Original Article | Clinical Studies

Connexin 43 expression in irradiated human neck skin: a prospective case-control study

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Background: Overexpression of connexin 43 (Cx43) is seen in chronic cutaneous wounds of humans, and downregulation of Cx43 expedites healing. Human epithelia that have received radiotherapy can also suffer from chronic wounds. Since increased expression of Cx43 was observed in murine skin following irradiation, we sought to confirm this in irradiated human neck skin because Cx43 inhibition may be a novel treatment of wounds in such tissue.

Methods: We prospectively recruited adult patients who underwent elective neck surgery between November 2017 and March 2018 in the Otorhinolaryngology Department of a tertiary hospital in Singapore. A sample size of five patients with prior radiotherapy to 25 controls without radiotherapy was planned a priori. Immunohistochemical staining of Cx43 was performed in the neck skin obtained from the patients and quantified under confocal microscopy. The association between demographic factors, comorbidities of the patients, and Cx43 expression was explored. Wound healing was assessed between 7 to 14 days postoperatively.

Results: Five patients received radiotherapy 9 months to 27 years before surgery. The expression of Cx43 was 94.2 *vs.* 146.4 pixel-area/nucleus in the epidermis with or without radiotherapy, respectively (P=0.39). The demographics and co-morbidities of the patients with or without radiotherapy were similar except for relative anemia in the patients who had radiotherapy (hemoglobin of 12.15 *vs.* 14.10 g/dL, P=0.02), but hemoglobin was not correlated with Cx43 expression (P=0.93). All skin incisions healed without dehiscence.

Conclusions: Previous radiotherapy may not alter Cx43 expression in human neck skin. Whether the same is true for chronic radiation-induced wounds or acute radiation-induced dermatitis should be investigated.

Keywords: Connexin 43 (Cx43); overexpression; human skin; radiation; neck

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Introduction

Connexins (Cxs) are gap junction proteins that facilitate intercellular communication in human tissues. Growing evidence supports its role in epithelial wound healing. In acute cutaneous wounds, connexin expression is dynamically orchestrated in tandem with the phases of wound healing (1). Inhibiting the expression of connexin 43 (Cx43), the most ubiquitous connexin in skin, soon after wounding expedites the closure of both incisional and excisional wounds and improves scarring (2). In the privileged healing of mucosal wounds, Cx26, Cx30, and Cx43 down-regulate more rapidly than in cutaneous wounds (3); in the nearly scarless healing of gingiva, Cx43 downregulates rapidly after wounding, promoting fibroblast migration and regulating the expression of wound healing-associated genes (4). In contrast, overexpression of Cx43 is associated with a variety of chronic wounds. In humans, diabetic, venous and pressure ulcers massively over-express Cx43 (5,6). Such overexpression is associated with reduced migration of keratinocytes at the wound edge. Treating the cutaneous wounds in streptozotocin-induced diabetic rats with antisense oligodeoxynucleotide (asODN) against Cx43 messenger RNA (mRNA) rescues healing and re-epithelialization (7), partly by reducing the recruitment of neutrophils, macrophages and by enhancing the formation of granulation tissue (2,8). Moreover, Cx43 down-regulation may reduce scar formation in deep cutaneous wounds (9). To translate these to patient care, recent randomized controlled clinical trials show that targeting the carboxyl terminus of Cx43 can augment the healing of chronic venous or diabetic ulcers and improve scarring in surgical incisions (10-13).

A type of wound that has received less attention than those above is radiation-induced wounds. Although exposure to a dose of environmental radiation sufficient to cause wounds is rare, ionizing radiation, or radiotherapy, is commonly used to treat human cancers. Being non-invasive and deeply penetrating, radiotherapy is a standard treatment in cancers such as nasopharyngeal carcinoma, and a definitive, adjuvant, or palliative treatment of many other malignancies such as squamous cell carcinoma of the head and neck. However, non-neoplastic tissue along the path of the ionizing radiation can be damaged during the treatment of cancer. This can manifest as cutaneous or mucosal wounds (14) that show impaired healing. Moreover, surgical resection of the cancer may still be required to salvage radiation failure or to treat new cancers arising in the irradiated tissue, necessitating an incision through the irradiated skin or mucosa. Such incisions are associated with higher rates of breakdown than those made in non-irradiated tissue (15). In the neck, the resultant wound can become life-threatening by exposing the carotid artery. Thus, methods that improve the healing of irradiated cutaneous or mucosal wounds are needed.

