Oral Diseases (2004) 10, 233–239

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Oral Biology/Periodontology

Immunohistochemical localization and expression of fibromodulin in adult rat periodontium and inflamed human gingiva

H Qian[1,2], Y Xiao[1,3], PM Bartold[4]

[1]*Department of Dentistry, University of Queensland, Brisbane, Australia;* [2]*School of Dentistry, Wuhan University, Wuhan, China;* [3]*Tissue BioRegeneration and Integration, Life Science, Queensland University of Technology, Brisbane, Australia;* [4]*University of Adelaide, CACDRC, Dental School, Adelaide, Australia*

OBJECTIVE: The aim of this study was to determine the distribution and expression of fibromodulin in adult rat periodontal tissues and inflamed human gingiva.

MATERIALS AND METHODS: The distribution of fibromodulin in rat molar periodontium and human gingival tissue was studied by immunohistochemistry. The expression of fibromodulin mRNA from human gingival fibroblasts, periodontal ligament fibroblasts and osteoblasts was studied by reverse transcription-polymerase chain reaction (RT-PCR). For comparative purposes, the distribution and mRNA expression of collagen types I and III, as well as the two small leucine-rich proteoglycans decorin and biglycan were also studied.

RESULTS: In the adult rat periodontium, fibromodulin was distributed in the suprabasal gingival epithelium, gingival and periodontal fibroblasts as well as their surrounding extracellular matrices. Strong expression was noted in the palatal gingival tissues and the interfaces of the periodontal ligament with alveolar bone and cementum. In human gingival tissues, staining of fibromodulin was detected in the connective tissue of inflamed gingiva associated with both gingivitis and periodontitis; whereas, weak staining for this molecule was noted in healthy gingival tissues. The expression of mRNA for fibromodulin was strongest in the cultured osteoblasts. Periodontal ligament fibroblasts showed only a weak level of expression for fibromodulin mRNA.

CONCLUSIONS: Fibromodulin is differentially expressed throughout the periodontium being primarily associated with collagen type I in non-mineralized sites. In addition fibromodulin showed an upregulation in inflamed gingival tissue.

Oral Diseases (2004) **10**, 233–239

Correspondence: PM Bartold, University of Adelaide, Colgate Australian Clinical Dental Research Centre, Dental School, From Road, Adelaide, South Australia, 5005, Australia. Tel: 61 8 8303 3435, Fax: 61 8 8303 6429, E-mail: mark.bartold@adelaide.edu.au

Received 28 August 2003; revised 13 November 2003; accepted 13 December 2003

Keywords: fibromodulin; immunohistochemistry; decorin; biglycan; type I collagen; type III collagen; periodontium

Introduction

Proteoglycans are large, highly anionic glycoproteins ubiquitous in all connective tissues. They are integral components of the extracellular matrix structure, and are also present on cell surfaces and within cell organelles (Bartold and Narayanan, 1998). Within the extracellular matrix, two classes of proteoglycans have been identified and characterized. One class is represented by large molecules, such as aggrecan (Neame and Sandy, 1994), versican (Zimmermann and Ruoslahti, 1989), and perlecan (Noonan and Hassell, 1993). These proteoglycans serve to maintain tissue hydration and contribute to the overall structural scaffolding in the extracellular matrix (Yanagishita, 1993). The other class is comprised of relatively small molecules, many of which contain leucine-rich repeat motifs in their protein cores. Five distinct members in this group have been identified and studied extensively: fibromodulin (Oldberg *et al*, 1989), biglycan (Neame, Choi and Rosenberg, 1989), decorin (Krusius and Ruoslahti, 1986), lumican (Blochberger *et al*, 1992) and chondroadherin (Neame *et al*, 1994). These small leucine-rich proteoglycans play important roles in binding to other matrix molecules such as collagens, serving either to aid fibrillogenesis or to act as bridging molecules between various tissue elements (Scott, 1988).

The amino acid sequence of bovine fibromodulin shows very close homology with both decorin and biglycan (Oldberg *et al*, 1989). This proteoglycan contains keratan sulphate with four potential substitution sites, all present in the leucine-rich region (Antonsson, Hinegård and Oldberg, 1991). Immunocytochemical localization of fibromodulin has identified a non-random distribution of fibromodulin on collagen fibrils and its presence in the gap region of collagen fibers

(Hedlund *et al*, 1994). There is considerable evidence that fibromodulin is involved in regulating the formation of the network of collagen fibrils through its interaction with collagen types I, II and XII (Hedbom and Heinegård, 1989; Font *et al*, 1996). Fibromodulin is also known to interact with transforming growth factor beta (TGF-β), which aids in the retention of this growth factor in tissues, acting in a 'reservoir' capacity for this growth factor within the matrix (Fukushima *et al*, 1993; Hildebrand *et al*, 1994).

