperkeratosis, and dermal inflammation resulting from exposure of the skin to high doses of ionizing radiation.¹⁶

Radiation exposure has been reported to increase production of total and active TGF- β within several hours after exposure and persisting for several months.^{5,7} Autoinduction of TGF- β in macrophages and neutrophils is Smad3 dependent, and selective loss of Smad3 expression may eliminate or reduce amplification and sustained TGF- β expression and chemotactic responses to TGF- β . This may be particularly important for fibrotic processes involving the skin after irradiation by reducing the initial influx of inflammatory cells and fibroblasts and ultimately reducing fibrosis.

Reductions in epidermal thickness and density of collagen deposition in the *Smad3* siRNA-treated group suggest that loss of Smad3 has altered keratinocyte and fibroblast signaling normally associated with ionizing radiation and resulting in fibrosis. The control of keratinocyte proliferation in vivo is complex and has been shown to be dependent on a paracrine interaction with fibroblasts in which interleukin 1 secreted by keratinocytes induces fibroblasts to secrete keratinocyte growth factor that then activates keratinocyte proliferation.^{20,21} Loss of Smad3 expression may disrupt this signaling interaction, thus reducing keratinocyte proliferation and ultimately limiting epidermal growth. Similarly, inhibition of Smad3 may decrease transcriptional activation of collagens 1, 3, 5, and 6 and other extracellular matrix proteins that are also Smad3 dependent, thereby reducing extracellular matrix deposition and dermal collagen organization. Fibrosis is characterized by the differentiation of fibroblasts into myofibroblasts that acquire contractile properties and secrete extracellular matrix proteins such as collagens. Although this conversion is temporary in normal wound healing, it remains chronically elevated in tissue fibrosis. Data suggest that Smad3 participates in the recruitment of fibroblasts to the site of injury and possibly also the differentiation of fibroblasts into myofibroblasts to secrete collagen.¹⁶

Although we have not directly quantified collagen levels, we hypothesized that the deleterious effects of radiation on the biomechanical profile of skin treated with *Smad3* siRNA will be lessened compared with control skin. Clinical quantification of fibrosis is somewhat subjective, with current practices dependent on clinician palpation scores and patient symptom questionnaires, and results are difficult to reproduce and follow up over time.²² In the absence of a criterion standard for measuring fibrosis, the Young modulus is a universally measurable physical characteristic of pliable material. The Young

modulus in the *Smad3* siRNA-treated irradiated skin samples was significantly greater than that of control irradiated skin and, perhaps more revealing, more closely resembled that of normal, nonirradiated skin. These data suggest that not only does Smad3 inhibition reduce the amount of matrix collagen deposition, but treatment also alters the biomechanical properties of irradiated skin and mitigates the loss of tissue elasticity that characterizes irradiation.

In conclusion, the increasing use of ionizing radiation for the treatment of head and neck malignant neoplasms and the resulting deleterious acute and chronic effects on skin make the study of wound healing and fibrosis in irradiated skin an important clinical problem. Smad3 is a key mediator in the fibrosis pathway, and targeted inhibition of Smad3 could have important clinical applications in radiotherapy. The topical siRNA delivery system proposed herein provides a novel strategy to potentially alleviate or even reverse the fibrotic effects of radiotherapy on the skin.

Submitted for Publication: May 6, 2009; final revision received November 11, 2009; accepted February 18, 2010.

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Author Contributions: Dr Saadeh had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Lee and Tutela contributed equally to this article. *Study concept and design:* Lee, Tutela, Zoumalan, Thanik, and Saadeh. *Acquisition of data:* Lee, Tutela, Zoumalan, Nguyen, and Varjabedian. *Analysis and interpretation of data:* Lee, Warren, and Saadeh. *Drafting of the manuscript:* Lee. *Critical revision of the manuscript for important intellectual content:* Tutela, Zoumalan, Thanik, Nguyen, Varjabedian, Warren, and Saadeh. *Obtained funding:* Zoumalan and Saadeh. *Administrative, technical, and material support:* Zoumalan, Thanik, Nguyen, and Varjabedian. *Study supervision:* Warren and Saadeh.

Financial Disclosure: None reported.

