Proceeding of the 22nd Annual Meeting of The Japanese Society of Oral Pathology
The 5th Meeting of Asian Society of Oral and Maxillofacial Pathology

Meeting Period: August 23-25, 2011
Meeting Venue: Centennial Hall, Kyushu University, School of Medicine, Fukuoka, Japan.
Organizing Committee: 22JSOP Chairperson: Professor Kunihisa Taniguchi,
Division of Pathology,
Department of Morphologidal Biology,
Fukuoka Dental College.
2-15-1 Tamura, Sawara-ku, Fukuoka, Japan
ASOMP Chairperson: Professor Takashi Takata
Graduate School of Biomedical Sciences,
Hiroshima University,
1-2-3 Kasumi, Minami-ku, Hiroshima, Japan

Program
Opening Address: K. Taniguchi (Fukuoka Dental College)
T. Takata (Hiroshima University)

Special Lecture:
Speaker: Y. Oda (Kyushu University)

ASOMP Session
Keynote lecture: A. Yamaguchi (Tokyo Medical and Dental University)
Educational Symposium: H. Maeda (Aichigakun University)
WM. Tilakaratne (University of Peradeniya)
CP. Chiang (National Taiwan University)
R. Zain (University of Malaya)

ASOMP Scientific Session: K. Hida (Hokkaido University)
KW. Chang (National Yang Ming University)
Y. Kudo (Hiroshima University)
SD. Hong (Seoul National University)
Y. Chen (Sichuan University)
YK. Chen (Kaohsiung Medical University)
N. Ishimaru (Tokushima University)

Scientific Symposium
Training Program: Autopsy by oral pathologists
Chairpersons: T. Kiyoshima (Kyushu University), K. Okamura (Fukuoka Dental College)
Speakers: M. Ehara (Asahi University), H. Suzuki (Kyushu University),
A. Ishikawa (Kyushu Dental College), T. Izumo (Saitama Cancer Center)

Public Symposium: Our approach to early detection of oral cancer using smear cytology
by general practitioners in collaboration with oral pathologists
Organizers: Y. Tanaka (Tokyo Dental College), J. Sekine (Shimane University)
Speakers: Y. Tanaka (Tokyo Dental College), H. Ishibashi (Shimane University),
J. Sekine (Shimane University),
K. Kuyama (Nihon University School of Dentistry at Matsudo),
N. Yada (Oita University), K. Takakura (Ichikawa Dental Association)

Case discussion: 8 Titles
Case Report: 1 Title (oral presentation)
16 Titles (poster)

General Session: 7 (oral presentation)
51 Titles (poster)

Closing Address: K. Taniguchi (Fukuoka Dental College)
Abstracts

ASOMP Session

Keynote lecture:
Histopathological characteristics of bisphosphonate-related osteomyelitis (osteonecrosis) of jaw
A. Yamaguchi
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Bisphosphonates (BPs) are potent inhibitors of osteoclastic bone resorption, and are widely used to prevent excess bone resorption in osteoporosis, cancer bone metastases, and multiple myeloma. There have been increasing reports showing that osteonecrosis of jaw infrequently occurred following the surgical treatments such as tooth extraction in patients who have been treated with BPs, which is called bisphosphonate-related osteonecrosis of the jaw (BRONJ).

There have been many reports on BRONJ, but the histopathological characteristics of BRONJ have not been well documented. We histopathologically investigated the characteristics of BRONJ in about 60 cases, which were kindly provided by 22 dental facilities in Japan. Almost cases contained various amounts of sequestrum (osteonecrosis) and many of these cases associated with bacterial growth. The vital bone tissues surrounding the sequestrum showed a typical histology compatible with osteomyelitis. We compared the histology of these areas to that of osteomyelitis patients who received no BP treatment. Basic histopathological findings were similar between these two groups except for osteoclast phenotype. Osteoclasts in BP-treated cases showed larger size and increased number of nuclei compared to those in non-BP-treated cases. BP-treated cases also often showed “floating osteoclast”, which are presumably nonfunctional osteoclasts detached from bone surface. These osteoclast phenotypes are compatible with that observed in bone biopsy specimens of alendronate-treated osteoporosis patients as reported by Weinstein, et al (N Engl J Med 2009).

