MOLECULAR ASPECTS OF SCLERODERMA

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TABLE OF CONTENTS

1. Abstract
2. Introduction
   2.1. Scleroderma fibroblasts
3. Growth factors and receptors
   3.1. TGF-beta vs CTGF
      3.1.1. TGF-beta may be responsible for the initiation of fibrosis
      3.1.2. CTGF expression correlates with the sclerotic phase of disease
      3.1.3. TGF-beta is a potent inducer of ECM in cultured fibroblasts
      3.1.4. The role of CTGF in ECM deposition and turnover is controversial
   3.2. TGF-beta receptors and Smads
      3.2.1. TGF-beta receptors are upregulated in scleroderma fibroblasts
      3.2.2. The role of Smads in scleroderma
   3.3. Other growth factors
4. Signaling pathways involved in collagen gene regulation
   4.1. MAP kinase family
   4.2. PKC-delta
   4.3. Sphingolipid signaling pathway
5. Transcriptional regulation of collagen type I gene
   5.1. The central role of Sp1 in basal collagen gene expression
   5.2. Sp1 mediates COL1A2 gene transcription in response to external stimuli
   5.3. Fli-1 may play a role in abnormal ECM deposition in scleroderma
   5.4. Other transcription factors that may contribute to scleroderma phenotype
   5.5. Current model for the COL1A2 gene regulation
6. Concluding remarks and perspectives
7. Acknowledgment
8. References

1. ABSTRACT

Scleroderma is a complex disease characterized by activation of the immune system, small-vessel vasculopathy, and fibrosis of the skin and other organs. This review is limited to the discussion of scleroderma fibroblast biology and the cellular and molecular mechanisms that contribute to the abnormal deposition of collagen. Selected aspects of abnormal extracellular matrix (ECM) regulation by scleroderma fibroblasts are critically reviewed. These include the role of transforming growth factor-beta (TGF-beta) and connective tissue growth factor (CTGF) and their receptors in the fibrotic process in scleroderma and the overview of the transcription factors involved in regulation of the human alpha2(I) collagen (COL1A2) gene.

2. INTRODUCTION

Scleroderma is a complex disease characterized by activation of the immune system, small-vessel vasculopathy, and fibrosis of the skin and other organs. Several excellent reviews have recently discussed various pathological aspects of scleroderma (1-4). This review will mainly focus on the mechanisms of ECM dysregulation in scleroderma with emphasis on the presently controversial issues and other aspects of ECM regulation not fully covered by other review articles.

2.1. Scleroderma fibroblasts

Scleroderma fibroblasts derived from skin biopsies of patients with active disease provide a useful in vitro model utilized by many scleroderma investigators. Over the years these studies have provided a wealth of information with potential relevance to fibrosis occurring in vivo. These fibroblasts produce elevated levels of collagen and other ECM proteins and exhibit altered expression of many other genes. There is an ongoing debate regarding the “origin” of scleroderma fibroblasts. Activation and/or selection of resident fibroblasts have been proposed (5,6). Transdifferentiation of other cell types, such as pericytes (7,8) or monocytes (9) into fibroblastic cells with an
activated phenotype has also been considered. Interestingly, studies from other experimental systems, including other fibrotic diseases, provide increasing evidence that epithelial (or endothelial) cells have the ability to transdifferentiate into fibroblasts (10). In addition, peripheral blood fibrocytes are a recently described population of cells that localize to areas of connective tissue deposition, including wound healing and fibrotic tissues (11). Together, these new developments suggest that activated fibroblasts isolated from scleroderma lesions may have originated from other cell types.

3. GROWTH FACTORS AND RECEPTORS

3.1. TGF-beta

TGF-beta and CTGF have emerged as leading candidates responsible for overproduction of ECM proteins in different fibrotic diseases. As discussed in this section, despite extensive studies, their specific contributions to the process of fibrosis, including scleroderma, are not fully understood.

