INVESTIGATIVE REPORT

Radiation Therapy Induces Tenascin Expression and Angiogenesis in Human Skin

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In analysing radiation-induced connective tissue changes, we studied tenasin expression, elastic fibres, angiogenesis and physio-mechanical properties in irradiated and contralateral healthy skin of radiotherapy-treated breast cancer patients. Skin biopsies were obtained from a radiotherapy-treated skin area and a corresponding non-treated skin area. Haematoxylin-eosin and Verhoeff stainings as well as immunohistochemical stainings for tenasin and factor VIII were performed. Epidermal and total skin thickness, together with the amount of elastic tissue calculated by computerized digital image analysis, were measured. Suction blisters were induced on both skin areas. Transepidermal water loss was analysed. Skin elasticity was also measured. Tenasin expression was found to be increased in irradiated human skin. In haematoxylin-eosin and factor VIII-stained sections, an increase in the number of blood vessels was detected. Although skin stiffness measured by an elastometer was increased in irradiated skin, no marked difference in the elastic fibres could be found between treated and non-treated skin. The increased tenasin expression could be due to activation of cytokines as a result of irradiation. An increase in angiogenesis could be caused by an activation of angiogenetic factors by irradiation or due to direct radiation damage on blood vessel walls. Our findings suggest that the effects of irradiation tend to accumulate in the dermal parts of skin. The higher skin stiffness values measured by elastometer in irradiated skin could be due to an accumulation of dermal connective tissue as a result of irradiation. Key words: angiogenesis; human skin; irradiation; tenasin.

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Tenasin is a large extracellular matrix glycoprotein. In fetal tissues it has been associated with morphogenesis. Its expression in most adult tissues is restricted or absent (5–7). In skin, tenasin is associated with wound healing, melanocytic tumors and premalignant and malignant lesions (8–11). In a previous study, tenasin expression was found to be increased in pig cutaneous tissue after high-dose gamma radiation (12). However, tenasin expression in X-irradiated human skin has not been previously studied.

The recoil properties of skin are largely due to elastin, although the amount of this protein in dermal connective tissue is low compared to that of collagen. A modification of collagen synthesis as a result of irradiation has previously been established in both animal models and human skin (13–15). Yet, elastic fibres in X-irradiated human skin have not been previously analysed.

In the present study, we analysed for the first time the distribution of tenasin expression in X-irradiated human skin and the effect of irradiation on dermal elastic fibres and angiogenesis. The histological findings and physio-mechanical properties of radiation-damaged skin were compared to those of non-treated skin.

MATERIALS AND METHODS

Subjects

Twenty women were randomly chosen who had been treated for breast cancer with mastectomy or breast-conserving surgery and radiation therapy (Table 1). The irradiated skin area was compared with the contralateral non-treated skin area, and the subjects thus acted as their own controls. Patients with a condition or medication affecting collagen synthesis were omitted from the study. Four of the subjects had received adjuvant therapy. Three had been treated with chemotherapy and one with hormone therapy.

Written informed consent was obtained from all the subjects and the study was approved by the Ethics Committee of the Faculty of Medicine and by Oulu University Hospital.

Histology and immunohistochemistry

A 4-mm biopsy was obtained under local anaesthesia from the irradiated skin area (at least 2 cm from the operation scar) and the contralateral non-treated skin area. Skin samples of 18 subjects were available for analysis. The specimens were fixed in 10% buffered formalin, embedded in paraffin and cut into 4-μm sections. Haematoxylin-eosin (HE) and Verhoeff stainings as well as immunohistochemical stainings for tenasin and endothelial cells were performed.

Monoclonal antitenasin 143DB7C8 (Locus, Helsinki, Finland) and polyclonal anti-human von Willebrand factor (factor VIII, FVIII) (Dako A/S, Denmark) were used. The sections for immunohistochemistry were pretreated with pepsin digestion for factor VIII. No pretreatment was given for antitenasin. The immunohistochemical stainings were performed using an ABC/HRP kit (Dako A/S, Copenhagen, Denmark) as previously described (21).

