Selectively Enhanced Procollagen Gene Expression in Sclerosing (Morphea-Like) Basal Cell Carcinoma as Reflected by Elevated Proα1(I) and Proα1(III) Procollagen Messenger RNA Steady-State Levels

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Sclerosing or morphea-like variant of basal cell carcinoma (BCC) is characterized by an extensive connective tissue stroma, and histopathology has suggested that the extracellular matrix is largely composed of collagen. In addition, fibronectin deposition has been proposed to modulate tumor growth in BCC. In this study, we examined the expression of genes coding for type I, III, and IV procollagens, as well as for fibronectin, in tissue from 10 patients with sclerosing BCC. For comparison, tissues from 5 patients with nodular BCC and 4 controls were examined. Total RNA was isolated by CsCl density gradient centrifugation, and messenger RNA (mRNA) steady-state levels were determined by slot-blot hybridizations with human sequence specific complementary DNAs (cDNAs). The abundance of type I procollagen mRNA in sclerosing BCC tissue was increased to 233.6 ± 36.7% of the controls (mean ± SEM). The corresponding value for type III procollagen mRNA in sclerosing BCC was 281.8 ± 54.8% of the controls. Consequently, the steady-state ratio of type I/III procollagen mRNAs in sclerosing BCCs (5.0 ± 1.2; mean ± SD) was within the control range. Thus, there is a coordinate increase in type I and type III procollagen mRNA levels in sclerosing BCC. In contrast, the values for type I and type III procollagen mRNAs in nodular BCC were not different from the controls. In addition, type IV procollagen and fibronectin mRNA levels were not different from the controls either in sclerosing or nodular BCCs, attesting to the selectivity of the increase in type I and III procollagen mRNA levels in sclerosing BCC. These observations may relate to the excessive deposition of the extracellular matrix stroma surrounding the tumor cells in sclerosing BCC. J Invest Dermatol 90:634–638, 1988

Sclerosing, or the morphea-like, variant of basal cell carcinoma (BCC) is characterized by a sclerosing appearance with indistinct borders [2]. Removal of the entire lesion is often difficult resulting in high recurrence rates. Histologically, sclerosing BCC is characterized by small islands of tumor cells embedded in a dense fibrous stroma [2,3]. Previous studies on other carcinomas have suggested that the stromal component plays an important role in the biology of tumor growth [4–6]. In the case of sclerosing BCC, the biologic role or the biochemical composition of connective tissue stroma has not been elucidated.

Type I and type III collagens are the two predominant genetically distinct collagen types in human dermis [7]. Type III collagen has a tissue distribution similar to type I collagen, but the ratio of type III to type I collagen varies from tissue to tissue, and alterations in this ratio have been reported in pathologic fibrotic conditions (for review, see Refs 8 and 9). In addition, fibronectin has been postulated to play a role in regulating the tumor growth [10,11]. Specifically, in the case of BCC, fibronectin matrix has been suggested to facilitate the growth of the tumor [12].

The isolation of cloned cDNAs for human procollagen and fibronectin sequences has permitted the development of techniques for accurate measurement of the corresponding mRNA levels in tissues. These procedures of molecular biology allow the detection of variations in the rates of gene expression, as reflected by the steady-state abundance of the mRNAs in tissues.

In the present study, we have explored collagen and fibronectin gene expression in sclerosing BCC, as reflected by type I, III, and IV procollagen, and fibronectin mRNA steady-state levels. For comparison, we have extended these studies to nodular BCC and control tissues.

MATERIALS AND METHODS

Tissue Material Sclerosing BCC tissue was obtained from 10 patients undergoing microscopically controlled surgery (Mohs micrographic surgery). The clinical diagnosis in each case was confirmed by histopathologic examination (Fig 1). For comparison, 5 patients with nodular BCC were examined; each of these cases had a
Figure 1. Characteristic histopathology of the sclerosing BCCs examined in this study. Note the dense stroma surrounding islands of tumor cells. (Hematoxylin-eosin stain; original magnification, 90X).

histologic picture classic for this type of BCC [3]. Also, normal control skin was obtained from cosmetic surgery procedures of the head and neck area.

The tumor tissue from sclerosing BCC was separated from the adjacent normal tissue using a dermal curette. Histologic examination of the curetted material demonstrated an abundance of stromal tissue mixed with aggregates of tumor cells. Part of the material was fixed in 2.5% glutaraldehyde for electron microscopy.

Assay of Procollagen and Fibronectin mRNA Levels For isolation of total RNA, the tissue specimens were homogenized in buffer containing 4 M guanidinium thiocyanate, 5 mM sodium citrate, 0.5% sarkosyl, 0.1 M 2-mercaptoethanol, and 0.1% Antifoam A (Sigma Chemical) [13]. The homogenates were layered on top of a 2.5-ml cushion of 5.7 M CsCl, and total RNA was recovered by centrifugation at 35,000 rpm for 16 h at 15°C using a Beckman SW 40.1 rotor [13]. The pellet containing RNA was dissolved in sterilized distilled water, and re-precipitated with 70% ethyl alcohol containing 0.4 M NaCl. The final pellet was dissolved in distilled water, and the concentration of total RNA was determined by absorbance at 260/280 nm.

