J Oral Pathol Med (2006) 35: 104–10

www.blackwellmunksgaard.com/jopm

Isolation and characterization of synovial cells from the human temporomandibular joint

H. Nagai, Y. Miyamoto, A. Nakata, S. Hatakeyama, Y. Iwanami, M. Fukuda

Division of Dentistry and Oral Surgery, Akita University Hospital, Akita, Japan

BACKGROUND: The synovial tissues with temporomandibular disorders (TMDs) often show chronic inflammatory changes and the synovial cells participate in the pathogenic processes of TMDs. The synovial membrane is composed of a synovial lining layer and a connective sublining layer. The synovial lining layer is made up of two kinds of cells: macrophage-like type A and fibroblastic type B cells. The aim of this study was to isolate and characterize synovial cells from the human temporomandibular joint (TMJ).

METHODS: Synovial cells were isolated using an explant culture method. Then, we characterized the cultured synovial cells (SGA2 cells) using immunocytochemistry.

RESULTS: SGA2 cells expressed the fibroblastic markers vimentin and prolyl 4-hydroxylase; they also expressed laminin and heat shock protein 27, all of which are markers of type B cells. However, some cells expressed the macrophage marker CD68. These CD68-positive cells simultaneously expressed laminin.

CONCLUSIONS: We isolated and cultured synovial type B cells from the human TMJ, and identified the presence of intermediate type synovial lining cells, having the phenotypic properties of both type A and type B cells, among the synovial lining cells.

J Oral Pathol Med (2006) **35**: 104–10

Keywords: synovial membrane; temporomandibular disorders; temporomandibular joint

Introduction

'Temporomandibular disorders' (TMDs) is a collective term embracing a number of clinical problems that involve the masticatory muscles, the temporomandibular joint (TMJ) and associated structures, or both (1). The most common signs and symptoms of TMD include joint sounds, limited jaw opening, deviations in the movement patterns of the mandible and masticatory muscles and/or TMJ pain. However, the pathophysiology of TMD has not been entirely elucidated. Several arthroscopic studies have demonstrated that synovial tissues in patients with TMD, especially those involved in the associated internal derangement and osteoarthritis, often show chronic inflammatory changes, including capillary hyperemia, increased vascularity, fibrosis, and synovial hyperplasia (2, 3). Biochemical analyses of the TMJ synovial fluids of patients with TMD have revealed that various inflammatory mediators, such as cytokines (4–8), free radicals (9), neuropeptides (10, 11), prostaglandins (12, 13), and matrix degrading enzymes (14, 15), are detectable. Furthermore, these inflammatory mediators have been detected in the synovial tissues of patients with TMD by immunohistochemical analysis (11, 13, 16–21).

The TMJ is a synovial joint, like all other articulating joints in the body and provides diarthrodial articulation between the mandibular condyle and the temporal bone. The synovial membrane covers all the intra-articular structures except the articular cartilage of the eminence and fossa, the mandibular condyle, and the articular disc (22). It is composed of a synovial lining layer and a connective sublining layer, and plays an important role in maintaining normal joint physiology and function (23–25). The functions of this membrane include the production of synovial fluid components, the provision and preservation of a non-adherent low-friction articular surface, and the furnishing of nutrients for the condyle and elimination of their waste products (23–26). Many electron microscopic studies have demonstrated that the synovial lining layer comprises two cell populations, macrophage-like type A cells and fibroblast-like type B cells (26–29). Type B cells synthesize and modify all extracellular matrix (ECM) and synovial fluid components (28), whereas type A cells predominantly eliminate degradation products, including fluid and fine particulate material, from the joint space and presumably from their ECM (29).

In this study, we isolated and cultured synovial cells derived from the human TMJ during joint surgery. We then examined and characterized these cultured synovial cells using immunocytochemistry. In this

Correspondence: Dr Hirokazu Nagai, Division of Dentistry and Oral Surgery, Akita University Hospital, Hondo 1-1-1, Akita 010-8543, Japan. Tel.: +81 18 884 6188. Fax: +81 18 884 6451. E-mail: hnagai@med.akita-u.ac.jp
Accepted for publication August 11, 2005

paper, we provide the first report of establishment of a synovial type B cell strain derived from the human TMJ as well as identification of the intermediate type synovial cells.