3.1.1. TGF-beta may be responsible for the initiation of fibrosis

A number of studies analyzed the presence of TGF-beta in lesional and non-lesional skin of patients with scleroderma. Elevated levels of TGF-beta have been reported in involved skin lesions of SSc patients (12-15). Furthermore, TGF-beta expression was shown to co-localize with collagen type I mRNA in involved skin of SSc patients (14). Subsequent studies, which analyzed TGF-beta1 expression during disease progression, indicated that TGF-beta1 is present in early inflammatory stage of the disease, prior to the onset of fibrosis (16). In a recent study, expression of all three TGF-beta isoforms at mRNA and protein levels were reexamined. It was found that all three isoforms were expressed in the inflammatory skin areas, but could not be detected in the sclerotic skin (17). Consistent with these observations, TGF-beta1 mRNA expression was shown to precede collagen expression in murine sclerodermatous graft-vs-host disease (Scl GVHD) considered an animal model of scleroderma (18). In the latter study, fibrosis was prevented by a neutralizing anti-TGF-beta antibody administered at day 1 and 6 after bone marrow transplant (18). In other experimental models of fibrosis, blockade of the TGF-beta pathway at the time of administration of the fibrotic stimulus was also successful in preventing development of fibrosis (19). Collectively, these in vivo studies support a critical role for TGF-beta in the initiation of the fibrotic process. However, the nature of the processes affected by TGF-beta that subsequently contribute to the development of fibrosis is presently unknown. One recently proposed possibility is that TGF-beta contributes to selection of an apoptosis resistant subset of fibroblasts (20, 21). These fibroblasts, which are also characterized by collagen overexpression, may persist in the lesional tissue and contribute to matrix deposition.

3.1.2. CTGF expression correlates with the sclerotic phase of the disease

The elevated expression of CTGF has been universally observed in various fibrotic disorders including scleroderma, keloids, glomerulosclerosis, hepatic fibrosis, atherosclerosis and others (22, 23). Significantly, a strong correlation between CTGF expression by lesional fibroblasts and skin sclerosis was observed in skin biopsies obtained from scleroderma patients (24, 25). Based on this observation, it was proposed that CTGF contributes to the maintenance of fibrosis. However, these studies did not examine whether fibroblasts that expressed CTGF also produced increased collagen levels. Taking into consideration the recently described properties of CTGF as an inducer of matrix metalloproteinases (MMPs, see below) and the fact that sclerotic lesions diminish spontaneously in scleroderma, the role of CTGF in the late stages of scleroderma is unclear. It is possible that persistent presence of CTGF in the late stages of the disease is associated with matrix degradation rather than with matrix deposition. To strengthen the case of CTGF as the profibrotic agent in scleroderma, co-localization of CTGF with cells actively producing collagen needs to be demonstrated.

The spatial and temporal relationship between TGF-beta and CTGF expression in vivo has not been adequately addressed. In the limited number of studies that investigated this issue, both growth factors appear to act in concert. For example, expression of CTGF overlaps with that of TGF-beta during wound healing (24). It is possible that under physiological conditions such as wound healing these two growth factors cooperate to effectively repair damaged tissue. However, this process appears to be dysregulated in fibrosis. As mentioned above, in scleroderma lesions, TGF-beta is expressed in the early inflammatory stage, while CTGF is highly expressed in the late sclerotic stage, where it may persist for many years (25). The inducer of CTGF in the late sclerotic stage is unknown. While initial in vitro studies have suggested that TGF-beta is an exclusive inducer of CTGF (24), recent studies have uncovered a number of additional stimuli acting via distinct signaling pathways capable of CTGF induction. These include high glucose (26), dexamethasone (27), Factor VIIa and thrombin (28,29), serotonin and LPA (30), and VEGF (31). Our recent work also suggests that a human cytomegalovirus immediate early gene product (HCMV IE1) is a potent inducer of CTGF (32). Consistent with these in vitro observations, a case of pseudoscleroderma was reported in which expression of CTGF colocalized with the COL1A1 in the absence of detectable TGF-beta (33). Together, in vivo observations suggest that TGF-beta is associated with the early stages of scleroderma, whereas CTGF is associated with late disease. However, the specific roles of these pleiotropic growth factors in the pathological process remain elusive.

3.1.3. TGF-beta is a potent inducer of ECM in cultured fibroblasts

The role for TGF-beta as a potent inducer of ECM synthesis in a variety of cells, including human fibroblasts, is well established. TGF-beta stimulates ECM accumulation through several mechanisms including direct induction of collagen transcription and mRNA stability (34,35) as well as inhibition of ECM degradative pathways (36). Recent studies have characterized some of the specific transcription factors contributing to collagen gene
Scleroderma Fibrosis

regulation by TGF-beta (37-39). This aspect of collagen gene regulation is discussed in section 5.