Since overexpression of Cx43 is seen in chronic cutaneous wounds in humans, and its inhibition rescues healing, it is possible that Cx43 overexpression plays a role in the impaired healing of irradiated human skin to wounding. That ionizing radiation causes Cx43 upregulation in the epidermis and dermis was observed in a murine model in which mice were irradiated to a cumulative dose of 90 Gy over 6 weeks at 3 Gy per fraction (16), resulting in a marked and dose-dependent increase in the expression of Cx43 associated with hypertrophy and hyperplasia of the epidermis. In a swine model of cutaneous radiation injury developed by XequelBio®, topical application of a mimetic peptide targeting the carboxyl terminus of Cx43 increased the probability and speed of wound closure compared to the vehicle gel (17). However, human studies on the molecular basis of cutaneous radiation-induced wounds are few. One study employing immunohistochemistry demonstrated that chronic cutaneous wounds on the irradiated neck of patients showed impaired expression of angiogenic vascular endothelial growth factor and basic fibroblast growth

Highlight box

Key findings

- Connexin 43 (Cx43) overexpression, seen in chronic cutaneous wounds, is not apparent in human neck skin that received radiotherapy in the past.

What is known and what is new?

- Chronic cutaneous or mucosal wounds can occur in patients who receive radiotherapy, a common treatment of head and neck cancer. Cx43 upregulation is seen in chronic human wounds such as diabetic, venous, or pressure ulcers. A murine study also showed dose-dependent increase in Cx43 expression in irradiated skin, implying that Cx43 upregulation may contribute to the impaired healing of irradiated human skin.
- In this case-control study of human neck skin, we find that Cx43 expression is not obviously increased by radiotherapy delivered 9 months to 27 years prior to surgery.

What is the implication, and what should change now?

- Future studies may focus on Cx43 expression in acute radiation-induced dermatitis which more closely mimics the animal models of cutaneous radiation injury.

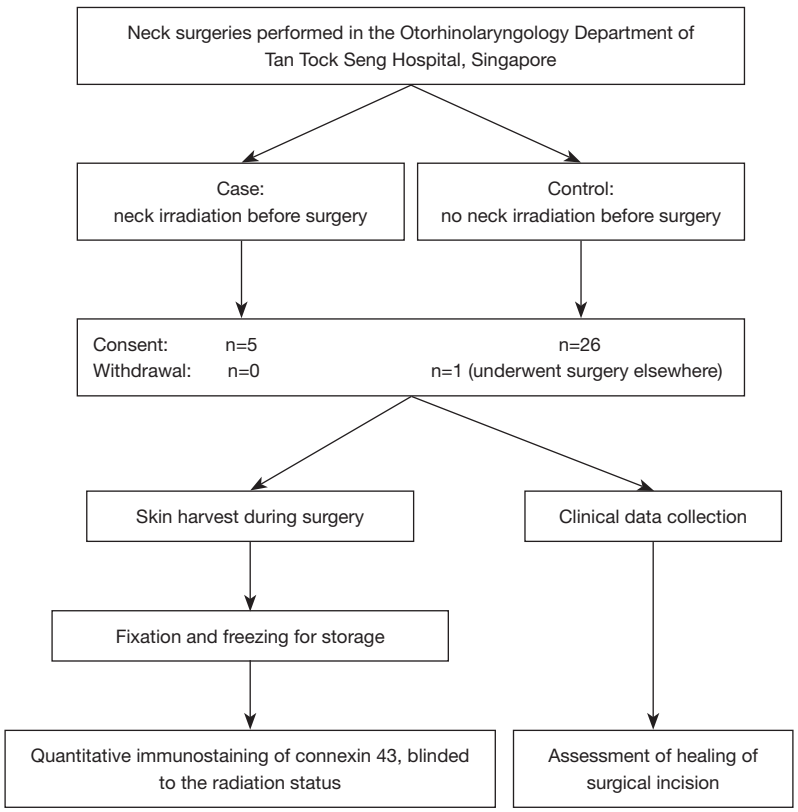

Figure 1 Study flowchart.

factor (18); keratinocytes from such wounds also expressed less transforming growth factor (TGF)-α and TGF-β1, fibroblast growth factor 1 and 2, keratinocyte growth factor, and hepatocyte growth factor than controls (19). Because the application of asODN to knock down Cx43 in cutaneous wounds increased TGF-β1 expression, promoted fibroblast migration and angiogenesis (8), we hypothesize that Cx43 overexpression may contribute to the impaired healing of wounds in irradiated tissue. Such overexpression may also persist after the completion of radiotherapy as impaired healing is observed chronically. For this purpose, we conducted a prospective case-control study to determine Cx43 expression in neck skin with or without previous radiotherapy. We present this article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-24-158/rc).