Materials and methods

Isolation and culture of synovial cells from the human TMJ

Human synovial tissue was obtained during joint surgery from a patient with condylar fracture (21-year-old woman) who had given her informed consent. Synovial tissue was excised from the retrodiscal tissue. It was washed with phosphate-buffered saline (PBS) in a vortex mixer to remove blood and maintained at 4°C in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St Louis, MO, USA) with penicillin (1000 U/ml), streptomycin (1000 μg/ml) and amphotericin B (2.5 μg/ml) for 60 min. Following antibiotic treatment, synovial tissue was cut and finely minced into 1–2 mm^3 pieces and plated onto 60 mm tissue culture dishes coated with type I collagen (Iwaki glass, Tokyo, Japan). They were incubated in a humidified atmosphere of 5% CO_2, 95% air at 37°C in culture medium which consisted of DMEM supplemented with 15% fetal bovine serum (FBS), penicillin (100 U/ml), streptomycin (100 μg/ml) and amphotericin B (0.25 μg/ml) with medium changes every 2 days. After 4 weeks, the cells grown from the explants had reached semi-confluence, and they were trypsinized and subcultured in culture medium.

Proliferation assay

To assess the proliferation rate of cultured synovial cells, cells were seeded at a concentration of 2×10^4 cells/well in 2 ml of culture medium into six-well plates (Corning, New York, NY, USA), and incubated in a humidified atmosphere of 5% CO_2, 95% air at 37°C. After the indicated days in culture, the cells were harvested using trypsin-ethylenediaminetetraacetic acid (EDTA) solution and the cell number was calculated.

Immunocytochemistry

For immunoperoxidase staining, cells were fixed in 4% paraformaldehyde in PBS for 10 min. After rinsing in PBS, cells were treated for 10 min with 3% H_2O_2 in methanol to inactivate endogenous peroxidases, and then treated with 10% powdered skimmed milk in PBS for 30 min to reduce non-specific background staining. After rinsing with PBS, cells were incubated for 60 min with primary antibodies. The primary antibodies were diluted as follows; mouse anti-vimentin monoclonal antibody (Dako, Copenhagen, Denmark) (30) was diluted at 1:200, mouse anti-prolyl 4-hydroxylase monoclonal antibody (Cosmo Bio, Tokyo, Japan) (31) at 1:200, mouse anti-CD68 monoclonal antibody (Dako) (32) at 1:100, mouse anti-von Willebrand factor monoclonal antibody (Dako) at 1:25, mouse anti-CD31 monoclonal antibody (Dako) at 1:25, mouse anti-CD4 monoclonal antibody (Nichirei, Tokyo, Japan) at 1:1, mouse anti-CD19 monoclonal antibody (Dako) at 1:100, mouse anti-heat shock protein 27 (Hsp27) monoclonal antibody (Wako, Osaka, Japan) (33) at 1:100, rabbit anti-laminin polyclonal antibody (Sigma) (34) at 1:100. After rinsing with PBS, cells were incubated for 30 min with the appropriate secondary antibody. The secondary antibodies used were horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG (Dako) and goat anti-mouse IgG (Dako), each at 1:200 dilution. Peroxidase activity was developed with 0.05% 3,3′-diaminobenzidine and 0.03% H_2O_2, and cells were counterstained with hematoxylin. For control study of the antibodies, the cells were treated with PBS, non-immunized mouse IgG1 (Dako), and normal rabbit IgG (Dako) instead of the primary antibodies and were confirmed to be unstained.

For immunofluorescence staining, cells were fixed in cold 50% acetone/50% methanol for 5 min. After rinsing with PBS, cells were incubated for 60 min with primary antibodies, diluted as described above. After rinsing with PBS, cells were incubated for 30 min with fluorescence conjugated secondary antibodies. Secondary antibodies were diluted as follows; fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG (Cappel, Irvine, CA, USA) was diluted to 1:300, and TRITC (tetra-methylrhodamine isothiocyanate) conjugated swine anti-rabbit IgG (Dako) to 1:100. After rinsing with PBS, cells were observed with a fluorescent microscope (Olympus, Tokyo, Japan). All staining procedures were performed at room temperature. All antibodies were diluted in 2% skimmed milk in PBS.

Results

Isolation and culture of synovial cells from the human TMJ

Synovial cells were isolated using an explant method followed by primary culture. Within 7 days after explantation, outgrowth of cells was observed from synovial tissue explants. Morphologically, outgrowing synovial cells appeared spindle-shaped. After 4 weeks in culture, the cells were semi-confluent and were transferred to new dishes. Of several cell strains, we selected one that consisted of uniformly spindle-shaped cells for further analysis and named it SGA2. They proliferated with a fibroblastic appearance (passage 2, Fig. 1a) and did not show any spontaneous changes in shape even in repeated culture (passage 20, Fig. 1b). SGA2 cells at passage 9 proliferate slowly and the doubling time is about 40 h (Fig. 2). No change in their proliferation rate was observed even at passage 20.

