Tenascin-C in Chronic Sclerosing Sialadenitis

Apostolos Epivatianos · Fotis Iordanidis ·
Dimitrios Andreadis · Savas Iordanidis ·
Athanasios Pouloupoulos · Anastasios Markopoulou

Received: 2 February 2011 / Accepted: 15 April 2011 / Published online: 11 May 2011
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Abstract Tenascin-C is an extracellular matrix glycoprotein that has been implicated in the development of fibrosis in certain chronic inflammatory/sclerosing conditions. This study was undertaken to expand our understanding of the processes involved in fibrosis that occurs in chronic sclerosing sialadenitis (CSS) by investigating the distribution of tenascin-C. Fifteen specimens of CSS with varying degrees of fibrosis and five normal submandibular glands were retrospectively examined immunohistochemically for the distribution of TNC. Linear deposition of TNC was found around collecting ducts in normal glands and around collecting ducts without surrounding fibrotic tissue in CSS; percentage incidences were not statistically different. In contrast, broader, band-like deposition of TNC was found in the fibrous tissue around collecting ducts in CSS with widespread degree of fibrosis compared to little or no fibrosis; the percentage incidence was statistically different. In addition, deposition of TNC was found around duct-like structures and extremely atrophic acini but, interestingly, however, was not found in fibrotic

interlobular septa. The results of this investigation suggest that TNC is likely involved in the fibrosis that occurs around collecting ducts in CSS.

Keywords Chronic sclerosing sialadenitis · Sialolithiasis · Tenascin-c · Fibrosis · Salivary glands

Introduction

Chronic sclerosing sialadenitis (CSS), also known as Kuttner tumor, is a benign, tumor-like fibro-inflammatory condition of the salivary glands, affecting the submandibular gland most commonly. Patients typically present in the 4th to 7th decades and males are affected slightly more often than females. The classic clinical presentation is firm and painful swelling of the affected gland, although some patients may be presented with asymptomatic swelling. Imaging studies generally show diffuse involvement with a “cirrhosis-like” appearance. Microscopic examination shows preservation of the lobular architecture with dense lymphoplasmacytic infiltration and lymphoid follicles with expanded germinal centers. Acinar atrophy is seen within the parenchyma and the lobules are separated by fibrous bands comprised of fibroblasts and chronic inflammatory cells.

The pathogenesis of CSS has not been fully elucidated. One possible pathogenic mechanism is obstructive electrolytic sialadenitis, which occurs secondary to a secretion disorder that obstructs small ducts [1]. Another possible mechanism previously suggested is that secretory inactivity in a normal gland leads to an accumulation of sialomicrooliths and, thus, foci of obstructive atrophy. This, in turn, becomes a haven for microbes that ascend Warton’s duct, cause chronic sialadenitis, and may subsequently lead to sialolithiasis [2].

A. Epivatianos · D. Andreadis (✉) · A. Pouloupoulos ·
A. Markopoulou
Department of Oral Medicine/Pathology, School of Dentistry,
Aristotle University of Thessaloniki, GR-54124 Thessaloniki,
Greece
e-mail: dandrea@dent.auth.gr; uvomorulin@hotmail.com

F. Iordanidis
Department of Histopathology, General Hospital
“Papanikolaou”, Thessaloniki, Greece

S. Iordanidis
Department of Oral and Maxillofacial Surgery,
School of Dentistry, Aristotle University of Thessaloniki,
Thessaloniki, Greece

In fact, CSS is associated with sialolithiasis in 29–83% of cases [3, 4]. A major clinicopathologic study on CSS revealed that there was increasing fibrosis and duration of symptoms and that fibrosis was related to inflammation, atrophy, and sialolithiasis [4]. The processes involved in fibrosis of CSS remain poorly understood as only a small number of cases have been reported [5–8].

More recently, increasing evidence suggests that CSS belongs to IgG4-related sclerosing disease, a systemic condition characterized by extensive IgG4-positive plasma cells and T-cell lymphocytic infiltration of various organs. Clinical manifestations of this disease include autoimmune pancreatitis, sclerosing cholangitis, retroperitoneal fibrosis, CSS, tubulointerstitial nephritis, inflammatory pseudotumors of the liver and lung, and lymphadenopathy [9].

Tenascin-C (TNC) is a hexameric extracellular matrix glycoprotein, and the best understood isoform of the tenascins. Each subunit of hexameric glycoprotein consists of a tenascin assembly domain, epidermal growth factor-like repeats, fibronectin type III-like repeats, and a C-terminal fibrinogen-related domain. Alternative splicing results in several different forms of tenascins (i.e., tenascin-C, -R, -X, -W) that contain variable numbers of fibronectin type III-like repeats [10–12]. TNC is expressed at high levels during embryonic development [13, 14], cell migration, tissue remodeling [15], and mechanical stress [16]. Furthermore, TNC has been shown to be involved in the development of fibrosis associated with chronic hepatitis [17, 18], systemic sclerosis, rheumatoid arthritis [19], pleural fibrotic diseases [20], chronic progressive nephropathy [21], and oral submucous fibrosis [22].