Our histopathological investigation indicated that BRONJ is a kind of osteomyelitis of jaw associating with modified osteoclast phenotype caused by BP action.

Introduction

H. Maeda
Department of Oral Pathology, School of Dentistry, Aichi-Gakuin University

Oral pathology in Asia; where are we?
WM. Tilakaratne
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Asia is a unique continent in the context of education compared to other parts of the world. The main reason for the above scenario is the economic diversity that we experience in Asia. We have a mixture of countries ranging from a few most developed countries on earth to some of the poorest. There is a significant gap in standards of education in different countries in Asia due to the direct relationship between the level of education and economic status. It is well reflected in the specialty of Oral Pathology with regards to number of reporting Pathologists, standards of diagnostic Pathology, infrastructure facilities and training methodology. It is unfortunate that a few countries in Asia are without a single qualified Oral Pathologist and all diagnostic services are performed by medically qualified Pathologists. It has been identified as a basic need to have at least a few Oral Pathologists to every country in a time that we discuss about standardization and accreditation of Oral Pathology across Asian countries. Therefore, it is the need of the hour to plan and implement a training programme where trainees from those countries without Oral Pathology services would benefit and start the specialty in their countries. That would be the stepping stone to standardization of Oral Pathology services in Asia. In that respect International Association of Oral Pathologists and Asian Society of Oral and Maxillofacial Pathologists should start working together to achieve the said goal. Unless, this is done sooner rather than later, the specialty of Oral Pathology in Asia may not achieve its targets.

Current status of education for the oral pathologist in Taiwan

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The oral pathology training program in Taiwan is a 4-year program that includes 2-year training of general dentistry, 1.75-year training of oral pathology, and 0.25-year training of general pathology. The 2-year training of general dentistry includes a rotation in 9 dental divisions. The trainee has to complete the minimal requirements that are set by each division. During the 3-month training in general pathology, each trainee has to see the whole procedure of 2 autopsy cases and learn how to write the autopsy reports for these 2 cases. In addition, he or she has to learn how to read the hematoxylin and eosin-stained and immunostained histological tissue sections and write or sign out the biopsy reports of at least 500 cases. During the 1.75-year training of oral pathology, each trainee has to examine and sign out at least of 300 oral and maxillofacial specimens. Furthermore, he or she has to attend the surgicopathological slide conference once a week, the clinicopathological conference once a week, and oral and maxillofacial surgery and oral pathology combined conference once a month. After finishing the 4-year oral pathology training program, each trainee is qualified to take the oral pathology specialist examination provided by the Taiwan Academy of Oral Pathology. Th is examination includes four writing tests for basic courses (oral histology and oral pathology), clinical courses (oral diagnosis, oral medicine, and oral and maxillofacial radiology), clinical slides (25-50 cases), and histopathological
slides (15 cases). After passing these 4 tests, the examinee has to pass the oral test to become a board-certified oral pathologist.

**Oral pathology specialty training in Asia - ensuring quality standards through strong partnerships**

RB. Zain¹, YJ. Fei²

¹University of Malaya
²National University of Singapore

Currently in Asia, only a few countries conduct the training of Oral Maxillofacial Pathologists (OMFPs). Curriculum for training has been individually developed by the respective training institutions with most benchmarking against international colleges in United Kingdom, USA and Australia/New Zealand. The move towards training standardization would mean first to have a strong governing body which would consists of special advisory committees for decisions on curriculum, training slots, teaching faculty, examinations and assessments.

A society such as the Asian Society for Oral Maxillofacial Pathology (ASOMP) may function in a similar fashion to the international colleges and a special advisory committee be set up consisting of experienced OMFPs from Asian countries that has such training programmes. The key to success would require such said society (i.e. ASOMP) to re-look at its functions and assesses the achievement of its objectives over the last 4 years while willing to certain changes towards strengthening the society.

There may be a need to start and maintains a foundation to help in the training especially for countries lacking in OMFPs. The base country for the training need to be selected and training may be as a joint efforts (partnerships) on a modular basis with multiple training centres (with sufficient teaching materials) in different countries. A concept of trainees’ rotation to different training centres and the roving teaching faculties whereby, selected centres for the trainees will also have the assistance of selected teaching faculties from other countries on a modular basis.