Methods

Study design

In the Otorhinolaryngology Department of Tan Tock Seng Hospital, we prospectively recruited adult patients who underwent surgery requiring an incision on the anterior or lateral neck from 9 November 2017 to 28 March 2018. The inclusion criteria are age ≥21 years and non-emergency surgeries. The exclusion criteria are (I) presence of a wound in the site of surgical incision; (II) lack of mental capacity to consent; (III) current pregnancy; (IV) history of non-radiation-induced dermatitis on the neck; (V) human immunodeficiency virus infection; (VI) active infection of the neck skin; (VII) connective tissue disease; (VIII) immune-modulating medications within 1 week of surgery. *Figure 1* shows the flowchart of the recruitment process. In each patient, after informed consent, we sought the donation of a 1 cm × 3 cm ellipse of skin from the site of their surgical incision (predominantly the lateral neck) for the determination of connexin expression. A sample size of five irradiated cases to 25 non-irradiated controls is considered adequate because fractionated irradiation of the murine skin similar to radiotherapy showed a visually compelling increase in Cx43 expression even between groups of 2–3 mice (16), indicating a large effect size. Moreover, irradiated human skin samples are hard to obtain,

thus a 1:5 ratio of case-to-control is intentionally designed to increase the statistical power per recommendation in epidemiology (20). This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics review board of National Healthcare Group (No. DSRB 2017/00383) and Nanyang Technological University of Singapore (No. IRB-2017-08-032), and informed consent was obtained from all individual participants.

Radiotherapy

In the five irradiated cases, the exact dose and plan of radiotherapy was available in one patient who received radiotherapy 9 months prior to surgery (adjuvant photon irradiation for laryngeal carcinoma, 2 Gy per day from Monday to Friday for 6 weeks without the addition of systemic therapy), and the skin specimen received 55.9 Gy of irradiation. The other cases received radiotherapy for the definitive treatment of nasopharyngeal carcinoma (two patients, 4 and 19 years ago, respectively, with concurrent chemotherapy in the patient treated 4 years ago), laryngeal carcinoma (one patient, 3 years ago), or postoperative radiotherapy for carcinoma of the oral tongue (one patient, 27 years ago). The dose of irradiation these skin specimens received could not be precisely calculated, but it is estimated to range from 20 to 70 Gy.

Immunostaining

The skin was excised from the patient at the start of the surgery, placed in 4% paraformaldehyde immediately and allowed to fix overnight, then snap frozen and stored at −80 ℃. It was then mounted in Tissue-Tek® O.C.T. (Torrace, CA, USA) and cryosections were cut at 10 µm thickness on a Leica® cryostat (Nussloch GmbH, Nussloch, Germany), placed on glass slides which were then stored at −20 ℃. Sections were rehydrated in phosphate-buffered saline (PBS) and blocked in 1% bovine serum albumin in PBS for 1 hour. They were then incubated in the primary antibody (rabbit anti-Cx43 1:1,000; Sigma C6219, St. Louis, MO, USA) overnight at 4 ℃. No primary antibody control (positive control) was included. Next day sections were washed twice in washing buffer (0.05% PBS/Tween 20) and then incubated in goat anti-rabbit Alexa Flour™ 488 1:500 (Thermo Fisher Scientific A11008, Waltham, MA, USA) for 1 hour in the dark at room temperature. Sections were then washed twice for 10 minutes, and the nuclei were counterstained with 4',6-diamidino-2-phenylindole (1:10,000; Life Technologies, Carlsbad, CA, USA), mounted with CitiFluor™ AF1 mounting medium (Electron Microscopy Sciences, London, UK) and sealed with nail varnish. Secondary antibody control (negative control) was performed to check for non-specific staining in the background. When present, these were of low intensity and were filtered out during the image analysis as follows.