The aim of the present study was to investigate the distribution of TNC in CSS in an attempt to understand the processes involved in fibrosis occurring in this chronic inflammatory condition.

Materials and Methods

Fifteen cases of CSS were retrieved from the archives of the Department of Oral Medicine and Oral Pathology of the School of Dentistry at Aristotle University of Thessaloniki. Common symptoms among these patients included pain, swelling, and discharge, and there was no evidence of Sjogren's syndrome, scleroderma, or rheumatoid arthritis. Sialolithiasis was diagnosed by means of radiography and histologic examination in all cases. All patients had given informed consent and the whole study was performed according to Helsinki II declaration. Five specimens of normal submandibular glands were selected from post-mortem material used in previous investigations [4, 7, 8].

Serial sections were cut at 4 µm-thickness from paraffin-embedded specimens and subsequently stained with

haematoxylin and eosin (H&E) and immunohistochemically for the detection of TNC. Endogenous peroxidase activity was quenched with 3% H₂O₂ for 10 min at room temperature. For antigen retrieval, the sections were pre-treated with 0.5 pepsin in phosphate-buffered saline (PBS), pH 1.8 at 37°C for 30 min (Dakocytomation, Glostrup, Denmark). After two PBS washes, nonspecific immunoglobulin uptake was blocked by incubation with 3% normal goat serum (Dakocytomation) for 20 min at room temperature. MAb DB7 (MU295-UC, Biogenex, San Ramon, CA, USA) was used at a 1:3000 dilution. Incubation with the primary antibody was performed overnight at 4°C. Tissue sections incubated for the same time with PBS were used as negative control. Sections from tonsil were used as positive control. After being washed three times with PBS, the slides were incubated with biotinylated goat anti-mouse secondary antibody at a 1:300 dilution for 30 min at room temperature, washed with PBS, followed by the peroxidase-conjugated Streptavidin (Biogenex) for 30 min at room temperature, and development by diaminobenzidine reaction. Slides were counterstained with aqueous haematoxylin, rinsed in tap water, and mounted with Supermount (Biogenex).

Fibrosis was graded as follows: 0, no or little fibrous tissue; 1, focal increase in fibrous tissue; 2, widespread moderate increase in fibrous tissue; and 3, widespread marked increase in fibrous tissue. Cases of Grades 0 and 1 were grouped together as cases with “no or localized fibrosis”, and cases with Grades 2 and 3 were grouped together as cases with “widespread fibrosis” [4, 7].

Detailed histologic examination of the salivary glandparenchyma carefully discriminated between acini, striated and collecting ducts, and duct-like structures, respectively. Acini were graded as normal or atrophic according to their size. In normal glands and CSS, intralobular ducts larger than striated ducts were grouped together with interlobular and extralobular ducts as “collecting ducts”. In CSS, extremely atrophic parenchyma containing structures resembling ducts were grouped together as “duct-like structures”.

In normal glands, the deposition of TNC was evaluated individually around acini, striated and collecting ducts, and in interlobular septa. According to the degree of fibrosis of CSS, the deposition of TNC was evaluated individually around normal or atrophic acini, duct-like structures, collecting ducts without periductal fibrosis, collecting ducts with periductal fibrosis and in normal or fibrotic interlobular septa. Deposition of TNC around normal or atrophic acini, duct-like structures and striated ducts was assessed by counting 100 of each parenchymal component and calculating the percentage showing deposition. Counts were performed using a microscope fitted with 10 × 10 mm ocular grid and 10 oculars. Starting from the top left hand corner of each lobule of specimen, successive fields were

assessed until 100 of each parenchymal component had been counted [23]. However, the number of collecting ducts with and without periductal fibrosis and interlobular septa in specimens was smaller than 100; therefore, the total number of each part was counted and used to calculate the percentage incidence showing deposition of TNC.

Histologic sections were examined by two of the authors (E.A. and I.F.) independently of each other. Sections were re-examined when there were differences in the measurements and discussion was occasionally necessary to establish uniformity. SPSS 10.0 software was employed for statistical analysis. The means were compared using Student's *t* test, at a level of significance $\alpha = 0.05$.

Results

Fibrosis was graded in all 15 cases of CSS according to no or little fibrosis (Grades 0–1) and widespread fibrosis (Grades 2–3). Results are summarized in Table 1. In normal glands, fine, linear deposition of TNC was present around collecting ducts (Fig. 1) and the percentage incidence was 90.48 ± 1.10 . Similarly, linear deposition of TNC was found in CSS around collecting ducts *without* periductal fibrosis. Moreover, there was no statistically

significant difference in the percentage incidence of linear deposition of TNC around collecting ducts without periductal fibrosis between CSS cases (Grades 0–3) and normal glands ($t = 0.562$, $t = 0.628$, $t = 0.18$, $P > 0.2$). The distribution of TNC and the percentage incidences in CSS are presented in Table 2.