Determination of the origin for cultured synovial cells

To investigate the properties of SGA2 cells, we performed immunocytochemical staining to detect marker proteins. The cells did not express CD31 (Fig. 3a) or von Willebrand factor (Fig. 3b), which are vascular endothelial cell markers, CD4 (Fig. 3c), which is a T cell and monocyte marker, or CD19 (Fig. 3d), which is a

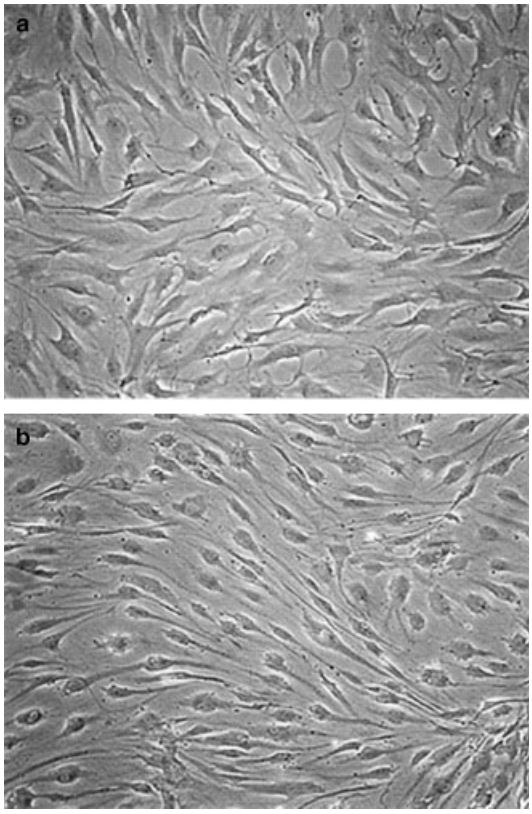

Figure 1 Phase-contrast micrograph of cultured synovial cells (SGA2 cells). The established SGA2 cells show almost exclusively spindle-shaped, fibroblastic morphology. They did not show any changes in shape even after repeated culture. (a) Second passage. (b) 20th passage. Original magnification × 100.

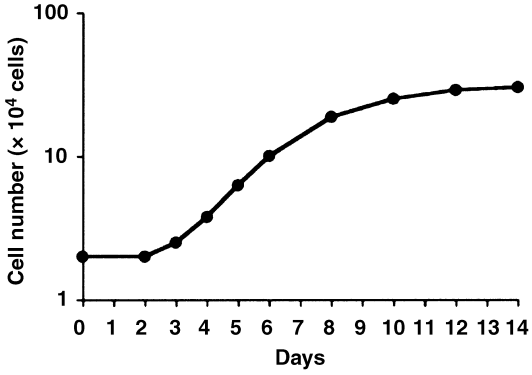

Figure 2 Growth assay. SGA2 cells were seeded at a concentration of 2×10^4 cells/well into six-well plates. After the indicated days in culture, the cell number was calculated. SGA2 cells proliferate slowly and the doubling time is about 40 h.

B-cell marker. All cells were found to express vimentin (Figs 3e and 4a) and prolyl 4-hydroxylase (Figs 3f and 4d), which are fibroblast markers.

To confirm whether SGA2 cells derived from synovial type A or type B cells, we examined their expression of laminin and Hsp27, which are type B-cell markers, and CD68, which is a macrophage (type A cell) marker. They were found to express laminin (Fig. 3g) and Hsp27 (Fig. 3h). A double immunofluoresence study showed that all cells express both laminin (Fig. 4b,e) and fibroblast markers (vimentin; Fig. 4a,c, prolyl 4-hydroxylase; Fig. 4d,f). However, about 20% cells expressed CD68 (Figs 3i and 4g). SGA2 cells with CD68 immunoreactivity simultaneously expressed laminin (Fig. 4h,i).

Discussion

In the present study, we isolated and cultured synovial cells derived from the human TMJ. The cultured synovial cell strain, designated SGA2 cells, showed spindle-shaped, fibroblastic morphology. By immunocytochemical analysis, all cells express vimentin and prolyl 4-hydroxylase, which are fibroblast markers, but do not express von Willebrand factor or CD31, which are vascular endothelial cell markers, CD4, which is a T cell and monocyte marker, or CD19, which is a B-cell marker. These results suggest that SGA2 cells have a fibroblastic phenotype.