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### Table I. Clinical data of the 20 subjects

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Time from treatment (months)</th>
<th>Clinical staging (TNM)*</th>
<th>Therapy</th>
<th>Dose (Gy)</th>
<th>Energy (MeV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42–75 (mean 55)</td>
<td>10–96 (mean 26)</td>
<td>T1N0M0 2 5</td>
<td>15</td>
<td>30–56 (mean 52)</td>
<td>6–10x (mean 7)</td>
</tr>
<tr>
<td>42–75 (mean 55)</td>
<td>10–96 (mean 26)</td>
<td>T1N1M0 2 5</td>
<td>15</td>
<td>30–56 (mean 52)</td>
<td>6–10x (mean 7)</td>
</tr>
<tr>
<td>42–75 (mean 55)</td>
<td>10–96 (mean 26)</td>
<td>T2N0M0 2 5</td>
<td>15</td>
<td>30–56 (mean 52)</td>
<td>6–10x (mean 7)</td>
</tr>
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<td>42–75 (mean 55)</td>
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<td>T1NXM0 2 5</td>
<td>15</td>
<td>30–56 (mean 52)</td>
<td>6–10x (mean 7)</td>
</tr>
</tbody>
</table>

TNM-staging – T: tumour size; N: involvement of the regional lymph nodes; M: metastasis. A: breast ablation; R: breast resection; x: photon energy; e: electron energy.

*Data on TNM-grading of one of the patients not available.

### RESULTS

#### Histology and immunohistochemistry

Collagen bundles and elastin fibres visualized by HE or Verhoeff stainings did not show any marked difference between irradiated and non-irradiated skin samples. Epidermal thickness was similar in both radiotherapy-treated and healthy skin samples (Table II).

In non-treated skin, the immunohistochemical reaction for antitenascin antibody was expressed primarily as a narrow band in the papillary dermis beneath the epidermis, in the blood vessel walls and adjacent to the cutaneous appendices. Expression of tenascin was not seen in epidermis, lower dermis or subcutaneous fat (Fig. 1). In irradiated skin, the staining for tenascin beneath the epidermis was more intense, thicker and continuous. Immunohistochemical staining showed a marked increase in tenascin expression in irradiated skin ($p = 0.026$).

In irradiated skin samples, an increase in the number of blood vessels in comparison with healthy skin was found (Fig. 2). The mean number of blood vessels was 19.2 in the upper dermis of irradiated skin and 14.7 in the upper dermis of non-treated skin. In the lower dermis, the corresponding figures were 8.2 in irradiated skin and 6.4 in non-treated skin (Fig. 3). In the upper dermis of irradiated skin, the number of blood vessels was markedly increased in FVIII-stained sections compared with non-treated skin ($p = 0.010$). In the lower dermis of irradiated skin, the number of blood vessels was also increased in irradiated skin samples, although the difference was not statistically significant ($p = 0.223$). In both HE and FVIII-stained sections, there appeared to be a greater number of large blood vessels in irradiated skin compared to non-irradiated skin.

### Computerized digital image analysis

The mean thickness of the epidermis was 45.9 µm in irradiated skin and 43.1 µm in non-treated skin ($p = 0.355$). In the upper (papillary) dermis, the proportional area of elastic fibres was 3.3% in irradiated skin and 3.1% in non-treated skin ($p = 0.805$). In the lower (reticular) dermis, the proportional area of elastic fibres was 5.6% in irradiated skin and 5.9% in non-treated skin ($p = 0.617$) (Table II). There was no marked difference in the morphology of elastic fibres between irradiated and non-irradiated skin.

### Physical parameters

The mean basal TEWL was 7 g/m² h in both treated and non-treated skin. The skin stiffness value was higher in the irradiated skin area (mean 15.1 MPa) than in the corresponding healthy skin area (mean 13.4 MPa) ($p = 0.026$) (Table II). There was also an increase in skin thickness in irradiated skin ($p = 0.004$) (Table II).