Fibromodulin may also potentially influence mineralization at the interface between hard and soft tissues and this has particular relevance for the periodontium, where it has been suggested that fibromodulin expression in bovine precementum indicates an inhibitory role in matrix mineralization (Cheng *et al*, 1996; Matias *et al*, 2003).

In light of the above, fibromodulin not only appears to influence mineralization but also matrix formation in general. Hence, this small proteoglycan is likely to be of considerable interest with regard to matrix formation and function in both normal and diseased states. Accordingly we have hypothesized that expression of this molecule varies with regard to both tissue location as well as inflammatory status. The aim of this study was, therefore, to determine the distribution and cellular source of fibromodulin within the periodontium and specifically study what changes inflammation might induce on its expression in the gingival tissues of the periodontium. Because of difficulty in obtaining block sections of healthy human periodontium, adult rat molars were extracted and block sections were stained to examine the distribution of fibromodulin throughout the periodontal tissues. The effect of inflammation on fibromodulin was assessed in biopsies of inflamed human gingiva obtained from healthy, gingivitis and periodontitis sites. The relationship of the immunohistochemical distribution, in normal gingival tissues, of fibromodulin and the well-characterized matrix components decorin, biglycan, collagen type I and collagen type III was also assessed.

Materials and methods

Animal model and human gingival tissue

The experimental protocol for this study was approved by The University of Queensland Human & Animal Experimentation Ethics Committee. Five male Lewis rats were housed in a light and temperature-controlled animal facility and fed food and water *ad libitum*. All rats were 12 weeks old and their body weight ranged from 230 to 260 g on the day of killing. Four patients (10 blocks of inflamed gingivitis and six blocks of periodontitis) and two healthy persons with three donor sites (six blocks of healthy gingival tissue) were included in this study. The inflamed and healthy gingival sites were diagnosed on clinical and histological criteria in each case.

Tissue preparation

Under general anesthesia (Nembutal; Boehringer Ingelheim Pty, Artarmon, NSW, Australia) rat mandibular molars, together with the surrounding alveolar bone and covering gingival tissues, were removed. These tissues were then washed in phosphate-buffered saline (PBS) for 5 min, then fixed in 4% paraformaldehyde in PBS at pH 7.4 for 12 h at room temperature. The specimens were demineralized thoroughly in 10% ethylenediaminothanetetraacetic acid disodium salt (EDTA) for 3–4 weeks. Demineralization was confirmed radiographically. The decalcified rat tissues and human gingival tissues were trimmed, dehydrated in graded ethanol (70, 90 to 100%), cleared in toluene and embedded in paraffin. Serial bucco-lingual sections (5 μm) were cut and mounted on glass slides precoated with 0.1% poly-L-lysine (Sigma, St. Louis, MS, USA). Histological assessment was carried out following routine hematoxylin and eosin staining.

Antibodies

A panel of well-characterized polyclonal antibodies against several small leucine-rich proteoglycans were used for immunohistochemical assessments. These included anti-fibromodulin (a gift from Dr AHK Plass, Shriners Hospital for Crippled Children, Tampa University, Tampa, FL, USA), anti-biglycan (LF-51, a gift from LW Fisher, National Institutes of Health, MD, USA) and anti-decorin (LF-30, a gift from LW Fisher, National Institutes of Health, MD, USA). Polyclonal antibodies against type I collagen and type III collagen (gifts from LW Fisher, National Institutes of Health, MD, USA) were from LF-67 and LF-71, respectively.

Immunohistochemistry

Prior to staining, the sections were deparaffinized in xylene and rehydrated in descending concentrations of alcohol and water. Before incubation with the primary antibodies, the sections were soaked in 3% H_2O_2 (Sigma) in PBS for 20 min to eliminate endogenous peroxidase activity and then blocked with 1:10 normal swine serum (DAKO, Carpinteria, CA, USA) in PBS and 0.1% bovine serum albumin (BSA) for 1 h at room temperature. Polyclonal antibodies against fibromodulin (1:2000), biglycan (1:2000), decorin (1:3000), type I collagen (1:7500), type III collagen (1:4000) were used as primary antibodies. All primary antibodies were diluted with 0.1% BSA and 0.01% NaN_3 in PBS. The sections were incubated in a humidity chamber at 4°C overnight. These sections were washed in PBS and incubated with the secondary antibody (biotinylated swine anti-mouse, anti-rabbit and anti-goat, DAKO LSAB+ kit, Carpinteria) for 15 min at room temperature and washed in PBS, and then incubated with horseradish peroxidase-conjugated streptavidin (DAKO LSAB+ kit) for 15 min. Specific immunostaining was visualized with 3,3′-diaminobenzidine tetrahydrochloride containing 0.015% H_2O_2 for up to 3 min, after which the sections were washed in PBS and rinsed in water. Sections were then counterstained with Mayer's hematoxylin for 20 s and rinsed in running water. Finally, the sections were dehydrated in ascending concentrations of alcohol, cleared in xylene and mounted using Depex mounting medium (BDH Laboratory Supplies, Poole, UK) for light microscopic examination. Photomicrographs were