For procollagen and fibronectin mRNA level determinations, serial dilutions of total RNA, in the range of 1 to 8 μg, were dotted onto nitrocellulose filters using a commercial vacuum manifold (Schleicher & Schuell). RNA was immobilized onto the filters by heating under vacuum at 78°C for 90 min. The filters were then pre-hybridized and hybridized with procollagen and fibronectin-specific cDNAs that were radioactively labeled by nick translation [14]. The cDNA probes used for specific hybridizations with type I, III, and IV procollagen mRNAs correspond to human α1(I) [15], α1(III) [16], and α1(IV) [17] procollagen sequences, and their estimated sizes are 1.2, 1.8, and 2.4 kb, respectively. The fibronectin cDNA corresponds to 3'-end of the molecule and is 1.4 kb in size [18]. The prehybridization and hybridization conditions [19] have been previously shown to exclude cross-hybridization between type I and type III procollagen cDNA probes and their corresponding mRNA species [20]. The specific mRNA-[32P]cDNA hybrids were visualized by autoradiography using X-ray cassettes equipped with intensifying screens, and the levels of the specific mRNAs were quantitated by scanning densitometry at 700 nm [21]. The procollagen mRNA levels were then expressed as absorbance units (U), based on scanning densitometry on the linear range of the values obtained from the autoradiograms, per mg total RNA.

For accurate type I/III procollagen mRNA ratio determinations, the absorbance values were corrected for the length of the corresponding cDNA probes; as indicated above, the sizes of the α1(I) and α1(III) procollagen cDNA probes used were 1.2 and 1.8 kb, respectively [15,16]. Furthermore, the values were corrected for the small differences in the final specific activity of the probes [22]. Finally, the observed ratios of α1(I)/α1(III) procollagen mRNAs, based on densitometric units, were multiplied by a factor of 1.5 due to the fact that type I procollagen is a heterotrimer with the chain
RESULTS

A total of 10 patients with sclerosing BCC were examined in this study. In each case, the clinical diagnosis was confirmed by histopathology (Fig 1). Electron-microscopic examination of the material removed from the lesions demonstrated an abundance of stromal tissue composed predominantly of collagen fibers (Fig 2A). The individual fiber architecture of collagen appeared normal. Elastic fibers with evidence of actinic damage were also present (Fig 2A). The connective tissue stroma was often noted in close proximity to tumor cells with prominent nuclei, pronounced rough endoplasmic reticulum, and an abundance of mitochondria (Fig 2B).

Total RNA was isolated under conditions that preclude degradation of mRNA [13], and type I and III procollagen mRNA levels were first determined by slot-blot hybridizations, as illustrated previously [20,21]. A representative experiment using tissue from 4 sclerosing BCC samples is shown in Table I. The results indicated that proα1(I) collagen mRNA level was increased about twofold over that in normal control skin.

Evaluation of the data from 3 separate experiments with 3 separate controls, on a total of 10 different tissue specimens of sclerosing BCC, revealed that the abundance of type I procollagen mRNA was 233.6 ± 36.7% (mean ± SEM) of the corresponding controls in each experiment (Fig 3A); this difference is statistically significant (p < 0.001). It should be noted that the values were expressed as percent, the values in matched controls in each experiment being 100%. The mean variation between separate control samples within a single experiment was ± 9.9% (range). In contrast, analyses of type I procollagen mRNA abundance in nodular BCC demonstrated a value of 109.6 ± 17.9% of the control; this difference was not statistically significant (p > 0.05) (Fig 3A).

Assay of type III procollagen mRNA in sclerosing BCC also demonstrated a significant increase over the control skin, the increase being 281.8 ± 54.8% of the control (p < 0.001) (Fig 3B). The value for type III procollagen mRNA in nodular BCC was 70.1 ± 19.2% of the control (p > 0.05; statistically not significant).

The steady-state ratio of type I/III procollagen mRNAs in sclerosing BCC, as shown in Table I, was 5.0 ± 1.2 (mean ± SD). This value was in the range of that noted in control skin, and also within the range of type I/III procollagen mRNA ratio measured previously in cultured human skin fibroblasts [20] (Table I). Thus, the results indicate that in sclerosing BCC there is a coordinate increase in type I and III procollagen mRNA steady-state levels.

To assess the specificity of the increases noted in type I and III procollagen mRNA levels, cDNA coding for α(I)IV of type IV procollagen and fibronectin sequences were also utilized in dot-blot hybridizations. These two probes have been shown to hybridize with a single 6.5 and 7.8 kb mRNA transcript, respectively [17,18]. The results obtained with total RNA isolated either from sclerosing or nodular BCC revealed no significant differences from the controls (Table II).

