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Expression of the Cell-Surface Heparan Sulfate Proteoglycan mRNA in Monkey Submandibular Gland. E. YAMAGATA, A. KAMADA and T. SAKAKI (Osaka Dental University, Osaka, Japan)

Cell-surface heparan sulfate proteoglycans belong primarily to two families of molecules, syndecans and glypicans, that differ significantly in core protein domain structure. They have been shown to participate in both matrix recognition and growth factor binding and thus may participate in cell regulation. We investigated the mRNA expression of cell-surface heparan sulfate proteoglycans in adult female monkey (*Macaca fascicularis*) submandibular glands using the RT-PCR technique. Agarose gel electrophoresis of the PCR products of the cDNA generated from RNA was carried out to demonstrate the expression of mRNA in syndecan-1, syndecan-2, syndecan-4 and glypican in this study. In order to compare the mRNA expression level among the cell-surface heparan sulfate proteoglycans, we measured changes in the relative intensity of PCR products with increasing thermal cycle number. The results demonstrated that the expression levels were syndecan-4, syndecan-1 and syndecan-2, glypican in descending order. Hence, it was indicated that the control of the expression patterns of the cell-surface proteoglycans may regulate the cellular function and behavior in the submandibular gland. This study was supported in part by a grant (09671913) from the Scientific Research Fund of the Japanese Ministry of Education.

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VAMP2-containing Complex at Secretory Granules in Parotid Acinar Cells. J. YOSHIGAKI*, Y. DOHKE, M. HARA-YOKOYAMA, S. FURUYAMA, H. SUGIYA (Dept. Physiol., Nihon Univ. Sch. of Dentistry at Matsudo, Chiba, Japan):

Amylase release from parotid acinar cells is mainly regulated by accumulation of intracellular cAMP. We previously reported that VAMP2, one of the SNARE proteins is specifically localized at secretory granules in rat parotid acinar cells and has an essential role in cAMP-regulated amylase secretion. We also found that VAMP2 makes complex with some unidentified protein(s) at secretory granules in the resting state. In the present study, we investigated whether the VAMP2-containing complex is pre-existing SNARE complex before priming. Although botulinum neurotoxin B (BNT-B) is a specific protease of VAMP2, VAMP2 in the SNARE complex is known to be resistant to BNT-B. We incubated solubilized granule membrane with activated BNT-B and performed immunoblotting analysis with anti-VAMP2 antibody. As a result, VAMP2 in solubilized granule membrane was efficiently cleaved by BNT-B. This result suggests that the VAMP2-containing complex is not pre-existing SNARE complex. This study was supported in part by a Grant-in-aid for Scientific Research (No. 09771550) from the Ministry of Education, Science and Culture of Japan.

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The relationship between the acid and alkaline phosphatase activity and the adherence of *Candida parapsilosis* to human buccal epithelial cells. *L. P. SAMARANAYAKE,[1] P. H. P. FERNANDO[2] & G. J. PANAGODA[2] ([1] Faculty of Dentistry, University of Hong Kong, Hong Kong, [2] University of Peradeniya, Sri Lanka)

Candida parapsilosis is an emerging fungal pathogen implicated in many diseases, especially in compromised hosts. Candidal colonization and infection depends on their initial ability to adhere to host surfaces, which in turn depends upon the host and the yeast cell wall components and the related biochemical mechanisms. Therefore we examined the potential pathogenic traits of 24 *C. parapsilosis* isolates, from both superficial and deep infections by evaluating and correlating their intracellular phosphatase activity measured with *para*-nitrophenyl phosphate (Onishi et al. *J. Biol. Chem.* 1979; **254**: 11943-52) and their adhesion to human buccal epithelial cells (Samaranayake et al. *J Med Microbiol* 1982; **15**: 511-517) Significant intraspecies differences were seen in both the alkaline and acid phosphatase activity as well as in their adhesion to buccal epithelial cells ($p < 0.0001$). Further the acid phosphatase activity of the superficial isolates was significantly greater (152%) than the systemic isolates ($p = 0.0352$). A highly significant positive correlation was also established between the yeast adhesion to buccal epithelial cells and, both the acid ($r = 0.88$, $p < 0.0001$) and alkaline ($r = 0.9$, $p < 0.0001$) phosphatase activity. These relationships, described for the first time, imply that the alkaline and acid phosphatases of *Candida* species may play a substantive role in potentiating their virulence.