taken to record the results. Controls for the performance of the immunostaining procedures included conditions where the primary antibody or the secondary (anti-mouse IgG) antibody was omitted and an irrelevant antibody (anti-CD-15), which should not have been present in the test sections, was used as a control. To assess the immunoreactivity of these antibodies, staining patterns and intensities were rated by three individual examiners according to our previously published study (Matias *et al*, 2003). Each round of staining included three to five serial sections.

Cell culture

Human gingival fibroblasts (GF), osteoblasts (OB) and periodontal ligament fibroblasts (PDLF) were obtained from Periodontal Connective Tissue Research Laboratory, School of Dentistry, University of Queensland. These cells had been isolated by explant culture from human healthy gingival, periodontal ligament and alveolar bone tissues as described previously (Worapamorn *et al*, 2000). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), 50 units ml^{-1} penicillin, 50 units ml^{-1} streptomycin and non-essential amino acids. Cells between the fourth and eighth transfer in culture were used.

Reverse transcriptase-polymerase chain reaction

The primers (Proligo, Lismore, NSW, Australia) designed for this study are listed in Table 1. Total RNA was isolated from the cell cultures using total RNA isolation reagent (ABgene House, Epsom, UK). The quantity and quality of isolated total RNA was determined using spectrophotometry and electrophoresis. To generate cDNA, 1 μg of total RNA was reverse transcribed in a 20-μl reaction using Oligo (dT) primer and M-MLV reverse transcriptase (Promega, Madison, WI, USA). mRNA for fibromodulin, decorin, biglycan, type I collagen, type III collagen and the house-keeping gene β2-microglobulin were amplified in the tube containing 2 μl cDNA, 10 pmol of each primer, 1 μl Red Tag DNA polymerase (Sigma) successively in 20 μl reaction mixture for 31–35 cycles. Different cycles and temperature used for different primers are listed in Table 2. The PCR products were analyzed on a 2% (w/v) agarose gel and visualized by staining with ethidium bromide and analyzed densitometrically using a NIH Gel image software (version 1.57) (National Institutes of Health, Bethesda, MD, USA). To compare the mRNA expression in OB, GF, PDLF, three cell lines from each cell type were cultured under the same conditions (same passage number, cell density and culture media). Each PCR reaction was duplicated with the same cDNA. The PCR reactions were run under different cycles from 29–35 and a linear relationship was identified between the cycle number (29–35) and densitometry of the PCR products in the agarose gels.

Results

Gingival localization

The immunolocalization results indicate the distribution of the components under study using polyclonal antibodies. No obvious difference was noted in the expression pattern between samples in the same group. Serial dilution of the primary antibodies was used to determine optimum staining potential and the results are summarized according to the general pattern of staining considered representative for each antibody. Immunohistochemical staining of adult rat molar periodontal tissues using a polyclonal antibody against fibromodulin showed specific distributions of fibromodulin in the gingival epithelium and gingival connective tissue. In the palatal gingival epithelium of both the human and rat tissues, keratinized suprabasal layers exhibited moderate staining with fibromodulin. In contrast, weak staining for fibromodulin was found in the stratified squamous keratinized layers of the epithelium. The basement membrane, non-keratinized

Table 1 Primers for RT-PCR

Gene	*Forward*	*Reverse*	*Product size (bp)*
Fibromodulin	AGCCTCATCGAGATCTGA	GGAGTGGGTGAAGTGGA	500
β2-microglobulin	CCCCCACTGAAAAAGATGAG	TCATCCAATCCAAATGCGGC	122
Decorin	GATCACCAAAGTGCGAA	CCAGAGAGCCATTGTCA	297
Biglycan	TGGAGAACAGTGGCTTTGAAC	TTCTCGATCATCCTGATCTGG	226
Collagen type I	GTGGGCTTCCTGGTGA	CTTTGGAGCCAGCTGGA	400
Collagen type III	GGTACTCCTGGTCTGCA	GAAGCCAGCAGCACCA	449