Funding/Support: This study was supported by CORE grant 2009 from the Plastic Surgery Educational Foundation/American Academy of Otolaryngology–Head and Neck Surgery Foundation.

Previous Presentation: This study was presented at the American Head and Neck Society 2009 Annual Meeting; May 31, 2009; Phoenix, Arizona.

REFERENCES

1. Dormand EL, Banwell PE, Goodacre TE. Radiotherapy and wound healing. *Int Wound J*. 2005;2(2):112-127.
2. Hopewell JW. The skin: its structure and response to ionizing radiation. *Int J Radiat Biol*. 1990;57(4):751-773.
3. Rodemann HP, Bamberg M. Cellular basis of radiation-induced fibrosis. *Radiation Oncol*. 1995;35(2):83-90.
4. Müller K, Meineke V. Radiation-induced alterations in cytokine production by skin cells. *Exp Hematol*. 2007;35(4)(suppl 1):96-104.
5. Martin M, Lefaix J, Delanian S. TGF- β 1 and radiation fibrosis: a master switch and a specific therapeutic target? *Int J Radiat Oncol Biol Phys*. 2000;47(2):277-290.

6. Border WA, Noble NA. Transforming growth factor β in tissue fibrosis. *N Engl J Med*. 1994;331(19):1286-1292.
7. Randall K, Coggle JL. Long-term expression of transforming growth factor TGF β 1 in mouse skin after localized β -irradiation. *Int J Radiat Biol*. 1996;70(3):351-360.
8. Shi Y, Massague J. Mechanisms in TGF- β signaling from cell membrane to the nucleus. *Cell*. 2003;113(6):685-700.
9. Flanders KC. Smad3 as a mediator of the fibrotic response. *Int J Exp Pathol*. 2004;85(2):47-64.
10. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF- β family signaling. *Nature*. 2003;425(6958):577-584.
11. Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF. Targeted disruption of Smad3 reveals an essential role in transforming growth factor β -mediated signal transduction. *Mol Cell Biol*. 1999;19(4):2495-2504.
12. Chen SJ, Yuan W, Lo A, Trojanowska M, Varga J. Interaction of Smad3 with a proximal Smad-binding element of the human α 2(I) procollagen gene promoter required for transcriptional activation of TGF- β . *J Cell Physiol*. 2000;183(3):381-392.
13. Verrecchia F, Chu ML, Mauviel A. Identification of novel TGF- β /Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. *J Biol Chem*. 2001;276(20):17058-17062.
14. Flanders KC, Major CD, Arabshahi A, et al. Interference with transforming growth factor- β /Smad3 signaling results in accelerated healing of wounds in previously irradiated skin. *Am J Pathol*. 2003;163(6):2247-2257.
15. Ashcroft GS, Yang X, Glick AB, et al. Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat Cell Biol*. 1999;1(5):260-266.
16. Flanders KC, Sullivan CD, Fujii M, et al. Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. *Am J Pathol*. 2002;160(3):1057-1068.
17. Chong AK, Satterwhite T, Pham HM, et al. Live imaging of Smad2/3 signaling in mouse skin wound healing. *Wound Repair Regen*. 2007;15(5):762-766.
18. Thanik VD, Greives MR, Lerman OZ, et al. Topical matrix-based siRNA silences local gene expression in a murine wound model. *Gene Ther*. 2007;14(17):1305-1308.
19. Thanik VD, Chang CC, Zoumalan RA, et al. A novel mouse model of cutaneous radiation injury. *Plast Reconstr Surg*. In press.
20. Maas-Szabowski N, Shimotoyodome A, Fusenig NE. Keratinocyte growth regulation in fibroblast cultures via a double paracrine mechanism. *J Cell Sci*. 1999;112(pt 12):1843-1853.
21. Maas-Szabowski N, Stark HJ, Fusenig NE. Keratinocyte growth regulation in defined organotypic cultures through IL-1-induced keratinocyte growth factor expression in resting fibroblasts. *J Invest Dermatol*. 2000;114(6):1075-1084.
22. Leung SF, Zheng Y, Choi C, et al. Quantitative measurement of the post-irradiation neck fibrosis based on the Young modulus: description of a new method and clinical results. *Cancer*. 2002;95(3):656-662.

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