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Cells from the skin of patients with systemic sclerosis secrete chitinase 3-like protein 1

Yuen Yee Ho [a], Murray Baron [b], Anneliese D. Recklies [a], Peter J. Roughley [a], John S. Mort [a,*]

[a] Shriners Hospital for Children, Department of Surgery, McGill University, 1529 Cedar Avenue, Montréal, Quebec H3G 1A6, Canada
[b] Department of Rheumatology, Jewish General Hospital, 3755 Cote Ste Catherine Road, Montréal, Quebec H3T 1E2, Canada

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ABSTRACT

Background: The chitinase-like protein, Chi3L1, is associated with increased fibrotic activity as well as inflammatory processes. The capacity of skin cells from systemic sclerosis (SSc) patients to produce Chi3L1, and the stimulation of its synthesis by cytokines or growth factors known to be associated with SSc, was investigated.
Methods: Cells were isolated from forearm and/or abdomen skin biopsies taken from SSc patients and normal individuals and stimulated with cytokines and growth factors to assess Chi3L1 expression. Chi3L1-expressing cells were characterized by immunohistochemical staining.
Results: Chi3L1 was not secreted by skin cells from normal individuals nor was its synthesis induced by any of the cytokines or growth factors investigated. In contrast, Chi3L1 secretion was induced by OSM or IL-1 in cells from all forearm biopsies of SSc patients, and endogenous secretion in the absence of cytokines was detected in several specimens. Patients with Chi3L1-producing cells at both the arm and abdomen had a disease duration of less than 3 years. Endogenous Chi3L1 production was not a property of the major fibroblast population nor of myofibroblasts, but rather was related to the presence of stem-like cells not present in normal skin. Other cells, however, contributed to the upregulation of Chi3L1 by OSM.
Conclusions: The emergence of cells primed to respond to OSM with increased Chi3L1 production appears to be associated with pathological processes active in SSc.
General significance: The presence of progenitor cells expressing the chilectin Chi3L1 in SSc skin appears to play a role in the initiation of the disease process.

1. Introduction

Systemic sclerosis (scleroderma, SSc) is a complex autoimmune disease with a highly variable array of clinical features, the most characteristic being an overproduction and excessive deposition of collagen in the skin and internal organs, with a progressive course and often fatal outcome. It is relatively rare, affecting between 50,000 and 100,000 North Americans, and up to 250,000 Europeans [1,2]. Although less common than other rheumatic diseases, it has one of the highest mortality rates [3].

Disturbances of both the immune and vascular systems are thought to contribute to the development of SSc. Endothelial alterations often occur early in the disease, followed by vascular damage that leads to a cascade of stimulatory changes culminating in tissue fibrosis [4]. This process involves T lymphocytes [5], monocytes, macrophages [6] and mast cells [7] as well as fibroblasts [8]. The activated cells secrete a variety of products, including growth factors, cytokines and their antagonists [9]. These substances cause inflammation and increased deposition of extracellular matrix (ECM) components, leading to progressive and widespread tissue fibrosis. The heterogeneity of various forms of SSc and the difficulty in discriminating between disease activity (aspects of the disease that vary over time and are potentially reversible spontaneously or with drug treatment) and disease damage (irreversible tissue injury that results from the disease) [10] complicate studies of SSc. At present there are no validated biomarkers which can be used to monitor disease progression. There is an extensive literature, however, investigating the relationship between many of the cytokines and effector molecules implicated in the various pathological processes associated with the development and progression of SSc [11–15].

The chitinase-like protein, Chi3L1 (YKL40, HCgp39), has been shown to be associated with increased fibrotic activity as well as inflammatory processes. Chi3L1 is upregulated in many pathological conditions [16–23].