Table 2 PCR cycles and temperature for primers

Primers	*PCR cycles*	*Denaturation temperature and time*	*Annealing temperature and time*	*Elongation temperature and time*
Fibromodulin	33	94°C for 1 min	50°C for 30 s	72°C for 1 min
β2-microglobulin	33	94°C for 1 min	60°C for 30 s	72°C for 1 min
Decorin	33	94°C for 1 min	50°C for 30 s	72°C for 1 min
Biglycan	35	94°C for 1 min	55°C for 2 min	72°C for 3 min
Collagen type I	31	94°C for 1 min	55°C for 2 min	72°C for 3 min
Collagen type III	31	94°C for 1 min	55°C for 2 min	72°C for 3 min

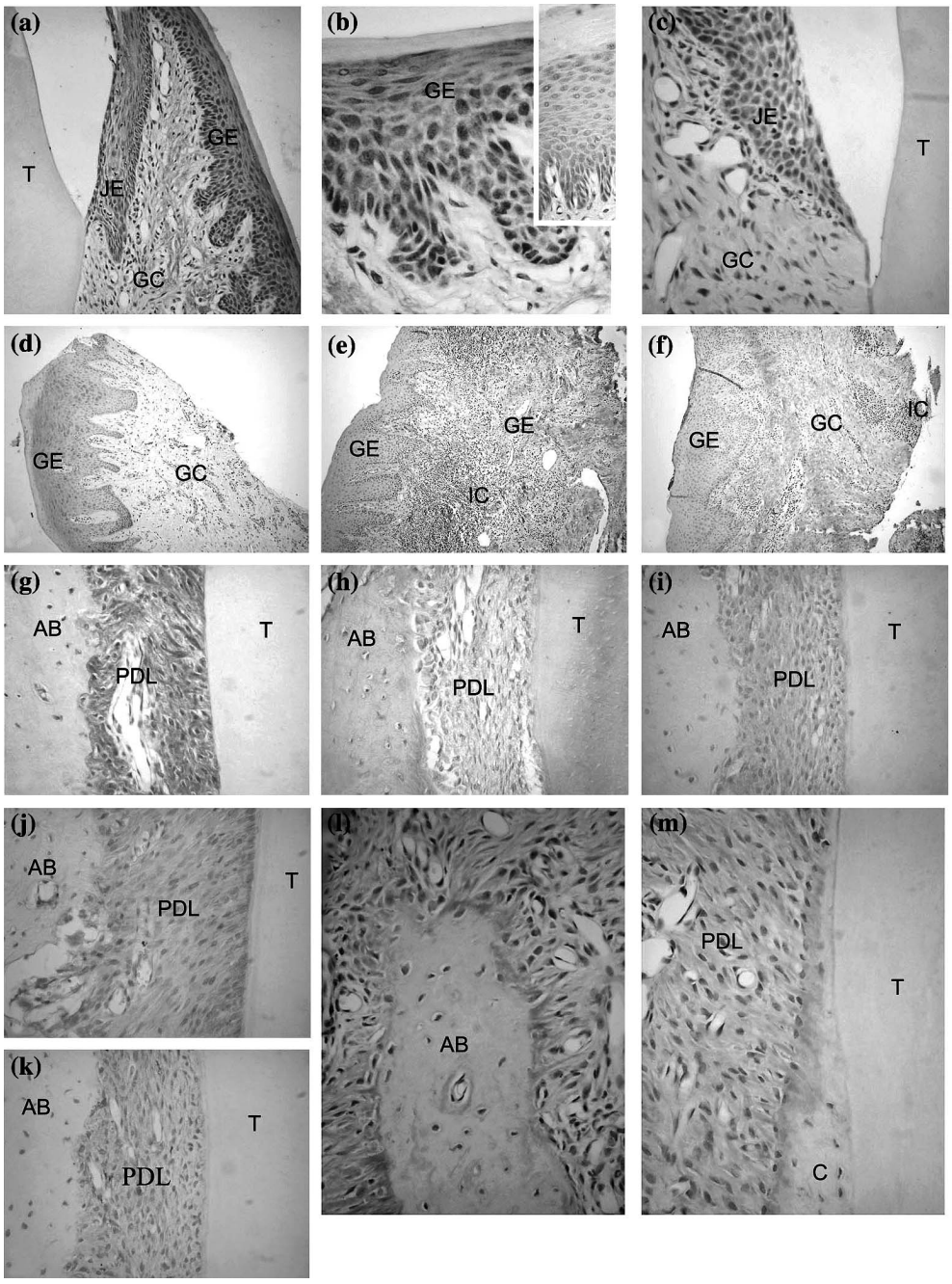

Figure 1 (**a**) Distribution of fibromodulin in rat gingival tissues. Fibromodulin was positively expressed in gingival fibroblasts and gingival connective tissue matrix. (**b**) Distribution of fibromodulin in rat gingival epithelium. Fibromodulin was distributed in the keratinized suprabasal layers of the gingival epithelium and negative staining was observed on the basement membrane. Negative control (using irrelevant antibody CD-15) is shown in the right-hand corner. (**c**) Distribution of fibromodulin in the areas of junction epithelium. Negative staining of fibromodulin was found in the areas of junction epithelium and underneath connective tissue. (**d**) Distribution of fibromodulin in human healthy gingival tissue. Fibromodulin was mainly distributed in epithelium with very faint staining in connective tissue. (**e** and **f**) Distribution of fibromodulin in human gingival tissue with gingivitis (**e**) and periodontitis (**f**). Very strong staining of fibromodulin was noted in the connective tissue, whereas, no obvious expression of fibromodulin was noted in inflammatory cells. (**g**–**k**). The expression of fibromodulin (**g**), type I collagen (**h**), type III collagen (**i**), biglycan (**j**), and decorin (**k**) in rat periodontal supporting apparatus. (**l**) Expression of fibromodulin in the alveolar bone of root furcation. (**m**) Expression of fibromodulin in cellular cementum. T, tooth; PDL, periodontal ligament; AB, alveolar bone; C, cementum; GE, gingival epithelium; GC, gingival connective tissue; JE, junction epithelium; IC, inflammatory cells [original magnification 200× for (**a**), (**d**), (**e**), (**f**), (**g**), (**h**), (**i**), (**j**), (**k**); 400× for (**b**), (**c**), (**l**), (**m**)]

cells of sulcular epithelium and cells of the junctional epithelium did not show any immunoreactivity with this antibody (Figure 1a–c).

Within the healthy human gingival connective tissue, no obvious staining was detected (Figure 1d). However, in inflamed gingival tissues from both gingivitis