Several researchers have isolated synovial cells with a fibroblastic phenotype from the TMJ, and investigated their intervention in the pathophysiology of TMD *in vitro* (35–37). However their origin is not clear, because of the lack of synovial lining cell-specific markers. In the present study, to confirm whether or not SGA2 cells are derived from the synovial lining layer, we examined their expression of Hsp27 and laminin. Heat shock proteins are synthesized by cells in response to various stress conditions and play a major role in protecting cells. Hsp27, a small heat shock protein, is an estrogen-responsive protein that is associated with estrogen receptor function and is phosphorylated and over-expressed in response to oxidative stress, thermal stress, inflammatory cytokines, and retinoic acid. In the murine TMJ, immunoreactivity for Hsp25, a homolog of human Hsp27, was present in type B cells (38, 39). Therefore, we examined the expression of Hsp27 in SGA2 cells. All cells were found to express Hsp27, indicating that they were derived from type B cells.

On the contrary, the synovial membrane has a basement membrane-like structure in synovial lining layer and the synovial lining layer shows an epithelial-like arrangement (23, 40–44). Several immunohistochemical studies have demonstrated that the synovial lining cells produce the constituents of basement membranes, such as laminin and type IV collagen (40–44). In the rat TMJ, Nozawa-Inoue et al. (44) demonstrated that a discontinuous basement membrane-like structure was present around the synovial type B cells, but not type A cells nor fibroblasts of a sublining layer under electron microscopic investigation. For this reason, we consider laminin to be a type B synovial lining cell marker. Our SGA2 cells expressed both laminin and fibroblast markers, suggesting that they were derived from type B cells.

It is generally accepted that the synovial lining layer contains two different cell populations: macrophage-like type A cells and fibroblast-like type B cells (24–29, 40). However, several studies have identified a third type of