3.1.4. The role of CTGF in ECM deposition and turnover is controversial

The role of CTGF as an inducer of ECM is less clear. Original studies by Grotendorst and collaborators using embryonic rat kidney cells (NRK fibroblasts) have demonstrated that CTGF is a mediator of the fibrogenic effects of TGF-beta including proliferation and ECM production (40). Using NRK fibroblasts these investigators have shown that blocking CTGF either by neutralizing antibody or by antisense oligonucleotides abolishes the stimulation of collagen by TGF-beta. Similar effects were observed in a wound healing model (40). Furthermore, it was shown that both TGF-beta induction of CTGF and subsequent CTGF induction of collagen are negatively regulated by cAMP. More recently Stratton et al (41) confirmed these observations using TGF-beta stimulated scleroderma and normal fibroblasts. Induction of cAMP with Iloprost resulted in inhibition of TGF-beta-induced CTGF and collagen. Surprisingly, in the latter study, increased CTGF production by scleroderma fibroblasts did not correlate with increased basal collagen production (41). Thus, CTGF may only function as a stimulator of collagen synthesis in conjunction with activation of TGF-beta signaling. In fact, recent studies using different approaches have suggested that CTGF and a closely related factor Cyr61 are inducers of MMPs (42). Adhesion of human dermal fibroblasts to either CTGF or Cyr61 resulted in formation of focal complexes and activation of FAK, paxillin and Rac kinases. In addition, a prolonged activation of MAPK and induction of MMP-1 (collagenase-1) and MMP-3 (stromelysin-1) mRNAs and proteins were observed. The effects of CTGF were mediated by alpha 6 beta 1 integrin and cell surface HSPGs (heparan sulphate proteoglycan).

Taken together, cell culture studies suggest that CTGF could have both pro-fibrotic and anti-fibrotic properties. There are a few possible explanations for these seemingly contradictory observations. i) As discussed earlier, induction of MMPs is mediated via the integrin receptors. A separate cell surface receptor(s) may mediate CTGF stimulation of ECM. However, despite intensive efforts, a classic, signaling CTGF-specific receptor has not been characterized. ii) The action of CTGF as either pro-fibrotic or anti-fibrotic agent may depend on the simultaneous activation of other signaling pathways such as TGF-beta or insulin/IGFs. For example, the original study by Grotendorst’s laboratory describing CTGF effects on ECM production utilized growth medium containing ITS (Insulin-Transferin-Selenium) and IGF-II (insulin growth factor-II) (40). We have also observed that human dermal fibroblasts incubated in ITS-supplemented medium demonstrate increased basal ECM production and increased responses to CTGF (43). On the other hand, studies by Chen et al (42) examining MMP induction by CTGF were performed in serum free IMDM medium. iii) Finally, specific responses to CTGF may depend on unidentified intrinsic properties of the target cell. For example, scleroderma fibroblasts respond to CTGF with increased collagen type I mRNA levels and an increased collagen promoter/reporter activity (43,44). It may also be relevant that scleroderma fibroblasts produce higher levels of endogenous CTGF (41,44,45). Whether autocrine expression of CTGF by scleroderma fibroblasts contributes to constitutive ECM overexpression by these cells remains to be determined. However, as mentioned in the previous section, there was no correlation between elevated collagen production and CTGF overexpression in a recent study by Stratton et al (41).

The mechanisms of action of CTGF, especially its possible profibrogenic effects, are still poorly understood. Lack of commercially available CTGF hampers the progress in this field and may be partially responsible for the inconsistent results reported by different laboratories.