Imaging and analysis

Stained sections were imaged by a Leica SP8 confocal microscope. Fluorophores were excited sequentially using 405 and 488 nm laser lines. Three regions of interest (ROIs) of identical size were demarcated in the epidermis per skin sample. All images were captured using identical laser powers, gain, and offset to allow for direct comparison and quantification. In-house quantification algorithms were used in ImageJ software (National Institute of Health, Bethesda, MD, USA). Because the images were 8-bit and 1,024×1,024 pixels, there were a total of 256 levels of color intensity [0–255] in each pixel. Thus, manual threshold values of 100 to 255 were set to filter out background noise. These thresholds were kept constant in generating a binary image for analysis. The readout was pixel-area per nucleus. The investigators were blinded to the radiation status of the skin specimen.

Additional clinical data

In addition to the details about the radiotherapy delivered to the skin, we collected the following clinical data from the electronic medical record at the time of surgery: patient's age, sex, ethnicity, location of the harvested skin, co-morbidities (diabetes mellitus, hypertension, hyperlipidemia, ischemic heart disease, stroke, chronic obstructive pulmonary disease, chronic renal impairment), pre-operative body mass index (BMI; weight in kg/height in m^2), serum hemoglobin concentration (g/dL), and the surgical closure technique. In diabetic patients, preoperative glycosylated hemoglobin A1c (HbA1c) level was also recorded when available. Healing of the neck incision was assessed by ruling out wound dehiscence on post-operative day 14 in patients who had radiotherapy and on day 7 in patients who did not have radiotherapy. The different timing is because surgical sutures are not removed from radiated tissue until 14 days postoperatively, but it can be removed earlier in non-irradiated tissue.

Statistical analysis

In each skin sample, the mean and the standard error of measurement of the Cx43 staining intensity in the three ROIs are calculated. The distribution of the mean in the radiation and control groups is then tested for normality using the Shapiro-Wilk test. Median and range are presented if normal distribution is not observed. Parametric continuous variables are compared by two-sample *t*-test. Non-parametric continuous variables are compared by Wilcoxon rank sum test if they are binary, or Kruskal-Wallis tests if they contain more than two groups. Categorical variables are compared by Fisher's exact test. Correlation between age, BMI, hemoglobin, and Cx43 expression is determined by Spearman's test. Missing data is not imputed. An alpha of 0.05 is sufficient to reject the null hypothesis. The above tests are performed on STATA (BE version 17.0, College Station, TX, USA).

Results

The patient demographics, laterality of the skin samples, BMI, and comorbidities did not differ significantly between those with and without radiotherapy (*Table 1*) except for serum hemoglobin concentration, which was significantly lower in patients who received radiotherapy (12.15 *vs.* 14.10 g/dL, P=0.02). With respect to the primary outcome (*Table 2*), the level of Cx43 expression in epidermis did not differ significantly between skin that received radiotherapy and those that did not (94.2 *vs.* 146.4 pixel-area/nucleus, respectively, Wilcoxon rank sum test, P=0.39). To check if serum hemoglobin concentration could confound this observation, the scatterplot of Cx43 on hemoglobin in the same individual showed no obvious relationship (Figure S1), and Spearman's correlation test showed no significance (P=0.93). Neither was age, sex, ethnicity, laterality of skin, diabetes mellitus, hypertension, hyperlipidemia, ischemic heart disease, stroke, chronic obstructive pulmonary disease, chronic renal impairment, or chronic liver disease associated with Cx43 expression (*Table 2*). Moreover, Spearman's test showed no correlation between Cx43 expression with age or BMI (P=0.38 and 0.24, respectively). The overall distribution of Cx43 expression in all 30 skin specimens appears bimodal, with the majority (24 out of 30) being 230.4 pixel-area/nucleus or lower and the rest being 296.5 pixel-area/nucleus or higher. This was observed in both radiated and non-radiated skin (*Figure 2*). Examples of such contrasting expression of Cx43 are shown in *Figure 3*. However, no association was found between the clinical, demographic variables and high or low Cx43 expression (*Table 3*). All skin incisions healed without dehiscence.