In the early stage, these suggestions would be more towards the training of OMFPs for countries in Asia without OMFPs. The countries with existing training programmes shall continue for their own country’s specialty requirements with efforts towards the use of similar curriculum. With time, there can be a convergence of all the OMFPs training in Asia towards having an Asian Fellowship Examination as additional Professional Qualifications to their respective academic qualifications.

**ASOMP Scientific Session:**

**A new insights into tumor endothelial cell**

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Tumor endothelial cells have been thought to be genetically normal. However tumor blood vessels have been shown to differ from their normal counterparts, for example, by changes in morphology. To develop novel antiangiogenic therapies, understanding crosstalk between blood vessels and tumor microenvironment is important.

We have reported that TECs are abnormal. TECs showed different gene profiles and angiogenic properties than normal endothelial cells (NECs). In addition, TECs were cytogenetically abnormal (Cancer Res 2004, Am J Pathol 2009) and resistant to paclitaxel or 5-FU. Furthermore, TECs showed stem cell characteristics, consisting with a previous report (Dudley et al. Cancer Cell 2008). The multidrug resistance 1 (MDR1) mRNA which encodes p-glycoprotein (P-gp) was upregulated in TEC compared with NEC. Tumorconditioned medium (tumor CM) induced drug resistance via upreguration of MDR1 gene in NEC (HMVEC).

It was suggested that TEC abnormality might be caused by the factors in tumor microenvironment.

**MicroRNA alterations in oral carcinoma - a linkage to hypoxia regulation**

KW. Chang¹², MM. Tsai¹, SC. Lin¹²

¹School of Dentistry, National Yang-Ming University, Taipei, Taiwan, ²Department of Stomatology, Veterans General Hospital

Oral squamous cell carcinoma (OSCC) is a worldwide disease. MicroRNAs (miRNAs) play important roles in the invasion and metastasis of OSCC cells. However, miRNAs in OSCC tumors is poorly explored. It was suggested that TEC abnormality might be caused by the factors in tumor microenvironment. Tumor endothelial cell abnormality might be caused by the factors in tumor microenvironment.

**Identificcation of novel invasion-related molecules in oral cancer**

Y. Kudo, T. Takata

Department of Oral and Maxillofacial Pathobiology, Graduate School of Biomedical Sciences, Hiroshima University

Recent cumulative evidences show that microRNAs (miRNAs) play important roles in the invasion and metastasis of various cancers. miRNA is consist of 21-24 nucleotides, bind to the 3’-UTR of specific target mRNA and suppresses translation or induces degradation of the target mRNA. Among them, miR200 family is well examined and is
involved in tumor progression via epithelial-mesenchymal transition (EMT). In fact, miR200 family regulates E-cadherin transcriptional repressor, ZEB1 and ZEB2. We previously established highly invasive clone (MSCC-Inv1) from parent OSCC cells (MSCC-1) by in vitro invasion assay. Interestingly, highly invasive clone showed EMT feature such as loss of E-cadherin. By comparing the gene expression profiles of these cells, we previously could identify several invasion-related molecules such as peristin, IFITM1 and Wnt-5b. Here we compared the gene expression profile of miRNAs between MSCC-1 and MSCC-Inv1 by microarray. Interestingly, several miRNAs including miR-200 family were downregulated in MSCC-Inv1 cells. We focused on some down-regulated miRNAs as a candidate of invasion related molecule in cancer. Now we are examining the detailed role of these miRNA in the invasion of cancer cells. In this symposium, we will show our recent findings of invasion-related molecules in oral cancer.