3.2. TGF-beta receptors and Smads

3.2.1. TGF-beta receptors are upregulated in scleroderma fibroblasts

There is evidence that the components of the TGF-beta signaling pathway may be dysregulated in scleroderma. Elevated mRNA levels of TGF-beta receptors type I and II (TGF-betaRI and -II) were observed in scleroderma fibroblasts, which correlated with elevated levels of collagen mRNA (46). Based on these results, it was suggested that elevated production of collagen by scleroderma fibroblasts is dependent on autocrine TGF-beta signaling. This possibility was further supported by demonstration of increased protein levels of TGF-betaRI and TGF-betaRII in scleroderma fibroblasts (47). Furthermore, the latter study has shown that a neutralizing anti-TGF-beta antibody significantly decreased collagen production by scleroderma fibroblasts, without an appreciable effect on collagen production by healthy dermal fibroblasts. Elevated TGF-beta receptor expression has also been observed in skin biopsies from patients with localized scleroderma (48). Our ongoing extended analyses of TGF-beta receptor expression in scleroderma and control fibroblasts confirmed the elevated levels of the TGF-betaRI protein in a large proportion of scleroderma fibroblasts. In contrast, the levels of TGF-betaRII were not consistently elevated (Gore and Trojanowska, unpublished data). To gain additional insights into the role of autocrine TGF-beta pathway in collagen upregulation by scleroderma fibroblasts, our laboratory utilized a kinase-deficient TGF-betaRII (deltakRII) overexpressed via an adenoviral vector. This treatment effectively diminished (by about 50%) basal collagen and fibronectin production by healthy fibroblasts, but was effective only in selected scleroderma fibroblast cell lines (49). Together, these findings suggest that the autocrine TGF-beta signaling may be responsible for the elevated production of ECM only in a proportion of scleroderma fibroblasts. Moreover, these data suggest that in some scleroderma cell lines constitutive collagen production may be independent of TGF-beta signaling. Alternatively, collagen levels in scleroderma cells may be decreased via a kinase-deficient TGF-betaRII (deltakRI) as it was recently demonstrated that only deltalRII abrogates TGF-beta-induced collagen expression in rat mesangial cells (50). Interestingly, disordinated expression of TGF-beta receptors has also been observed in other fibroproliferative disorders.
Scleroderma Fibrosis

including hepatic fibrosis and atherosclerosis (51,52). Further studies are needed to delineate the functional significance of the altered expression levels of the TGF-beta receptor subunits in fibrotic disorders.

3.2.2. The role of Smads in scleroderma is unknown

Smads are downstream mediators of the TGF-beta signaling pathway (53). Smad3 has been shown to mediate induction of COL1A2 (37-39) and CTGF (54) in normal dermal fibroblasts. However, the Smad-response element does not contribute to the elevated expression of the CTGF promoter in scleroderma fibroblasts (54). Moreover, the expression levels of Smad3, Smad4, and Smad7 did not differ between scleroderma and healthy control fibroblasts (54). On the other hand, the increased presence of nuclear Smad3 has been observed in SSC fibroblasts in the absence of exogenous TGF-beta stimulation (4). Whether Smads are specifically involved in the abnormal phenotype of scleroderma fibroblasts remains unknown.

3.3. Other growth factors

Besides TGF-beta and CTGF, other growth factors, including pro-IL-1alpha, PDGF, IL-4, Oncostatin M (OSM), IL-13, and others most likely contribute to the pathogenesis of scleroderma by either directly contributing to ECM production or by affecting other cellular functions (reviewed in 4). Recent studies also suggest that thrombin may directly contribute to induction of the myofibroblast phenotype in scleroderma lung fibroblasts (55, 56).

4. SIGNALING PATHWAYS INVOLVED IN COLLAGEN GENE REGULATION

4.1. MAP kinase family

The three major groups of the mammalian MAP kinase family include extracellular signal-regulated kinases (ERKs), c-jun N-terminal or stress activated protein kinases (JNK/SAPKs), and p38 kinases (57). There are numerous studies that link specific kinases to collagen gene regulation in various experimental systems (58, 59). Since their effects depend on experimental conditions and appear to be cell context specific, no clear conclusions can be made at this point about the role of each of these pathways in ECM deposition and turnover. Studies utilizing human dermal fibroblasts are briefly reviewed here because of their potential relevance to scleroderma. In general, activation of ERK1/2 and JNK/SAPKs inhibit collagen production in dermal fibroblasts albeit via distinct mechanisms, whereas activation of p38 is stimulatory. Thus, ERK 1/2 signaling cascade negatively regulates basal and TGF-beta-induced expression of type I collagen through inhibition of alpha1(I) and alpha2(I) collagen mRNA stability (60). Induction of stress activated protein kinases, JNK/SAPKs, inhibit collagens type I and III at the transcriptional level (61). Furthermore, c-Jun is one of the mediators of UV-induced collagen repression in dermal fibroblasts in vitro, and in vivo in human skin (61). The role of these inhibitory pathways in scleroderma has not been determined.