Discussion

This is a rare study in human that sought to determine whether Cx43 would be overexpressed in skin that received radiotherapy. One challenge in the design of the study is in the adjustment of confounders of Cx43 expression when comparing radiotherapy cases to controls. Besides the rare congenital skin diseases caused by mutations in the connexin-encoding genes (21), psoriasis and eczema can also perturb Cx43 expression (22), and diabetes was associated with decreased epidermal and increased dermal Cx43 expression in streptozotocin-treated rats (7). Thus, gross signs of dermal disease at the skin harvest site were exclusion criteria in this study. To minimize unknown confounders, we also excluded patients with immunosuppressive medications, autoimmune conditions, and obtained skin from the neck of adults only. Interestingly, we did not find diabetes mellitus to be significantly associated with Cx43 expression level in the epidermis. This may be because the diabetes in the patients of this study could be milder than the diabetes in animal models (glycosylated HbA1c level is available in five out of the six diabetic patients with values of 5.6, 6.2, 6.7, 7.5, and 12.1 mmol/L, respectively, indicating satisfactory glycemic control in four out of five patients). Regarding the difference in the serum hemoglobin concentration between the case and control groups, it likely reflects a higher prevalence of anemia in patients who were treated for head and neck cancer than in those who were not. This difference may not be due to radiotherapy alone but may also be contributed by the major surgical resection or chemotherapy some patients had for their cancer, or dysphagia-related malnutrition. Moreover, data on hemoglobin is missing in about 20% of irradiated and non-irradiated patients because medically fit patients undergoing minor surgeries would not require a hemoglobin measurement. Thus, this observation should be interpreted with caution. Nevertheless, in subjects with data, the lack of correlation between serum hemoglobin concentration and Cx43 in the same patient reduces the risk of hemoglobin as a confounder of the relationship between Cx43 expression and irradiation.

Our result shows that Cx43 expression in skin that received radiotherapy months to years ago is similar to skin that has not received radiotherapy. To be exact, Cx43

Table 1 Patient characteristics

Characteristics	Previous radiotherapy (n=5)	No radiotherapy (n=25)	P value
Age (years)	62.5 (6.0)	59.4 (14.0)	0.63
Sex			0.64
Male	4 [80]	16 [64]	
Female	1 [20]	9 [36]	
Ethnicity			0.76
Chinese	4 [80]	19 [76]	
Malay	0	3 [12]	
Indian	1 [20]	2 [8]	
Others	0	1 [4]	
Laterality of neck skin			0.55
Left	2 [40]	15 [60]	
Right	3 [60]	8 [32]	
Central	0	1 [4]	
Bilateral	0	1 [4]	
BMI (kg/m^2)	21.7 (4.08)	26.0 (5.45)	0.11
Hemoglobin (g/dL)	12.15 (12–13.1)†	14.10 (10.7–15.7)†	0.02
Diabetes mellitus			0.55
Yes	0	6 [24]	
No	5 [100]	19 [76]	
Hypertension			>0.99
Yes	1 [20]	7 [28]	
No	4 [80]	18 [72]	
Hyperlipidemia			>0.99
Yes	1 [20]	7 [28]	
No	4 [80]	18 [72]	
Ischemic heart disease			>0.99
Yes	0	4 [16]	
No	5 [100]	21 [84]	
Stroke			0.31
Yes	1 [20]	1 [4]	
No	4 [80]	24 [96]	
Chronic obstructive pulmonary disease			>0.99
Yes	0	1 [4]	
No	5 [100]	24 [96]	

Table 1 (*continued*)

Table 1 (*continued*)

Characteristics	Previous radiotherapy (n=5)	No radiotherapy (n=25)	P value
Chronic renal impairment			0.18
Yes	2 [40]	3 [12]	
No	3 [60]	22 [88]	
Chronic liver disease			>0.99
Yes	0	1 [4]	
No	5 [100]	24 [96]	

Data are presented as mean (SD), n [%], or median (range). †, serum hemoglobin concentration in 1 radiated and 6 non-radiated patients was unknown. BMI, body mass index; SD, standard deviation.