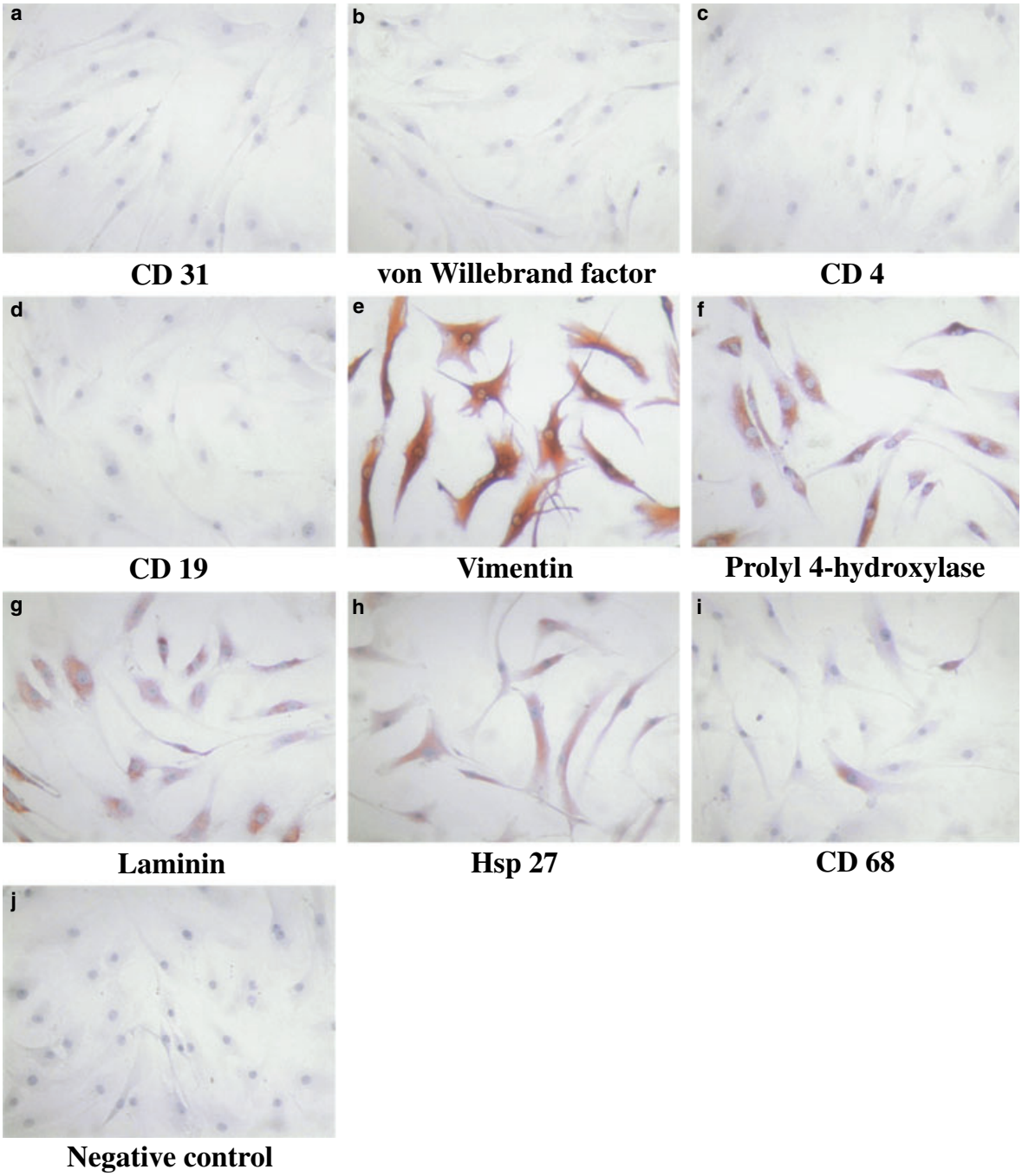

Figure 3 Immunoperoxidase staining of SGA2 cells. CD31 (a) and von Willebrand factor (b), which are vascular endothelial cell markers, CD4 (c), which is a T cell and monocyte marker, and CD19 (d), which is a B-cell marker, showed negative staining. All cells were positive for vimentin (e) and prolyl 4-hydroxylase (f), which are fibroblast markers, and laminin (g) and heat shock protein (Hsp27; h), which are synovial type B-cell markers. Some cells express CD68 (i), which is a macrophage marker. (j) Negative control. Original magnification × 200.

intermediate synovial lining cells (27, 45). It is thought that these three types of synovial lining cells originate from the same cell lineage and become differentiated under the influence of local micro-environmental conditions. Our present study shows that some cells express CD68, which is a macrophage maker, and that CD68-positive cells simultaneously express laminin, indicating that CD68-positive cells share both phenotypic properties of macrophages and type B cells. It has been suggested that CD68-positive cells may be this intermediate cell type, and that SGA2 cells contain intermediate type cells, expressing both type A- and type B-cell markers. Our SGA2 cells may possess interesting characteristics which prove the presence of intermediate type cells.

This is the first report of the isolation and culture of synovial type B cells from the human TMJ, and also the first to demonstrate that intermediate type