In contrast, p38MAPK is activated by TGF-beta and cooperates with TGF-beta in stimulation of collagen protein and mRNA levels, and COL1A2 promoter activities in dermal fibroblasts (62). Interestingly, in human fibroblasts, TGF-beta induction of p38 is prolonged with the early peak at 2 hours and the activated state persisting for up to 24 hours. The kinetics of p38 activation correlate well with the persistent stimulatory effects of TGF-beta on collagen mRNA levels (63), suggesting an important role for p38 in collagen gene regulation. The transcription factors involved in collagen gene regulation that are targeted by p38 are currently unknown. However, p38 levels, as well as kinetics of p38 activation by TGF-beta, are similar in SSC and healthy skin fibroblasts, suggesting that elevated collagen production by SSC fibroblasts is not due to the alteration of this pathway (62).

4.2. PKC-delta

Recent studies have implicated PKC-delta in collagen gene regulation in dermal fibroblasts (64). Using a specific pharmacological inhibitor of PKC-delta, rottlerin, Jimenez et al have demonstrated inhibition of basal collagen type I protein, mRNA, and promoter levels in scleroderma and healthy skin fibroblasts. The cis-response element and cognate transcription factor mediating inhibitory effects of rottlerin on the COL1A1 promoter remain to be characterized. Further studies are also needed to directly support the role of PKC-delta in the collagen gene regulation. Significantly, scleroderma fibroblasts express higher levels of PKC-delta suggesting that this pathway may directly contribute to scleroderma phenotype.

4.3. Sphingolipid signaling pathway

Sphingolipids, in addition to their role as structural molecules of the plasma membrane, are now recognized as important bioactive mediators of a variety of cellular processes (65, 66). In particular, biologic functions of ceramide, sphingosine, and their phosphates have been extensively studied. Recent studies have suggested that ceramide may be involved in the regulation of collagen metabolism. It was shown that exogenously added C2-ceramide inhibits collagen gene expression in hepatic stellate cells and dermal fibroblasts (67, 60). Induction of the ERK pathway by C2-ceramide was shown to mediate collagen gene inhibition (60). In contrast, our recent observations suggest that endogenous ceramide (or a non-sphingoid metabolite), generated by the action of sphingosine 1-phosphate phosphatase is a coactivator of the TGF-beta signaling pathway (Sato and Trojanowska, unpublished data). These observations suggest that sphingolipids may have a dual role in collagen gene regulation and that metabolites with both stimulatory and inhibitory functions can be formed endogenously. The nature of the interaction between the sphingolipid and the TGF-beta signaling pathways and a possible role of sphingolipid signaling in scleroderma remains to be elucidated.

5. TRANSCRIPTIONAL REGULATION OF COLLAGEN TYPE I GENE

Collagen type I, a triple helix of two COL1A1 and one COL1A2 chains, is a primary component of the connective tissue and one of the most abundant physiologic
Scleroderma Fibrosis

proteins. Its overexpression is a hallmark of scleroderma and other diseases characterized by fibrosis. In recent years, our understanding of collagen type I gene regulation has increased significantly. In addition to the mapping of cis-response elements in the COL1A2 and COL1A1 promoters, several transcription factors involved in regulation of these promoters have been identified. More importantly, it has been found that in scleroderma fibroblasts several of these transcription factors differ either in their expression levels or in levels of post-translational modifications, strongly suggesting that the alteration of transcriptional control mechanisms contribute directly to the activated state of scleroderma fibroblasts. To fully understand collagen gene regulation, it is necessary to complete characterization of the relevant transcription factors and to delineate the functional significance of their post-translational modifications, as well as the nature of the signaling pathways that induce them. Ultimately, these studies should lead to a better understanding of the specific mechanisms that are responsible for collagen dysregulation in scleroderma.