Not surprisingly, collecting ducts *with* periductal fibrosis showed a broad, band-like deposition of TNC (Figs. 2–3). Furthermore, the difference in percentage incidence of deposition of TNC around collecting ducts with periductal fibrosis was statistically significant between Grades 0–1 and Grades 2–3 ($t = 19.75$, $P < 0.0001$). In cases of fibrosis Grades 0–1, absence of TNC around acini and striated ducts was found, whereas in cases of Grades 2–3, deposition of TNC around duct-like structures and extremely atrophic acini was present (Figs. 3–4). In CSS, notably, deposition of TNC was not seen in either normal or fibrotic interlobular septa.

Discussion

Our results showed linear deposition of TNC around the collecting ducts of normal submandibular glands, which is in agreement with previous reports [24–26]. It has been proposed that the deposition of TNC around excretory ducts of normal human major and minor salivary glands is related to the function of responding to mechanical stress provoked by saliva transport [26]. A recent study [16] demonstrated that TNC induced by mechanical stress may enhance tissue tension and local stiffness, and cells may counteract this tension through reduced adhesion with the extracellular matrix.

Searching the literature, we found only one study concerning the expression of TNC in CSS [27]. Souza et al. [27] examined two cases of CSS, one of which was associated with sialolithiasis, and cited light expression of TNC in areas of periductal fibrosis and more intense expression around ducts. Recall that our findings showed broad, band-like deposition of TNC in collecting ducts with periductal fibrosis. Their results are partly in agreement with our results, and the disagreements possibly are due to the limited number of cases.

The results of the current study showed no significant differences in the percentage incidence of linear deposition of TNC around collecting ducts *without* periductal fibrosis between normal glands and CSS, regardless of degree of fibrosis (Grades 0–3). This finding suggests that the deposition of TNC around these ducts in response to mechanical stress is not altered during the early pathogenesis of CSS. However, the significant difference in TNC deposition around *fibrosed* collecting ducts in CSS between Grades 0–1 and Grades 2–3 suggests involvement of TNC in the local development and progression of glandular fibrosis.

Table 1 Degrees of fibrosis in 15 cases of chronic sclerosing sialadenitis

Grade of fibrosis	Number of cases
Grade 0	3
Grade 1	4
Grade 2	4
Grade 3	4

Fig. 1 Normal submandibular gland. Tenascin-C antibody highlights fine, linear deposition of tenascin-C around collecting ducts. Streptavidin-biotin method ($\times 100$)

Table 2 Percentage incidence of deposition of tenascin-C in different parenchymal parts according to the degrees of fibrosis in chronic sclerosing sialadenitis

Fibrosis	Number of cases	Acini	Striated ducts	Collecting ducts without periductal fibrosis	Collecting ducts with periductal fibrosis
Grades 0–1 (no or little fibrosis)	7	0%	0%	89.23 ± 5.12	1.92 ± 1.3
Grades 2–3 (widespread fibrosis)	8	3.62 ± 1.97*	44.21 ± 20.10**	90.93 ± 6.57	79.17 ± 11

* Extremely atrophic acini, ** Duct-like structures

Fig. 2 a. Chronic sclerosing sialadenitis with Grade 1 (no or localized) fibrosis. Fibrosis is seen around two collecting ducts (arrows). (Haematoxylin and eosin, $\times 100$). **b.** Tenascin-C antibody in

the same area shows a broader, band-like deposition of tenascin-C around the two fibrotic collecting ducts. Streptavidin–biotin method ($\times 100$)

Fig. 3 Chronic sclerosing sialadenitis with Grade 3 (widespread) fibrosis. Note the increased, band-like deposition of tenascin-C in the fibrotic tissue surrounding collecting ducts and in the stroma around duct-like structures. Streptavidin–biotin method ($\times 100$)

Fig. 4 Chronic sclerosing sialadenitis associated with Grade 3 (widespread) fibrosis. Note the deposition of tenascin-C around duct-like structures and one extremely atrophic acinus (arrow). Streptavidin–biotin method ($\times 200$)

In fibrosis, the extracellular matrix is continuously being remodeled and many of its proteins are increased and disarranged [20, 28]. TNC has both adhesive and anti-adhesive properties [20, 29–31] and may be involved in the development of fibrosis around collecting ducts in CSS by modulating the adhesion of fibroblasts and myofibroblasts

in the extracellular matrix. Myofibroblasts are present around larger ducts, likely providing a supportive function, and play a limited role in the development of fibrosis in CSS [8].

In our study, we found deposition of TNC around duct-like structures and extremely atrophic acini in cases of CSS

with widespread degree of fibrosis (Grades 2–3). The duct-like structures represent extremely atrophic striated ducts and are present in advanced cases of CSS [4]. TNC around these ducts and atrophic acini is possibly involved in the remodeling of the surrounding stroma. Matrix metalloproteinase-mediated catabolism of the preexisting extracellular matrix may promote the formation of a provisional matrix that is enriched by TNC [32, 33]. Ductal, acinar, and stromal cells in CSS express metalloproteinases [6], but their role has not been fully elucidated. The precise role of TNC around duct-like structures and extremely atrophic acini in CSS will have to be explored in further studies.

Lastly, and interestingly, our study did not show staining for TNC in the fibrotic interlobular septae. This suggests that TNC is not involved in the development of fibrosis occurring in interlobular septae of CSS.

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