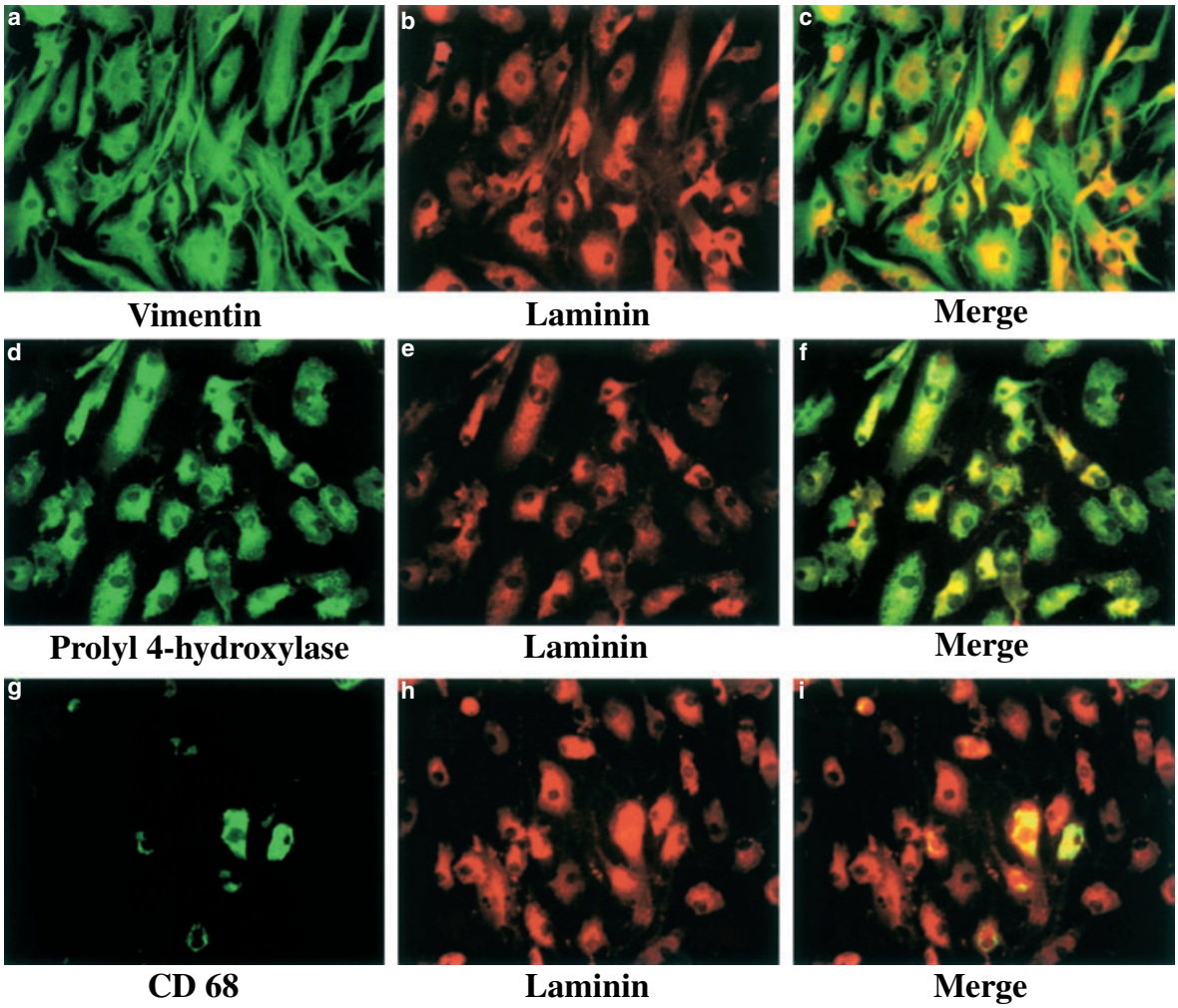

Figure 4 Double immunofluorescence staining of SGA2 cells. Micrographs show the double immunofluorescence detection of vimentin (a, green), prolyl 4-hydroxylase (d, green), CD68 (g, green), laminin (b, e and h, red), with the respective merged images (c, f and i). Original magnification × 200.

cells can be identified as the third type of synovial lining cells. It is clear that the synovial cells play an important role in the onset or progression of TMD, in particular the internal derangement and osteoarthritis, and we believe that our cultured synovial cells provide an important insight into the elucidation of the pathophysiology of the TMD.

References

1. Okeson JP. *Orofacial pain: guidelines for assessment, diagnosis and management*, 3rd edn. Chicago: Quintessence Publishing Co Inc., 1996; 113–58.
2. Holmlund AB, Axelsson S. Temporomandibular arthropathy: correlation between clinical signs and symptoms and arthroscopic findings. *Int J Oral Maxillofac Surg* 1996; **25**: 178–81.
3. Israel HA, Diamond B, Saed-Nejad F, Ratcliffe A. Osteoarthritis and synovitis as major pathoses of the temporomandibular joint: comparison of clinical diagnosis with arthroscopic morphology. *J Oral Maxillofac Surg* 1998; **56**: 1023–7.
4. Sandler NA, Buckley MJ, Cillo JE, Braun TW. Correlation of inflammatory cytokines with arthroscopic findings in patients with temporomandibular joint internal derangements. *J Oral Maxillofac Surg* 1998; **56**: 534–43.
5. Kubota E, Kubota T, Matsumoto J, Shibata T, Murakami KI. Synovial fluid cytokines and proteinases as markers of temporomandibular joint disease. *J Oral Maxillofac Surg* 1998; **56**: 192–8.
6. Shafer DM, Assael L, White LB, Rossomando EF. Tumor necrosis factor-alpha as a biochemical marker of pain and outcome in temporomandibular joints with internal derangements. *J Oral Maxillofac Surg* 1994; **52**: 786–91.
7. Emshoff R, Puffer P, Rudisch A, Gassner R. Temporomandibular joint pain: relationship to internal derangement type, osteoarthrosis, and synovial fluid mediator level of tumor necrosis factor-alpha. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; **90**: 442–9.
8. Nishimura M, Segami N, Kaneyama K, Suzuki T, Miyamaru M. Proinflammatory cytokines and arthroscopic findings of patients with internal derangement and osteoarthritis of the temporomandibular joint. *Br J Oral Maxillofac Surg* 2002; **40**: 68–71.