5.1. The central role of Sp1 in basal collagen gene expression

Sp1 is a ubiquitously expressed zinc finger transcription factor implicated in the regulation of numerous viral and cellular genes (68). Studies using the Sp1 inhibitor mithramycin, as well as other strategies aimed at inhibiting Sp1 expression and function, suggest the crucial role for this factor in basal collagen gene regulation in healthy and scleroderma fibroblasts (69, 70). While Sp1 universally functions as a transcriptional activator, another ubiquitously expressed member of the Sp/KLF (Kruppel-like factor) family, Sp3, can function either as an activator or as a repressor depending on the promoter and cellular context (68). Extensive analyses of the human COL1A2 and COL1A1 promoters characterized several Sp1/Sp3 binding sites in the promoter regions of both genes (71-74). Interestingly, although the organization of both promoters differs significantly, similar transcription factors seem to be involved in their coordinate regulation. The specific role of Sp1 and Sp3 in regulation of each promoter is not clear yet. For example, both Sp1 and Sp3 activate COL1A2 promoter activity in Drosophila Schneider cells (74) and in human dermal fibroblasts (Trojanowska, unpublished observations). On the other hand, Sp3 inhibits Sp1-stimulated COL1A1 promoter activity in Drosophila Schneider cells (75).

Scleroderma and control healthy fibroblasts express similar levels of Sp1 and Sp3 proteins. However, Sp1 is more phosphorylated in scleroderma fibroblasts (69). Furthermore, increased Sp1 phosphorylation correlates with increased levels of COL1A2 mRNA. While it is tempting to speculate that increased Sp1 phosphorylation contributes to the scleroderma phenotype, many questions remain unanswered. Where do the differentially phosphorylated sites map within Sp1? What is the functional consequence of this phosphorylation? What is the nature of the signaling pathway involved in increased Sp1 phosphorylation in scleroderma fibroblasts? It may be relevant that recent studies have shown that serum induces Sp1 phosphorylation in dermal fibroblasts, which correlates with increased collagen mRNA levels (76). A novel kinase activity has been postulated to phosphorylate the C-terminal segment of Sp1 in response to serum stimulation (77). It may also be relevant that activity of Sp1 is regulated by serine/threonine phosphatases (78). Since multiple cellular kinases have already been implicated in Sp1 phosphorylation in various experimental systems (78), further studies are clearly needed to define the specific pathway relevant to Sp1 phosphorylation in scleroderma.

5.2. Sp1 mediates collagen gene transcription in response to external stimuli

TGF-beta modulation of gene transcription is mediated by Smads, which act in conjunction with other transcription factors in a tissue and promoter specific manner (53). It has been shown that Smad3 binds to the CAGA motif within the COL1A2 promoter and that overexpression of Smad3 leads to induction of this promoter (37,38). Furthermore, cooperation between Sp1 and Smad3 is required for the full TGF-beta response (39). Other inducers of COL1A2 gene expression such as serum and OSM also require intact Sp1 binding sites within the COL1A2 promoter for their effects (76,79). One may speculate that other factors cooperate with Sp1 to stimulate COL1A2 transcription in response to these signals. The nature of these factors is presently unknown. Thus Sp1 is not only an activator of basal collagen type I transcription, but also the effector of distinct signaling pathways that lead to collagen gene activation. It should be noted that basal collagen gene transcription in vitro represents an activated state induced by fibroblast adhesion to plastic and propagation in the presence of serum. Furthermore, recent evidence suggests that Sp1 is also a mediator of the inhibitory pathways regulating COL1A2 transcription (39,80,81). The function of Sp1 as a mediator of inhibitory pathways is discussed in the next section.

5.3. Fli-1 may play a role in abnormal ECM deposition in scleroderma

Ets transcription factors are critical mediators of ECM remodeling with a wide spectrum of target genes (82). Recent studies indicate that Fli-1 is an inhibitor of basal COL1A2 transcription. While the specific mechanisms involved in Fli-1 inhibition of collagen are not fully understood at present, the inhibitory function of Fli-1 appears to be Sp1 dependent (80). Furthermore, both Fli1 and Ets1 are antagonists of the profibrogenic effects of TGF-beta (83). Our recent observations suggest that Fli-1 is underexpressed in scleroderma fibroblasts and in addition exhibits abnormal post-translational modifications (unpublished observations). Since Fli-1 is an inhibitor of basal and TGF-beta-induced ECM production, decreased expression of Fli-1 in scleroderma fibroblasts may directly contribute to the increased collagen production by these cells. The functional significance of post-translational modifications of Fli-1 is currently under investigation. Interestingly, Ets-1 has been shown to be elevated in synovial fibroblasts in vitro and in vivo and may contribute to the pathology of rheumatoid arthritis (84). We have not observed consistent differences in Ets-1 protein expression...
levels or modification patterns between scleroderma and control fibroblasts (unpublished observations).