Abbreviations: Chi3L1, chitinase 3-like protein 1; DAPI, 4′,6-diamidino-2-phenylindole; ECM, extracellular matrix; IL, interleukin; mRSS, modified Rodnan skin score; OSM, oncostatin M; PDGF, platelet-derived growth factor; αSMA, α-smooth muscle actin; SBTI, soybean trypsin inhibitor; SSc, systemic sclerosis (scleroderma); TIE2, tyrosine kinase with Ig and EGF homology domains-2; TGFβ, transforming growth factor-β

* Corresponding author at: Genetics Unit, Shriners Hospital for Children, 1529 Cedar Avenue, Montréal, Quebec H3G 1A6, Canada. Tel.: +1 514 282 7166; fax: +1 514 842 5581.
E-mail address: jmort@shriners.mcgill.ca (J.S. Mort).

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Elevated serum levels of Chi3L1 are associated with poor prognosis, shorter recurrence-free interval and low overall survival [16,17] in patients with a broad range of cancers, including breast [16] and colorectal cancers [17]. Patients with diseases characterized by inflammation and tissue fibrosis, including rheumatoid arthritis [18], osteoarthritis [19], pneumonia [20], liver cirrhosis [21] and systemic sclerosis (SSc) [22–24] are also reported to have elevated serum Chi3L1 levels. Chi3L1 belongs to the family of mammalian chitinase-like proteins, which share primary sequence homology and three-dimensional structure with the family 18 glycohydrolases [25] but lack chitinolytic activity. The lack of catalytic activity is due to two amino acid substitutions in the active site region of the protein, the most critical one being substitution of the catalytic glutamate residue with leucine [26].

Chi3L1 is produced by macrophages, synovial cells and chondrocytes from arthritic joints [27], and neutrophils [28]. It has been shown to act synergistically with insulin-like growth factor (IGF-1) in fibroblasts to stimulate cell growth [29]. The protein also has mitogenic effects on chondrocytes and synovial cells and promotes proteoglycan synthesis in these cells [30]. Furthermore, Chi3L1 has been shown to be a migration and adhesion factor for vascular smooth muscle cells, which suggests a role in angiogenesis [31]. Previous work has demonstrated that Chi3L1 can modulate the response of connective tissue cells to inflammatory cytokines, such as IL-1 or TNF-α [32]. Chi3L1$^{-/-}$ mice display an exaggerated inflammatory response in a lung injury model [33], supporting this observation. The detailed molecular mechanisms by which Chi3L1 exerts its biological effects are not known.

In SSc, dermal fibroblasts are one of the main effector cells involved in the development of fibrotic lesions, and their biological activity is regulated by a variety of inflammatory cytokines and growth factors. Chi3L1 had been implicated in other pathologies leading to excessive fibrosis [34], and thus its production might be upregulated in affected tissues in patients with SSc. The goals of the present work were to investigate the capacity of skin cells from SSc patients and healthy individuals to synthesize Chi3L1 and to assess the regulation of this process by growth factors and cytokines shown to be associated with this disease.

2. Materials and methods

2.1. Patients, controls, and skin biopsies

Full-thickness biopsies were obtained by an experienced rheumatologist (MB) from the skin of the distal forearm and abdomen of 41 SSc patients recruited from the Canadian Scleroderma Research Group (CSRG) Registry. Similar skin biopsies were also obtained from 10 healthy control individuals. To be eligible for the Registry, patients must have a diagnosis of SSc made by the referring rheumatologist, be age ≥18 years, and be fluent in English or French. Registry patients undergo an extensive clinical history, physical evaluation, and laboratory investigations, and complete a series of self-report questionnaires. Clinical sclerodermatous involvement of skin from biopsy sites was determined by an experienced rheumatologist (MB). Patients and individuals for control biopsies provided written informed consent, and the sample collection and analysis protocols were approved by the McGill University Institutional Review Board. Patients were classified as having limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc (dcSSc) according to the classification by LeRoy et al. [35]. Disease duration was calculated from the date of appearance of the first non-Raynaud's symptom of SSc. This is based on both cutaneous and systemic symptoms, including respiratory symptoms, finger ulcers, inflammatory arthritis, telangiectasia, skin tightening anywhere, fatigue, puffy extremities (hands or feet), weight loss, heartburn or dysphagia and erectile dysfunction.