9. Takahashi T, Kondoh T, Ohtani M, Homma H, Fukuda M. Association between arthroscopic diagnosis of temporomandibular joint osteoarthritis and synovial fluid nitric oxide levels. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999; **88**: 129–36.
10. Holmlund A, Ekblom A, Hansson P, Lind J, Lundeberg T, Theodorsson E. Concentrations of neuropeptides substance P, neurokinin A, calcitonin gene-related peptide, neuropeptide Y and vasoactive intestinal polypeptide in synovial fluid of the human temporomandibular joint. A correlation with symptoms, signs and arthroscopic findings. *Int J Oral Maxillofac Surg* 1991; **20**: 228–31.
11. Suzuki T, Segami N, Nishimura M, Sato J, Nojima T. Bradykinin expression in synovial tissues and synovial fluids obtained from patients with internal derangement of the temporomandibular joint. *Cranio* 2003; **21**: 265–70.
12. Murakami KI, Shibata T, Kubota E, Maeda H. Intraarticular levels of prostaglandin E2, hyaluronic acid, and chondroitin-4 and -6 sulfates in the temporomandibular joint synovial fluid of patients with internal derangement. *J Oral Maxillofac Surg* 1998; **56**: 199–203.
13. Quinn JH, Kent JH, Moise A, Lukiw WJ. Cyclooxygenase-2 in synovial tissue and fluid of dysfunctional temporomandibular joints with Internal derangement. *J Oral Maxillofac Surg* 2000; **58**: 1229–32.
14. Kanyama M, Kuboki T, Kojima S, et al. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids of patients with temporomandibular joint osteoarthritis. *J Orofac Pain* 2000; **14**: 20–30.
15. Ishimaru JI, Oguma Y, Goss AN. Matrix metalloproteinase and tissue inhibitor of metalloproteinase in serum and lavage synovial fluid of patients with temporomandibular joint disorders. *Br J Oral Maxillofac Surg* 2000; **38**: 354–9.
16. Kardel R, Ulfgren AK, Reinholt FP, Holmlund A. Inflammatory cell and cytokine patterns in patients with painful clicking and osteoarthritis in the temporomandibular joint. *Int J Oral Maxillofac Surg* 2003; **32**: 390–6.
17. Suzuki T, Segami N, Kaneyama K, Nishimura M, Nojima T. Specific expression of interleukin-1 beta in temporomandibular joints with internal derangement: correlation with clinical findings. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999; **88**: 413–7.
18. Sato J, Segami N, Nishimura M, et al. Expression of interleukin 6 in synovial tissues in patients with internal derangement of the temporomandibular joint. *Br J Oral Maxillofac Surg* 2003; **41**: 95–101.
19. Nagai H, Kumamoto H, Fukuda M, Takahashi T. Inducible nitric oxide synthase and apoptosis-related factors in the synovial tissues of temporomandibular joints with internal derangement and osteoarthritis. *J Oral Maxillofac Surg* 2003; **61**: 801–7.
20. Homma H, Takahashi T, Seki H, Ohtani M, Kondoh T, Fukuda M. Immunohistochemical localization of inducible nitric oxide synthase in synovial tissue of human temporomandibular joints with internal derangement. *Arch Oral Biol* 2001; **46**: 93–7.
21. Takahashi T, Homma H, Nagai H, et al. Specific expression of inducible nitric oxide synthase in the synovium of the diseased temporomandibular joint. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; **95**: 174–81.
22. Dijkgraaf LC, De Bont LG, Boering G, Liem RS. Structure of the normal synovial membrane of the temporomandibular joint: a review of the literature. *J Oral Maxillofac Surg* 1996; **54**: 332–8.
23. Dijkgraaf LC, De Bont LG, Boering G, Liem RS. Function, biochemistry, and metabolism of the normal synovial membrane of the temporomandibular joint: a review of the literature. *J Oral Maxillofac Surg* 1996; **54**: 95–100.
24. Nozawa-Inoue K, Amizuka N, Ikeda N, Suzuki A, Kawano Y, Maeda T. Synovial membrane in the temporomandibular joint–its morphology, function and development. *Arch Histol Cytol* 2003; **66**: 289–306.