5.4. Other transcription factors altered in scleroderma

The heterotrimeric CCAAT binding factor CBF/NF-Y is a ubiquitous transcription factor involved in regulation of many genes (85). Its role in activation of the murine collagen type I gene is well established. CBF/NF-Y is also a strong contributor to the basal promoter activities of human COL1A1 and COL1A2 genes (73,86). Significantly, increased binding of CBF/NF-Y to the proximal CCAAT box in the COL1A1 promoter was observed in nuclear extracts obtained from scleroderma fibroblasts (86).

Another factor, potentially involved in upregulation of the collagen type I gene by scleroderma fibroblasts is c-myb (87,88). C-myb expression is induced by TGF-beta in dermal fibroblasts, and an elevated expression of c-myb has been demonstrated in unstimulated scleroderma fibroblasts. The specific role of c-myb in collagen gene regulation is presently not well defined.

5.5. The role of CBP/p300 in collagen gene regulation

CREB binding protein (CBP) and p300 function as transcriptional coactivators, which are critical to expression of many genes (89). The precise role of these factors in transcriptional process is still not fully understood. One of their proposed functions is to bridge promoter-specific transcription factors to components of the basal transcription machinery. Furthermore, CBP/p300 possess intrinsic histone acetyltransferase (HAT) activity and also associate with other HATs such as PCAF, thus playing a direct role in histone acetylation. Moreover, CBP/p300 may also acetylate transcription factors. Similar to phosphorylation, acetylation of transcription factors has recently been recognized as an important mode of gene regulation.

Another aspect of the CBP/p300 regulatory function is that expression of these multifunctional proteins in limiting quantities leads to competition between different transcription factors involved in specific cellular programs. With regard to the COL1A2 promoter, it has been demonstrated that the antagonistic effects of Interferon-gamma (IFN-gamma) and TGF-beta involve competition between Stat-1-alpha and Smad3 for limiting amounts of CBP/p300 (90,91). The inhibitory effect of the TNF-alpha-induced c-Jun and JunB proteins may also be mediated via sequestration of CBP/p300 (92).

5.6. Current model for COL1A2 gene regulation

Our current knowledge of collagen gene regulation is primarily based on the analyses of the proximal promoter regions. With the recent characterization of the evolutionary conserved far-upstream enhancer of the human COL1A2 gene (93), the spectrum of transcription factors involved in regulation of collagen genes may soon increase. The data presently available indicate that Sp1 (Sp3) and CBF/NF-Y play a major role in activating the proximal COL1A2 promoter (Figure 1). Sp1 also appears to play a critical role in mediating the actions of other co-activators and co-repressors of this promoter. Among the co-activators, only the Smad3/CBF complex...
Scleroderma Fibrosis

has been relatively well characterized. There are also several known repressors of the COL1A2 gene. In addition to Stat-1-alpha and Jun proteins discussed in the previous section, C/EBP-beta and NF-kappaB have been characterized as mediators of the TNF-alpha repression of the collagen gene (81,94). Furthermore, NF-kappaB has also been shown to inhibit COL1A1 promoter in a Sp1-dependent manner (95). Both IFN-gamma and TNF-alpha are capable of antagonizing the stimulatory effects of TGF-beta and may play important roles in regulating the balance between ECM synthesis and degradation. Finally, Fli-1 appears to repress the basal collagen level (80).

6. CONCLUDING REMARKS AND PERSPECTIVES

It is well recognized that precisely controlled regulation of ECM turnover is critical for the normal function of all tissues. In scleroderma the balance between ECM deposition and degradation is tilted towards enhanced ECM deposition resulting in fibrosis of skin and other internal organs. The activated fibroblast (myofibroblast) is believed to play a critical role in this process. This review has focused on the aspects of ECM regulation which differ in scleroderma. Not discussed here are the influence of the internal organs. The activated fibroblast (myofibroblast) is capable of antagonizing the stimulatory effects of TGF-beta and may play important roles in regulating the balance between ECM synthesis and degradation. Finally, Fli-1 appears to repress the basal collagen level (80).

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8. REFERENCES


Scleroderma Fibrosis


Scleroderma Fibrosis


Scleroderma Fibrosis


Scleroderma Fibrosis


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