2.2. SSc clinical outcomes

Using standardized definitions, the recruiting rheumatologist (MB) reported whether or not the patients had active or healed digital ulcers, interstitial lung disease and pulmonary hypertension. Disease activity was measured using the Valentini Scleroderma Disease Activity Index (SDAI) [36,37], consisting of 10 variables with weights ranging from 0.5 to 2.0 and resulting in a total score ranging from 0 to 10. Variables being measured with the SDAI include modified Rodnan skin score (>14), scleredema, change in skin symptoms in the last month, digital necrosis, change in vascular symptoms in the last month, arthritis, lung diffusion capacity <80% predicted, change in cardiopulmonary symptoms, erythrocyte sedimentation rate >30 mm/h and hypocomplementemia [36,37]. Disease severity was measured using physician global assessments of disease severity (scales ranging from 0 to 10) [38]. Predictors of disease severity have been shown to include skin involvement, severity of Raynaud's phenomenon, shortness of breath, gastrointestinal symptoms and pain, number of fingertip ulcers, tender and swollen joints, creatinine, and fatigue [38].

2.3. Cell isolation and culture

Full thickness skin biopsies were collected at bedside and placed into Falcon tubes containing 10 ml Dulbecco's modified Eagle medium (DMEM) (Gibco BRL, Grand Island, NY, USA). To separate epidermis from dermis 0.5% dispase (Invitrogen, Burlington, Canada) was added and the skin biopsies were incubated at 37 °C, in 5% CO_2 for 2 h. Cells were isolated by incubation of dermal samples overnight at 37 °C under gentle mixing in a solution of 0.2% collagenase H (Sigma, St Louis, MO, USA) in DMEM, and 100 U/ml penicillin, and 100 μg/ml streptomycin (Schering Inc., Pointe Claire, Canada). DMEM with 5% fetal calf serum (FCS) was added to neutralize the activity of collagenase. The mixture was centrifuged for 5 min at 500 *g*. The cell pellet was resuspended with 1 ml DMEM, and cell viability was assessed by Trypan Blue exclusion. Cells were then plated in T-75 culture flasks (Becton Dickinson, Franklin Lakes, NJ, USA) and maintained in 12 ml DMEM supplemented with 5% FCS and antibiotics at 37 °C in a 5% CO_2/95% air atmosphere. Culture media were changed every 3 days until cells reached 90% confluency. At this point the cells were sub-cultured at a ratio of 1:3 by trypsinization. It is expected that these cell preparations would be enriched in fibroblasts, but they could also contain any other cell types capable of adherence to tissue culture plastic.

2.4. Stimulation of skin cells by cytokines and growth factors

Skin cells were plated in T-25 culture flasks at 4×10^5 cells/flask and were cultured to near confluence in DMEM supplemented with 5% FCS and antibiotics, followed by 24 h of incubation in serum-free conditions before stimulation with growth factors or cytokines. Cells were either left untreated or treated with 10 ng/ml recombinant interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-17 (IL-17), oncostatin M (OSM), transforming growth factor-β (TGFβ) or platelet-derived growth factor (PDGF) (R&D Systems, Minneapolis, MN, USA) in 3.5 ml DMEM for 48 h. Conditioned media were collected and secreted proteins were analyzed by SDS-PAGE followed by western blotting.

2.5. Soybean trypsin inhibitor (SBTI) biotinylation

Soybean trypsin inhibitor (SBTI, MW 24 kDa) was used as a control for the efficiency of acetone precipitation of cell culture media prior to SDS-PAGE. The protein was biotinylated with sulfosuccinimidyl-6-(biotin-amido) hexanoate (Sulfo-NHS-LC-Biotin; Pierce, Rockford, IL, USA), following the manufacturer's instructions, to allow detection in western blots with the streptavidin–biotin system used for detection of Chi3L1. Excess Sulfo-NHS-LC-Biotin was removed by overnight dialysis (Nominal MWCO: 12,000–14,000; Fisher Scientific, Ottawa, Canada) into 10 l of 100 mM Tris, pH 7.6 at 4 °C. Ten ng biotinylated SBTI was sufficient for use as a control for detection by western blotting.