Table 3 Summary of immunoreactivity in the periodontal ligament, cementum and alveolar bone for fibromodulin

Tissue/cell	*Fibromodulin*	*Decorin*	*Biglycan*	*Collagen type I*	*Collagen type III*
Periodontal ligament fibroblasts	++	++	++	++	++
Periodontal ligament matrix	++	++	++	++	+
Cementoblasts	–	++	++	+	+
Cementocytes	–	–	–	–	–
Cementoid	–	+	+	++	++
Acellular cementum	–	–	–	+++	–
Osteoblasts	++	+	++	+?	++
Osteocytes	–	–	++?	+?	–
Osteoclasts	?	?	?	?	?
Osteoid	–	–	+	++	+

+, weakly positive staining; ++, moderately positive staining; +++, strongly positive staining; +?/++?, some areas positive staining; ?, no presenting cells in the sections; –, no positive staining.

(Figure 1e) and periodontitis lesions, gingival fibroblasts and gingival connective tissue matrix demonstrated strong staining patterns for fibromodulin (Figure 1f). No obvious staining was detected around the infiltrating inflammatory cells.

Periodontal supporting apparatus localization

A summary of the distribution of fibromodulin in the periodontal tissues is given in Table 3. Fibromodulin was strongly expressed by periodontal ligament, fibroblasts, particularly those in areas close to cementum and bone surfaces. In the periodontal ligament, strong staining was noted in the fiber bundles (Sharpey's fibers) inserting into alveolar bone and cementum (Figure 1g). Expression of fibromodulin was also noted on osteoblasts lining the crestal part and inner surface of the alveolar bone proper. An intense staining of filament-like structures was observed in the extracellular matrix connecting to the alveolar bone located in the furcation areas (Figure 1l). No staining was evident on osteocytes or osteoid (Figure 1g). Fibromodulin was expressed by cementoblasts lining the root surface, and in newly formed cementoid. Cementocytes, acellular cementum and mature cementum were not stained (Figure 1m,g).

Compared with fibromodulin, type I collagen stained strongly in the cementum, alveolar bone matrix and periodontal ligament (Figure 1h). The expression of type III collagen in the periodontium was not as strong as that noted for type I collagen (Figure 1i). In contrast to fibromodulin, the staining for type I and III collagen was not particularly strong in the region of Sharpey's fiber insertion into bone and cementum. Biglycan and decorin showed a slightly stronger expression in the areas close to root surface. Biglycan appeared to stain the cells lining the root surfaces and also showed a co-localization with collagen fiber bundles (Figure 1j) while decorin showed more predilection towards an extracellular distribution (Figure 1k).