Table 2 Association between radiation status, clinical factors, and Cx43 expression in human neck skin

Clinical factors	Cx43 expression in pixel-area/nucleus, median (range)	P value	Standard error of measurement
Radiation		0.39	
Yes (n=5)	94.2 (21.9–302.9)		12.94
No (n=25)	146.4 (21.1–447.4)		32.23
Age (years)		0.54	
<50 (n=6)	81.15 (21.1–169.3)		14.78
≥50 but <60 (n=12)	149.15 (32.4–412.4)		21.27
≥60 but <70 (n=5)	120 (61.3–426.3)		31.23
≥70 (n=7)	180.8 (21.9–447.4)		36.69
Sex		0.73	
Male (n=20)	130.3 (21.9–447.4)		30.01
Female (n=10)	152.2 (21.1–230.4)		24.02
Ethnicity		0.53	
Chinese (n=23)	148.3 (21.1–447.4)		33.76
Malay (n=3)	55.2 (42.9–102)		12.76
Indian (n=3)	173.3 (37.4–296.5)		31.23
Others (n=1)	146.4 (N/A)		2.01
Laterality of neck skin		0.96	
Left (n=17)	148.3 (21.1–426.3)		31.23
Right (n=11)	140.6 (32.4–447.4)		12.94
Central (n=1)	169.3 (N/A)		73.4
Bilateral (n=1)	120.0 (N/A)		56.99
Diabetes mellitus		0.76	
Yes (n=6)	116.8 (32.4–426.3)		25.28
No (n=24)	147.4 (21.1–447.4)		29.60

Table 2 (*continued*)

Table 2 (*continued*)

Clinical factors	Cx43 expression in pixel-area/nucleus, median (range)	P value	Standard error of measurement
Hypertension		0.07	
Yes (n=8)	177.1 (92.9–447.4)		35.23
No (n=22)	111 (21.1–426.3)		19.93
Hyperlipidemia		0.40	
Yes (n=8)	157.0 (32.4–447.4)		32.50
No (n=22)	133.2 (21.1–426.3)		25.51
Ischemic heart disease		0.25	
Yes (n=4)	234.9 (42.9–447.4)		43.27
No (n=26)	130.3 (21.1–426.3)		25.51
Stroke		0.16	
Yes (n=2)	241.9 (180.8–302.9)		44.23
No (n=28)	130.3 (21.1–447.4)		25.51
Chronic obstructive pulmonary disease		0.69	
Yes (n=1)	158 (N/A)		41.36
No (n=29)	140.6 (21.1–447.4)		27.96
Chronic renal impairment		0.93	
Yes (n=5)	173.3 (21.9–302.9)		31.23
No (n=25)	140.6 (21.1–447.4)		27.96
Chronic liver disease		0.60	
Yes (n=1)	92.9 (N/A)		9.55
No (n=29)	146.4 (21.1–447.4)		31.23
Presence of any comorbidity		0.22	
Yes (n=19)	148.3 (21.9–447.4)		33.8
No (n=11)	75.2 (21.1–384.5)		14.58

Cx43, connexin 43; N/A, not applicable.

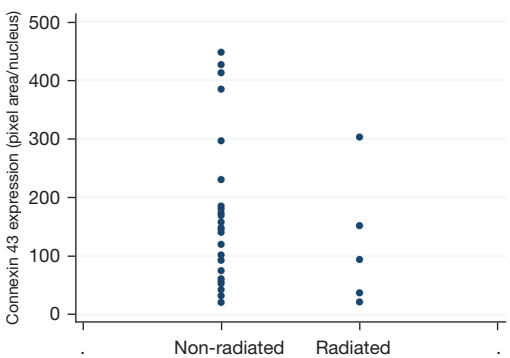

Figure 2 Distribution of Cx43 expression in adult neck skin with or without previous radiotherapy. Cx43, connexin 43.

measured 37.4, 151.9, 94.2, 21.9, and 302.9 pixel-area/nucleus in skins that received radiotherapy 9 months, 3 years, 4 years, 19 years and 27 years ago, respectively. This implies that the completion of radiotherapy as recent as 9 months ago did not influence Cx43 expression compared to the completion of radiotherapy much earlier. Certainly, the lack of precise dose of radiation the skin samples received in most cases can cast doubt on the validity of this conclusion. However, it is the standard of care to irradiate bilateral neck in the treatment of nasopharyngeal carcinoma; certainly, the larynx—a midline neck structure—was irradiated in the patient with laryngeal carcinoma, and