25. Iwanaga T, Shikichi M, Kitamura H, Yanase H, Nozawa-Inoue K. Morphology and functional roles of synoviocytes in the joint. *Arch Histol Cytol* 2000; **63**: 17–31.
26. Graabaek PM. Characteristics of the two types of synoviocytes in rat synovial membrane. An ultrastructural study. *Lab Invest* 1984; **50**: 690–702.
27. Barland P, Novikoff AB, Hamerman D. Electron microscopy of the human synovial membrane. *J Cell Biol* 1962; **14**: 207–20.
28. Wilkinson LS, Pitsillides AA, Worrall JG, Edwards JC. Light microscopic characterization of the fibroblast-like synovial intimal cell (synoviocyte). *Arthritis Rheum* 1992; **35**: 1179–84.
29. Mapp PI, Revell PA. Ultrastructural characterization of macrophages (type A cells) in the synovial lining. *Rheumatol Int* 1988; **8**: 171–6.
30. Osborn M, Debus E, Weber K. Monoclonal antibodies specific for vimentin. *Eur J Cell Biol* 1984; **34**: 137–43.
31. Bai Y, Muragaki Y, Obata K, Iwata K, Ooshima A. Immunological properties of monoclonal antibodies to human and rat prolyl 4-hydroxylase. *J Biochem (Tokyo)* 1986; **99**: 1563–70.
32. Pulford KA, Rigney EM, Micklem KJ, et al. KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. *J Clin Pathol* 1989; **42**: 414–21.
33. Ingolia TD, Craig EA. Four small drosophila heat shock proteins are related to each other and to mammalian alpha-crystallin. *Proc Natl Acad Sci U S A* 1982; **79**: 2360–4.
34. Vater R, Cullen MJ, Harris JB. The expression of vimentin in satellite cells of regenerating skeletal muscle in vivo. *Histochem J* 1994; **26**: 916–28.
35. Ogura N, Tobe M, Sakamaki H, et al. Interleukin-1beta increases RANTES gene expression and production in synovial fibroblasts from human temporomandibular joint. *J Oral Pathol Med* 2004; **33**: 629–33.
36. Tanimoto K, Suzuki A, Ohno S, et al. Effects of TGF-beta on hyaluronan anabolism in fibroblasts derived from the synovial membrane of the rabbit temporomandibular joint. *J Dent Res* 2004; **83**: 40–4.
37. Sambajon VV, Cillo JE Jr, Gassner RJ, Buckley MJ. The effects of mechanical strain on synovial fibroblasts. *J Oral Maxillofac Surg* 2003; **61**: 707–12.
38. Andoh E, Kawano Y, Ajima H, Nozawa-Inoue K, Kohno S, Maeda T. Expression of 25 kDa heat shock protein by synovial type B cells of the mouse temporomandibular joint. *Arch Oral Biol* 2001; **46**: 947–54.
39. Nozawa-Inoue K, Ohshima H, Kawano Y, Yamamoto H, Takagi R, Maeda T. Immunocytochemical demonstration of heat shock protein 25 in the rat temporomandibular joint. *Arch Histol Cytol* 1999; **62**: 483–91.
40. Schneider M, Voss B, Rauterberg J, et al. Basement membrane proteins in synovial membrane: distribution in rheumatoid arthritis and synthesis by fibroblast-like cells. *Clin Rheumatol* 1994; **13**: 90–7.
41. Konttinen YT, Li TF, Xu JW, et al. Expression of laminins and their integrin receptors in different conditions of synovial membrane and synovial membrane-like interface tissue. *Ann Rheum Dis* 1999; **58**: 683–90.

42. Pollock LE, Lalor P, Revell PA. Type IV collagen and laminin in the synovial intimal layer: an immunohistochemical study. *Rheumatol Int* 1990; **9**: 277–80.
43. Dodge GR, Boesler EW, Jimenez SA. Expression of the basement membrane heparan sulfate proteoglycan (perlecan) in human synovium and in cultured human synovial cells. *Lab Invest* 1995; **73**: 649–57.
44. Nozawa-Inoue K, Ajima H, Takagi R, Maeda T. Immunocytochemical demonstration of laminin in the synovial lining layer of the rat temporomandibular joint. *Arch Oral Biol* 1999; **44**: 531–4.
45. Fell HB, Glauert AM, Barratt ME, Green R. The pig synovium. I. The intact synovium in vivo and in organ culture. *J Anat* 1976; **122**: 663–80.

Acknowledgement

This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.