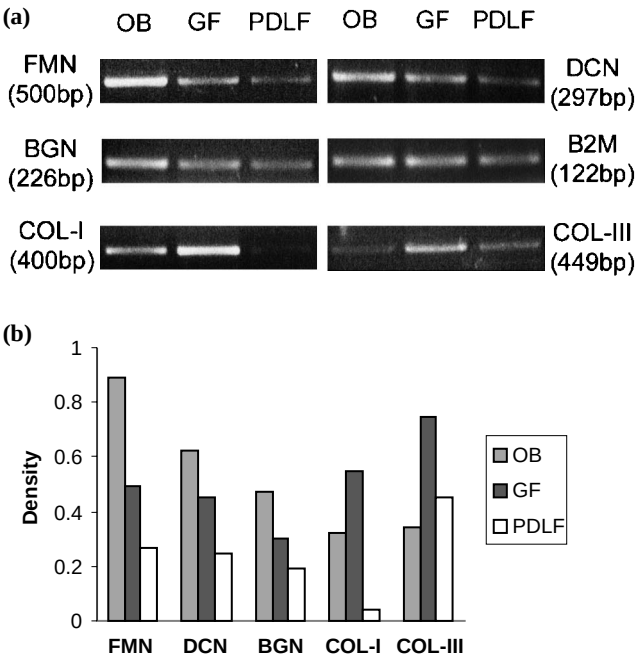

Figure 2 (**a**) Fibromodulin, decorin, biglycan, type I collagen, type III collagen and β-2 microglobulin mRNA expression by osteoblasts, gingival fibroblasts and periodontal ligament fibroblasts. (**b**) Fibromodulin, decorin, biglycan, type I and type III collagen mRNA levels by osteoblasts, gingival fibroblasts and periodontal ligament fibroblasts

Reverse transcriptase-polymerase chain reaction

The mRNA expression for fibromodulin, decorin, biglycan, type I collagen, type III collagen and β2-microglobulin by osteoblasts, gingival fibroblasts and periodontal ligament fibroblasts is shown in Figure 2a. Quantitation of the mRNA levels showed that the osteoblasts had the highest level of expression of fibromodulin in these three types of cells. It was noted that cells with a higher expression of fibromodulin also expressed higher mRNA levels for decorin and biglycan. Type I and type III collagens mRNA levels were highest in the gingival fibroblasts compared with the osteoblasts and periodontal ligament fibroblasts.

Discussion

In this study concerning the distribution of fibromodulin in periodontal tissues we have extended previous studies which have reported the distribution of fibromodulin in tooth cementum (Cheng *et al*, 1996; Ababneh, Hall and Embery, 1998, 1999; Watanabe and Kubota, 1998). We have demonstrated that fibromodulin is widely distributed in adult periodontal connective tissues and its mRNA can be detected in three major types of periodontal cells namely gingival fibroblasts, periodontal ligament fibroblasts and osteoblasts. In addition, altered expression of fibromodulin was noted under inflammatory conditions.

Different patterns of fibromodulin expression were noted in the epithelial and gingival connective tissues of rat and human tissues. In healthy human gingival

connective tissue very faint staining was detected, whereas, in rat gingival connective tissue expression of fibromodulin was detected in the subepithelial gingival connective tissue and showed site specificity with intensive staining in the palatal side. The reason for the different expression of fibromodulin between human and rat gingiva is not clear. It could be that the human gingival biopsies used in this study were collected from the buccal side which for unknown reasons appears to express fibromodulin less strongly (Matias *et al*, 2003). It may also be possible that the antibody against fibromodulin used in this study recognizes antigens with different affinities for different species.

Strong staining of fibromodulin was noted in inflamed human gingival connective tissue compared with healthy gingival tissue. As all the human gingival tissues collected for this study were from the buccal surface the differences noted in the expression of fibromodulin between different disease status is presumed to be associated with the presence or absence of inflammation. The strong expression of fibromodulin in inflamed gingival connective tissue indicated an active role of this type of proteoglycan in re-organization of collagen fibrils during the inflammation. Such findings are consistent with findings in other tissues in which increased expression of fibromodulin has been noted following induction of inflammation and subsequent granulation tissue formation (Venkatesan *et al*, 2000). Furthermore, the expression of fibromodulin by granulation tissue fibroblasts isolated from inflamed human gingiva has been noted to express elevated basal levels of fibromodulin as well as other proteoglycans such as biglycan and versican (Häkkinen *et al*, 1996).