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Oral infectivity of *Candida* species in a healthy and immunocompromised animal model *Y. H. SAMARANAYAKE and L. P. SAMARANAYAKE (Oral Bio-sciences, Faculty of Dentistry, University of Hong Kong, Hong Kong)

Little is known of the pathogenic potential of different *Candida* species in healthy and compromised animal models. Therefore we investigated the oral colonisation and infectivity of *C. albicans* and *C. krusei* in healthy and immunocompromised Sprague-Dawley rats. A total of 15 rats, were allocated into three groups and were fed on a carbohydrate-rich and a tetracycline laced diet. These animals were orally inoculated using standard suspensions of clinical isolates of *Candida*. Fungal colonisation was then assessed by swabbing the full mouth in a standard manner at regular intervals and, infectivity was evaluated by routine histopathologic techniques (Samaranayake et al., *J Med Microbiol* 1994; **41**: 250-8). After a three week period of intermittent oral inoculation of healthy rats, both species demonstrated variable oral colonisation although the load of *C. albicans* was significantly greater ($p<0.05$) than *C. krusei*. Nonetheless, neither species induced candidal infection during this period. Subsequent immunosuppression of the rats, by intramuscular cyclophosphamide initiated *C. albicans* infection of the dorsal lingual mucosa especially around the conical papillae after a few weeks in all animals in this group while similar lesions due to *C. krusei* were seen in only 37% of the latter group. Histological changes resembling mucosal candidosis were observed in the lingual mucosa of rats infected with both *Candida* spp. Further, both species produced fungal hyphae that penetrated the epithelium, although *C. albicans* hyphae tended to be relatively more profuse. In conclusion, these results confirm the clinical observation that *C. krusei*, though an innocuous commensal, is able to transform into an invasive pathogen under immunosuppression. Further, the SD animal model is useful for further studies on the ralative infectivity of different *Candida* species.

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S-1

Biochemical Approach to Synovial Fluid associated with Temporomandibular Joint Disorders
Takanori SHIBATA*[1] and Masao NAGUMO[2](Department of Dentistry and Oral Surgery, School of Medicine, Yamagata University[1],Second Department of Oral & Maxillofacial Surgery,School of Dentistry,Showa University[2],Japan)

Recent improvements in techniques for detecting trace amounts of biologic molecules in small volume of synovial fluid have lead to analysis on various inflammatory and cartilage degradation markers in the diseased temporomandibular joint(TMJ). Then,the first international symposium on TMJ synovial fluid analysis, which was named "Biochemical Changes in Synovial Fluid Associated With Pathology of the Temporomandibular Joint", was held in 75th general session of the International Association for Dental research at Orlando in 1997. After this symposium, in Japan, a lot of newly research groups have grown in this field. They have been focusing and analyzing on various molecules in TMJ synovial fluid , e.g. interleukin 1-β,tumor necrosis factor-α, Interferon-γ, matrix metalloproteinase 1 and 3, tissue inhibitor matrix metalloproteinase 1 and 3 As inflammatory markers , and Chondroitin sulfate, Hyaluronic acid, Keratan sulfate, procollagen II propeptide, pyridinoline and deoxypyrodinoline as cartilage degradation markers.

The purpose of this symposium is to report on the current state of knowledge regarding the biochemical changes in the synovial fluid associated with TMJ disorders, to discuss their role in the joint pathology, to provide data regarding the possibility as molecular markers of disease, to assess the advance of research in this field during the last two years, and to accelerate the advance of research in this field.