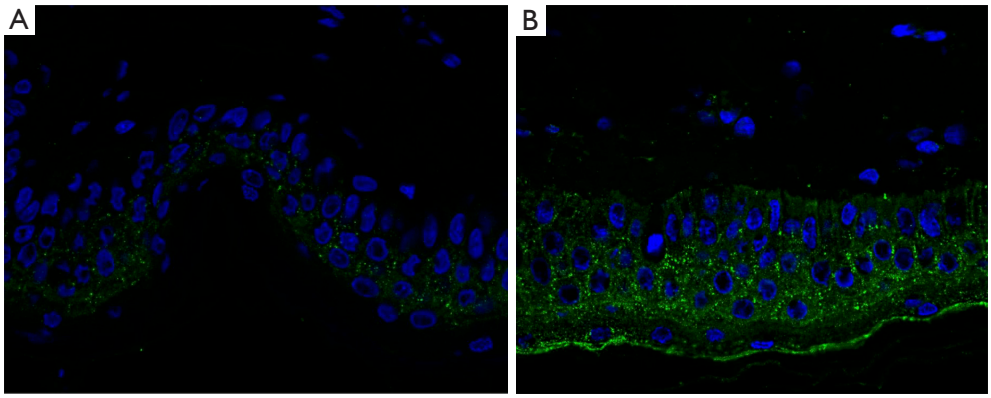

Figure 3 Images of Cx43 expression in the epidermis of human neck skin. (A) Low expression level of 21.1 pixel-area/nucleus. (B) High expression level of 447.4 pixel-area/nucleus. The magnification was 40 times (40×). Cx43 stains green; nuclei stain blue. Cx43, connexin 43.

Table 3 Association between clinical factors and high or low Cx43 expression in human neck skin

Characteristics	High Cx43 expression (296.5–447.4 pixel-area/nucleus; n=6)	Low Cx43 expression (21.1–230.4 pixel-area/nucleus; n=24)	P value
Age (years)	63.8 [11.7]	58.9 [13.4]	0.42
Sex			0.07
Male	6 (100.0)	14 (58.3)	
Female	0	10 (41.7)	
Ethnicity			>0.99
Chinese	5 (83.3)	18 (75.0)	
Malay	0	3 (12.5)	
Indian	1 (16.7)	2 (8.3)	
Others	0	1 (4.2)	
Laterality of neck skin			>0.99
Left	4 (66.7)	13 (54.2)	
Right	2 (33.3)	9 (37.5)	
Central	0	1 (4.2)	
Bilateral	0	1 (4.2)	
BMI (kg/m^2)	23.1 [2.6]	25.8 [5.8]	0.28
Hemoglobin (g/dL)	13.7 [1.0]	13.6 [1.4]	0.88
Diabetes mellitus			>0.99
Yes	1 (16.7)	5 (20.8)	
No	5 (83.3)	19 (79.2)	
Hypertension			0.65
Yes	2 (33.3)	6 (25.0)	
No	4 (67.7)	18 (75.0)	

Table 3 (*continued*)

Table 3 (*continued*)

Characteristics	High Cx43 expression (296.5–447.4 pixel-area/nucleus; n=6)	Low Cx43 expression (21.1–230.4 pixel-area/nucleus; n=24)	P value
Hyperlipidemia			0.65
Yes	2 (33.3)	6 (25.0)	
No	4 (67.7)	18 (75.0)	
Ischemic heart disease			0.17
Yes	2 (33.3)	2 (8.3)	
No	4 (67.7)	22 (91.7)	
Stroke			0.37
Yes	1 (16.7)	1 (4.2)	
No	5 (83.3)	23 (95.8)	
Chronic obstructive pulmonary disease			>0.99
Yes	0	1 (4.2)	
No	6 (100.0)	23 (95.8)	
Chronic renal impairment			>0.99
Yes	1 (16.7)	4 (16.7)	
No	5 (83.3)	20 (83.3)	
Chronic liver disease			>0.99
Yes	0	1 (4.2)	
No	6 (100.0)	23 (95.8)	
Presence of any comorbidity			0.37
Yes	5 (83.3)	14 (58.3)	
No	1 (16.7)	10 (41.7)	

Data are presented as mean [SD] or n (%). BMI, body mass index; Cx43, connexin 43; SD, standard deviation.