The clinical skin reactions among the subjects included...
Table II. Mean epidermal thickness, skin thickness, proportional area of elastic fibres in skin biopsies and skin elasticity of irradiated skin area and contralateral non-treated skin area of the 20 patients (SD)

<table>
<thead>
<tr>
<th>Epidermis (μm)</th>
<th>Skin thickness (mm)</th>
<th>Elastic fibres (%)</th>
<th>Elasticity (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>H</td>
<td>I</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epidermis</td>
<td>Skin thickness</td>
</tr>
<tr>
<td>pd</td>
<td>rd</td>
<td>I</td>
<td>H</td>
</tr>
<tr>
<td>45.9*</td>
<td>43.1</td>
<td>1.88*</td>
<td>1.63</td>
</tr>
<tr>
<td>(13.1)</td>
<td>(6.7)</td>
<td>(0.35)</td>
<td>(0.21)</td>
</tr>
</tbody>
</table>

I: irradiated skin area; H: corresponding healthy skin area; Pd: papillary dermis; Rd: reticular dermis.

*Only 18 patients were examined with respect to elastic fibres and epidermal thickness.

*p = 0.004 for difference versus healthy skin.

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**DISCUSSION**

In this study, tenasin expression was studied immunohistochemically in irradiated and normal human skin. For the first time, an increase of tenasin expression in X-irradiated human skin was demonstrated.

The role of tenasin in vivo is as yet not clear. Although the expression of tenasin in normal adult human skin is generally low (7), tenasin expression has previously been found to be increased under certain pathological conditions, such as scleroderma (19), hyperproliferative skin diseases (20), tumours (11) and wound healing (8).

The increased tenasin expression in irradiated skin could be due to the activation of cytokines as a result of irradiation. Transforming growth factor β (TGF-β) is a cytokine that has been shown to increase deposition of tenasin by fibroblasts (21). In several animal models, ionizing radiation has been shown to induce TGF-β, which has been implicated as a central cytokine in both tissue repair and fibrotic conditions (13, 22, 23).

Especially at early stages of wound healing, tenasin accumulates into the upper dermis. The fact that tenasin deposition was observed in irradiated skin even several years after treatment confirms the hypothesis that irradiation-induced damage is analogous to long-lasting wound healing.

As a side effect of irradiation, telangiectasiae are found frequently (1), and in chronic radiodermatitis blood vessel damage has been described (24). In the present study, the number of blood vessels was increased in both the upper and lower dermis of irradiated skin in comparison with non-treated skin. Also, there appeared to be more large-calibre blood vessels in the dermis of irradiated skin. The present findings could be due to the activation of angiogenetic factors, such as TGF-β, as a result of irradiation or due to the direct damage of vascular walls by irradiation.

There was no difference in the proportional area or morphological appearance of elastic fibres between X-irradiated and non-treated skin. The reason for the higher values measured by elastometer in treated skin could be an accumulation of connective tissue in irradiated skin, as demonstrated previously (15). The increased amount of collagen in irradiated skin could be due to the activation of growth factors, such as TGF-β (3, 4). Also, we have previously found skin type I collagen cross-linking.

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*Fig. 1. Immunohistochemical staining for tenasin. Tenasin expression is increased in irradiated skin (A) compared to non-treated skin (B).*
Fig. 2. Immunohistochemical staining for blood vessels (F VIII). The number and diameter of blood vessels is increased in irradiated skin (A) compared to non-treated skin (B).

to be increased as a result of radiotherapy in these same patients (25). A modulation of collagen cross-linking could also contribute to the tensile properties of skin in these subjects.

The integrity of epidermis measured by TEWL was not changed as a result of X-irradiation. Measurement of epidermal thickness by computerized image analysis showed no marked difference between irradiated and non-irradiated skin. Both epidermal thickness and epidermal function also seemed to be unaffected by radiation therapy. However, in our previous work, both skin thickness and skin collagen synthesis were found to be increased as a result of radiotherapy. The data have been published in a separate article (15). These findings suggest that the effect of X-irradiation is more visible in the dermal than in the epidermal parts of skin.

In conclusion, these findings indicate increased tenasin expression in irradiated skin similar to that seen in wound healing. Slightly enhanced angiogenesis in irradiated skin was also detected. Epidermis and dermal elastic fibres were found to be unaffected by irradiation, but an increase in the skin stiffness values measured by an elastometer was detected, implicating modification of dermal connective tissue components other than elastin as a result of irradiation.

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REFERENCES