S-2

Clinical Assessment for Joint Effusion of the TMJ
K. KOBAYASHI* (Department of Oral Radiology, Tsurumi University School of Dental Medicine, Yokohama, Japan)

The purpose of this presentation was to find out a possible correlation between the MR image of the temporomandibular joint (TMJ) suffering from joint effusion and the existing symptomatic joint pain and internal derangement. In 1992, Westesson and Brooks suggested that the joint effusion was closely asscociated with joint pain. However, joint effusion is considered to correlate other some facters, too. For1129 TMJ's with clinical symptoms of the TMJ disorders, sagittal and coronal T2* and T2 weighted images were obtained. The image finding for joint effusion were correlated with disc position, disc configulation and pain. The cases were classified into three groups by the extent of anterior disc displacement. The TMJ's with anterior disc displacement showed a higher frequency of joint effusion than the TMJ's with the disc normally positioned. ($p<0.05$) Among the joints with the anterior disc displacement without reduction, the biconcave group showed a higher frequency of joint effusion than the disc deformity group. ($p<0.05$ by chi-square test) In conclusion, the MR image evidence for joint effusion in the TMJ was associated with the presence of disc displacement , disc configulation and TMJ pain, for it was unspecific findings.

S-3

Analysis of proinflammatory mediators in synovial fluids of the TMJ.
T. TAKAHASHI* (Division of Dentistry and Oral Surgery, Akita University School of Medicine, Akita, Japan)

Various inflammatory mediators, including arachidonic acid metabolites, cytokines, glucosaminoglycan components, protenases, neuropeptides, and free radicals are found in SF from patients with TMD. In this study, to investigate how these mediators are involved in the pathology of synovitis and cartilaginous degeneration and in clinical features such as pain, a proinflammatory cytokine, IL-1β and a gasseous free radical, nitric oxide (NO), were analyzed using synovial lavage fluid samples (SF) and compared with clinical signs and symptoms as well as arthroscopic findings. The levels of nitrites (NO_2) (>0.22 pmol/L) were measured by chemiluminescence assay and expressed as NO, and IL-1β (>1.5 pg/ml) was assayed by ELISA. Measurable levels of IL-1β were found in SF from patients, not in SF from healthy asymptomatic controls. IL-1β was detected from TMJs with severe synovitis and/or cartilaginous degeneration. Furthermore, significantly higher levels of NO were seen in the patients with TMD. In addition, the levels of NO were higher in painful joints than in pain-free joints. The levels of NO were also well correlated with the degree of cartilaginous degeneration. There was also a positive correlation between the levels of NO and IL-1β. These findings indicate that increased levels of IL-1β, and NO are involved in the pathogenesis of osteoarthritic changes of the TMJ. These findings also suggested that many proinflammatory mediators seems to be involved in TMJ pathosis in a quite complex manner. The analysis of these proinflammatory mediators may shed a light into the clinical management of the TMJ diseases.

S-4

Assay of cartilage metabolite in synovial fluid of TMJ
Kenji KAKUDO* (Second Department of Oral and Maxillofacia Surgery, Osaka Dental University, Japan) :

To clarify the mechanisms of cartilage degradation of temporomandibular joint (TMJ), we investigated the molecular size profile of hyaluronic acid (HA) and the activity of its degradation enzyme, *N*-acetyl -,β-glucosaminidase (NAG), examined in the synovial fluid (SF) collected from patients with internal derangement of TMJ (ID group) or osteoarthritis of TMJ (OA group) diagnosed by MRI and X-ray examination and normal subjects useing by indirect aspiration technique. Secondly, the joint effusion was diagnosed by MRI and matrix metalloproteinase(MMPs) level were assyed by ELISA and its activities were determined by enzymograpy and Western blot analysis in SF samples obtained by direct aspiration technique(Shibata,T. et al:1995). The molecular size of HA differed among the three groups. It was greatest for the normal group, followed by the ID group and the OA group, in that order, reflecting the degree of progression of temporomandibular disorders (TMD). The specific activity of NAG was lowest in the normal group, was greater in the ID group and greatest in the OA group, showing a negative correlation between NAG activity and the molecular size of HA. MMP-3 was detected in all SF samples obtained by direct aspiration technique and its level in ID group was greater than in OA group. In conclusion, the assay of caltilage metabolite in SF of TMJ was useful for diagnosis of TMD.