the oral cavity or upper anterolateral neck or both were irradiated in the patient with tongue cancer. In all these patients, the laterality of the skin harvest was concordant with the site of irradiation; and since it was not possible to spare the overlying skin from irradiation when these patients were treated, we are able to provide a range of radiation dose these skin samples received (20–70 Gy). Because even 15 Gy of X-irradiation given at 3 Gy per day, Monday to Friday, increased Cx43 expression in the epidermis of mice (16), we could expect that the skin samples in our study would be able to show an obvious difference in Cx43 expression between cases and controls. Nevertheless, the limited sample size lacks power to detect a small effect of radiotherapy on Cx43 expression. However, the similar distribution of Cx43 expression in the cases and controls makes this association less likely.

Clinically, the manifestation of radiation effect on skin is bimodal. The acute phase results in a dermatitis that can ulcerate through the skin, but this typically resolves within 4 weeks of completion of the radiotherapy. The chronic phase ensues in a milder manner characterized by the gradual development of telangiectasia, fibrosis, and atrophy (14). As the chronic effects are relentless, the irradiated skin samples in this study should reflect this. Thus, our finding suggests that Cx43 expression is probably not a hallmark of the chronic cutaneous effects of radiotherapy. Moreover, no clear association existed between Cx43 expression and age, sex, ethnicity, or common chronic medical conditions. This supports that Cx43 expression in skin is probably altered mainly after wounding (1-4,9,23,24). Considering these,

it is possible that the acute radiation-induced dermatitis may more likely involve Cx43 overexpression, consistent with the hyperplastic and hypertrophic skin observed in the murine model (16). This acute dermatitis can be akin to burns as the irradiated skin can turn erythematous, swell, peel, exude, and ulcerate. A study on skin samples obtained from human burn wounds showed that elevated Cx43 expression in dermal fibroblasts may play a role in the progression of such wounds through the spreading of apoptosis (25). Moreover, asODN against Cx43 expedited the healing of partial thickness skin burns in mice (26). However, acute radiation-induced dermatitis is a painful condition that also heals quite promptly. This causes challenge in consenting patients for the donation of their skin for research during the occurrence of the dermatitis. Considering this, it is prudent to conduct additional animal studies to determine the effect of inhibiting Cx43 expression on acute radiation-induced dermatitis before translation to humans. Regarding this, the proprietary compound aCT1, a mimetic peptide of Cx43 developed by XequelBio®, shows promise in a swine model (17). This compound interacts with the binding partners of Cx43, promotes the switch of Cx43 from hemichannels to gap junction channels, thus enhancing the intercellular communication while reducing the hemichannel activities that lead to inflammation (27).

Our study has several other limitations. First, despite radiotherapy, all cutaneous wounds healed without dehiscence. Thus, the skin obtained may differ from those present in chronic radiation wounds. Second, we did not perform a positive control on the primary antibody because we saw that the distribution of Cx43 in our skin samples was as expected in the epidermis, a pattern established in the last two decades (21). Off-target staining might have occurred, but it would likely affect all skin samples and not bias the analysis. Finally, the skin samples are obtained from Asians of predominantly Chinese ethnicity. Whether these results are generalizable to non-Asians should be determined.

Conclusions

Cx43 over-expression is not apparent in human neck skin that received radiotherapy in the past. Future studies may choose to investigate the role of connexin in chronic radiation-induced wounds or acute radiation-induced dermatitis.

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None.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-24-158/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-24-158/dss

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics review board of National Healthcare Group (No. DSRB 2017/00383) and Nanyang Technological University of Singapore (No. IRB-2017-08-032), and informed consent was obtained from all individual participants.

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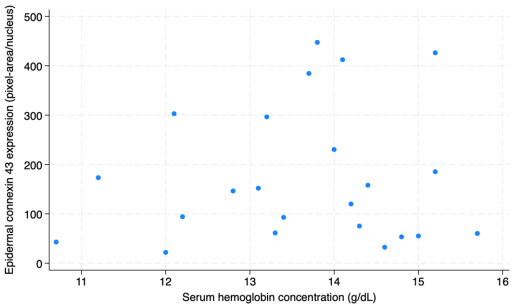

Figure S1 Scatterplot of the co-relation between serum hemoglobin concentration and Cx43 expression in epidermis. Cx43, connexin 43.

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