Table I. Assay of Type I and Type III Procollagen mRNA Levels in Sclerosing Basal Cell Carcinoma Tissue

| Tissue           | Procollagen mRNA Levels (U/mg total RNA) | Type I/III Ratio *
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Sclerosing BCC 1</td>
<td>300</td>
<td>42</td>
</tr>
<tr>
<td>Sclerosing BCC 2</td>
<td>194</td>
<td>38</td>
</tr>
<tr>
<td>Sclerosing BCC 3</td>
<td>244</td>
<td>58</td>
</tr>
<tr>
<td>Sclerosing BCC 4</td>
<td>146</td>
<td>39</td>
</tr>
<tr>
<td>Sclerosing BCC 5</td>
<td>221 ± 57</td>
<td>44.8 ± 9.2</td>
</tr>
<tr>
<td>Control skin</td>
<td>108</td>
<td>19</td>
</tr>
<tr>
<td>Human skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroblasts</td>
<td></td>
<td>5.2 ± 2.1</td>
</tr>
</tbody>
</table>

* Calculated on the basis of densitometric units of 700 nm, and corrected for the specific activity and the length of the cDNA probes and for the α-chain composition of Type I and III procollagens.

Table II. Assay of Type IV Procollagen and Fibronectin mRNA Levels in Sclerosing and Nodular Basal Cell Carcinoma Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fibronectin mRNA</th>
<th>Type IV Procollagen mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclerosing BCC</td>
<td>63.0 ± 11.9</td>
<td>98.0 ± 9.6</td>
</tr>
<tr>
<td>Nodular BCC</td>
<td>74.0 ± 9.5</td>
<td>90.8 ± 23.4</td>
</tr>
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* Values are expressed as percent of controls, based on densitometric analysis, as described in Table I and Fig 3 (mean ± SEM of 4 parallel determinations on 4 subjects in each group).
DISCUSSION

The results of this study indicate a coordinate increase in type I and type III procollagen mRNA steady-state levels in sclerosing BCC tissue, while no significant alterations were observed in nodular BCC tissue. Thus, sclerosing BCC tissues, which contain an abundant connective tissue stroma, actively express both type I and type III procollagen genes. Previous studies have demonstrated that, in many situations, the assay of procollagen mRNA levels reflects collagen synthesis, and a concordance between the steady-state levels of procollagen mRNAs and the production of the corresponding procollagen polypeptides has been demonstrated [8,9]. Thus, the increase in both type I and type III procollagen mRNA levels potentially reflects an increased collagen synthesis and may determine the biochemical composition of the sclerosing BCC stroma. In contrast to type I and III procollagen mRNA abundance, the levels of type IV procollagen and fibronectin mRNAs were not different between the sclerosing or nodular BCC and the control skin. In other systems, such as in scleroderma fibroblast cultures [22], the fibronectin mRNA levels are often coordinate with those for type I and III procollagen. The observation of uncoordinate changes in sclerosing BCC fibronectin mRNA attests to the specificity of the increase noted in type I and III procollagen mRNA levels.

It is of interest to note that our results do not parallel the previous findings in scar tissue or keloids. Specifically, in studies on scar tissue, the relative amounts of type III collagen have been suggested to be increased [23,24]. In keloids, on the other hand, the concentration of type I collagen is significantly increased, the ratio of the distinct collagen types I/III being significantly elevated, as compared with normal human skin [21].

An abundant stroma is a characteristic feature of sclerosing BCC and constitutes the major part of the tumor volume. Histologically, collagen deposition is evident and more abundant than in other types of BCC [3]. Previous biochemical studies involving BCC have emphasized collagen degradation [25–27] and have concentrated on the nodular type of BCC. Recently, it has been demonstrated that BCC tumor cells are capable of producing cytokines that stimulate collagenase production in human skin fibroblast cultures [28]. An analogous situation in terms of collagen production could exist in sclerosing BCC. For example, sclerosing BCC tumor cells could elaborate factors that enhance collagen production by adjacent stromal fibroblasts. The reasons for the lack of such reaction in nodular BCC are not clear. Nevertheless, the enhancement of collagen production in sclerosing BCC could occur at the pretranslational level of gene expression, since our studies were able to demonstrate elevated steady-state levels of type I and type III procollagen mRNAs in the lesion. Thus, sclerosing BCC may offer a useful model to study tumor-stromal interactions in further detail.

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REFERENCES


**PANAMERICAN PIGMENT CELL BIOLOGY MEETING**

The meeting will be held in Minneapolis, Minnesota, June 24–26, 1988. Abstracts are due by March 15, 1988. Correspondence to: Dr. Richard A. King, c/o Pan American Cell Meeting, Box 485 UMHC, University of Minnesota, 420 Delaware St., Minneapolis, MN 55455.