Previous studies which have shown that fibromodulin has a specific tissue distribution in bovine precementum and pericementocyte areas and that this proteoglycan may regulate cementum mineralization (Cheng *et al*, 1996). However, to date no expression has been found in human cementum (Ababneh *et al*, 1999). In the present study we found that, as noted for bovine tissues, rat precementum and pericementocytes were stained for fibromodulin. No staining for fibromodulin was noted in cellular and acellular cementum.

Strong staining of fibromodulin in association with fibrillar collagens connecting alveolar bone and cementum tissue suggests that fibromodulin might interact with collagens at the interface of soft tissue and hard tissue (Hedbom and Heinegård, 1989; Hedlund *et al*, 1994). The periodontal ligament collagen fibrils inserted into the alveolar bone and cementum also stained very strongly for fibromodulin. However, collagen fibrils in the mineralized tissues such as those found in the alveolar bone and cementum area did not stain for fibromodulin. As proteoglycans associated with type I collagen might inhibit mineralization in soft connective tissues, we speculate that fibromodulin may have a regulatory role in early formation of the attachment fibers at the bone–periodontal ligament and cementum–periodontal ligament interfaces. Fibromodulin could aid this process by inhibiting mineralization and then with its subsequent removal, by hitherto unidentified processes, mineralization could take place leading to the fibers becoming embedded in a mineralized matrix.

Fibromodulin is a collagen-binding proteoglycan and is widely distributed in many connective tissues. It has been reported that for each collagen molecule there is at least one fibromodulin binding site. However, these sites are limited in number and are highly specific (Hedbom and Heinegård, 1989). Staining for fibromodulin could be found in similar areas to staining for type I collagen. As type III collagen had a diffuse distribution throughout the tissues, it is difficult to speculate, from this immunohistochemical study whether fibromodulin associates with type III collagen. The characteristic binding of fibromodulin to fibrillar collagens suggests that fibromodulin may play a role in the development and regeneration of periodontal tissues.

Previous studies have revealed that decorin binds to fibrillar collagens and inhibits collagen fibrillogenesis *in vitro* (Vogel *et al*, 1984). Although both fibromodulin and decorin were expressed in periodontal soft connective tissues, they were distributed to different sites within the periodontium. For instance, fibromodulin was localized mainly in the areas close to the palatal aspect of the gingiva, while strong expression of decorin was found in areas closest to the gingival sulcus. These findings are consistent with a previous report that decorin and fibromodulin bind to different sites on fibrillar collagens (Hedbom and Heinegård, 1993). In contrast to decorin, biglycan is very similar to fibromodulin in its primary structure, but it has different functional properties from fibromodulin. It has no effect on binding to fibril-forming collagens and collagen fibrillation (Bianco *et al*, 1990) and hence it was not surprising to find this proteoglycan had a different distribution to fibromodulin in the periodontal connective tissues.

In conclusion, in this study we have demonstrated that fibromodulin is widely distributed in the periodontal connective tissues associating itself with a number of well characterized extracellular matrix components as well as showing altered expression under inflammatory conditions. While this proteoglycan is expressed by a number of different types of cells residing within the periodontal connective tissues, the precise role of this proteolgycan in these tissues and its interactions with particular matrix components awaits further characterization.

Acknowledgements

This work was supported by a grant from the National Health and Medical Research Council of Australia. We thank Dr AHK Plass (Shriner's Hospital for Crippled Children, Tampa University, Tampa, FL, USA), for donating the anti-fibromodulin antibody and Dr LW Fisher, (National Institutes of Health, MD, USA) for providing the anti-biglycan (LF-51), anti-decorin (LF-30), anti-type I collagen (LF-67) and anti-type III collagen (LF-71) antibodies. H. Qian was supported by the China Scholarship Program. Y Xiao was supported by a Fellowship from NHMRC of Australia ID199925 and an Early Career Research Grant from the University of Queensland.

References

Ababneh KT, Hall RC, Embery G (1998). Immunolocalization of glycosaminoglycans in ageing, healthy and periodontally diseased human cementum. *Arch Oral Biol* **43:** 235–246.

Ababneh KT, Hall RC, Embery G (1999). The proteoglycans of human cementum: immunohistochemical localization in healthy, periodontally involved and ageing teeth. *J Periodont Res* **34:** 87–96.

Antonsson P, Hinegård D, Oldberg Å (1991). Posttranslational modifications of fibromodulin. *J Biol Chem* **266:** 16859–16861.

Bartold PM, Narayanan AS (1998). Proteoglycans. In: Bartold PM, Narayanan AS, eds. *Biology of periodontal connective tissues*. Quintessence Press: Chicago, pp. 121–147.

Bianco P, Fisher LW, Young MF, Termine JD, Robey PG (1990). Expression and localization of two small proteoglycans biglycan and decorin in developing human skeletal and non skeletal tissues. *J Histochem Cytochem* **381:** 1549–1563.

Blochberger TC, Vergnes JP, Hempel J, Hassell JR (1992). cDNA to chick lumican (corneal keratan sulfate proteoglycan) reveals homology to the small interstitial proteoglycan gene family and expression in muscle and intestine. *J Biol Chem* **267:** 347–352.

Cheng H, Caterson B, Neame PJ, Lester GE, Yamauchi M (1996). Differential distribution of lumican and fibromodulin in tooth cementum. *Connective Tissue Res* **34:** 87–96.

Font B, Eichenberger D, Rosenberg LM, van der Rest M (1996). Characterization of the interactions of type XII collagen with two small proteoglycans from fetal bovine tendon, decorin and fibromodulin. *Matrix Biol* **15:** 341–348.

Fukushima D, Butzow R, Hidlebrand A, Ruoslahti E (1993). Localization of transforming growth factor beta binding site in betaglycan. Comparison with small extracellular matrix proteoglycans. *J Biol Chem* **268:** 22710–22715.

Häkkinen L, Westermarck J, Kähäri VM, Larjava H (1996). Human granulation-tissue fibroblasts show enhanced proteoglycan gene expression and altered response to TGF-β1. *J Dent Res* **75:** 1767–1778

Hedbom E, Heinegård D (1989). Interaction of a 59-kDa connective tissue matrix protein with collagen I and collagen II. *J Biol Chem* **264:** 6898–6905.

Hedbom E, Heinegård D (1993). Binding of fibromodulin and decorin to separate sites on fibrillar collagens. *J Biol Chem* **268:** 27307–27312.

Hedlund H, Mengarelli-Widholm S, Heinegård D, Reinholt FP, Svensson O (1994). Fibromodulin distribution and association with collagen. *Matrix Biol* **14:** 227–232.

Hildebrand A, Romaris M, Rasmussen LM *et al* (1994). Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. *Biochem J* **302:** 527–534.

Krusius T, Ruoslahti E (1986) Primary structure of an extracellular matrix proteoglycan core protein deduced from cloned cDNA. *Proc Natl Acad Sci USA* **83:** 7683–7687.

Matias MA, Li H, Young WG, Bartold PM (2003). Immunohistochemical localization of fibromodulin in the periodontium during cementogenesis in the rat molar. *J Periodont Res* **38:** 502–507.

Neame PJ, Sandy JD (1994). Cartilage aggrecan. Biosynthesis, degradation and osteoarthritis. *J Fla Med Assoc* **81:** 191–193.

Neame PJ, Choi HU, Rosenberg LC (1989). The primary structure of the core protein of small, leucine-rich proteoglycan (PGI) from bovine articular cartilage. *J Biol Chem* **264:** 8653–8661.

Neame PJ, Sommarin Y, Boynton RE, Heinegård D (1994). The structure of a 38-kD leucine-rich protein (chondroadherin) isolated from bovine cartilage. *J Biol Chem* **269:** 21547–21554.

Noonan DM, Hassell JR (1993). Perlecan. The large low-density proteoglycan of basement membranes: structure and variant forms. *Kidney Int* **43:** 53–60.

Oldberg Å, Antonsson P, Lindblom K, Heinegård D (1989). A collagen-binding 59-kd protein (fibromodulin) is structurally related to the small interstitial proteoglycan PG-S1 (biglycan) and PG-S2 (decorin). *EMBO J* **8:** 2601–2604.

Scott JE (1988). Proteoglycan-fibrillar collagen interactions. *Biochem J* **252:** 313–323.

Venkatesan N, Ebihara T, Roughley PJ, Ludwig MS (2000). Alterations in large and small proteoglycans in bleomycin-induced pulmonary fibrosis in rats. *Am J Respir Crit Care Med* **161:** 2066–2073.

Vogel KG, Paulsson M, Heinegård D (1984). Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. *Biochem J* **223:** 587–597.

Watanabe T, Kubota T (1998). Characterization of fibromodulin isolated from bovine periodontal ligament. *J Periodont Res* **33:** 1–7.

Worapamorn W, Li H, Haase HR, Pujic Z, Girjes AA, Bartold PM (2000). Cell surface proteoglycan expression by human periodontal cells. *Connective Tissue Res* **41:** 57–68.

Yanagishita M (1993). Function of proteoglycans in the extracellular matrix. *Acta Pathol Jpn* **43:** 283–293.

Zimmermann DR, Ruoslahti E (1989). Multiple domains of the large fibroblast proteoglycan, versican. *EMBO J* **8:** 2975–2981.