**The role of Cripto-1 in the tumorigenesis and progression of oral squamous cell carcinoma**

SD. Hong

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Oral squamous cell carcinoma (OSCC), the most common malignancy of the oral cavity, remains a lethal disease in over 50% of cases diagnosed annually, due mostly to late detection of this cancer in its advanced stages despite the easy accessibility of the oral cavity for regular examinations. Cripto-1 is a member of the epidermal growth factor (EGF) - CFC protein family and is involved in the activation of several different signaling pathways during embryonic development and cellular transformation. Although the Cripto-1 protein is overexpressed in several human cancers including breast, colon, cervix, gastric, and pancreatic cancer, no prior study has evaluated Cripto-1 expression in OSCC. Therefore, our aims in this study were to examine Cripto-1 expression in clinical samples of OSCC patients using immunohistochemistry, to analyze the correlation between Cripto-1 expression and clinicopathologic parameters, and to identify the oncocgenic roles of Cripto-1 in OSCC cell lines. Both epithelial dysplasia (73.3%) and OSCC (55.5%) tissue samples showed significantly higher expression of Cripto-1 than normal mucosa (20%) (p = 0.031). In the OSCC samples, there was a significant correlation between Cripto-1 expression and the histological differentiation of OSCC (p= 0.015) and a high PCNA index (p= 0.011). The in vitro cell proliferation assays demonstrated that recombinant human Cripto-1 (rhCripto-1) induced both SCC-4 and SCC-25 cells to proliferate as compared with control cells (p=0.05 and p=0.01, respectively). In in vitro migration assays, treatment of SCC-4 and SCC-25 cells with rhCripto-1 protein induced a 2.4-fold and 1.7-fold increase in cell migration, respectively (p=0.000 and p=0.008, respectively). Taken together, our data suggest that Cripto-1 plays a role in the malignant transformation of the oral mucosa and is involved in the tumorigenesis and progression of OSCC by promoting the growth and migration of malignant cells.

**Aberrant Wnt-1/beta-catenin signaling and WIF-1 deficiency are important events in salivary gland adenoid cystic carcinoma progression**

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Objective: In this study we investigated Wnt-1, beta-Catenin, E-cadherin and WIF-1 expressions in formalin fixed paraffin embedding samples of salivary glands adenoid cystic carcinoma(SACC) and SACC cell lines. We disclosed the potential relationship of Wnt-1/beta-catenin pathway, WIF-1 and SACC biological behaviors.

Methods: Expressions of Wnt-1, beta-Catenin, E-cadherin and WIF-1 in human SACC cell lines, ACC-2, ACC-M and T-ACC-M were detected by RT-PCR and western blot. T-ACC-M was collected by Transwell invasion chambers coated with Matrigel and owned high metastasis ability. We also evaluated these factors in SACC tissue microarray by immunohistochemistry. The data were analyzed using SPSS10.

Results: Wnt-1 overexpression was a frequent event in SACC and hints a close relationship between Wnt-1 and tumour cell which have a high invasion and metastasis activity. Beta-catenin stability in cytoplasm and translocation into cell nuclei activated canonical Wnt signaling pathway and implicated high invasion and poor prognosis of SACC. WIF-1 deficiency or down-regulation indicated high metastasis and high recurrence and poor prognosis of SACC. There was a strong correlation between Wnt-1/beta-catenin signaling pathway activation and WIF-1 deficiency. E-cadherin expression was resembled beta-Catenin pattern, which means the dysregulation of E-cadherin-beta-Catenin complex may result in loss of intercellular adhesion, and possible consequently induce tumour cell invasion and tumour progression.

Conclusions: This study indicated that alterations of the correlative components in canonical Wnt pathway might contribute to SACC invasion, metastasis and poor prognosis. Furthermore our findings reinforced the therapeutic potential of inhibiting Wnt signaling pathway via such strategies as reversing WIF-1 inactivation.

Key Words: adenoid cystic carcinoma; Wnt-1; beta-catenin

**Study on different cryopreservations and hepato- genic differentiation of human dental pulp stem cells**

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This study is firstly aimed to determine if human dental pulp stem cells (hDPSCs) can be derived from a diseased but...
vital tooth after cryopreservation of the intact teeth and the isolated dental pulp tissue respectively. It is also intended to demonstrate that the hDPSCs derived from cryopreserved dental pulp tissues are able to differentiate into hepatocyte-like cells.

Materials: Fifty tooth samples were divided into: Group A (n=20) - Freshly derived dental pulp tissues; Group B (n=20) - Liquid nitrogen (LN2) stored dental pulp tissues; Group C (n=10) - LN2 stored intact teeth; and Group D - Hepatogenic differentiation (6 samples randomly selected each from Groups A & B).

Methods & Results: The male to female ratio of all the 50 tooth samples was 1:1.78 with an average age to be 25.5 year-old ranging from 6 to 74 year-old. Groups A and B had a 100% successful rate on hDPSCs isolation respectively whilst Group C had only a 20% successful rate. Groups A to C demonstrated selfrenewal properties and similar multipotent potential characteristics of adipogenic, chondrogenic, and osteogenic differentiation. Th ere was no statistical significance on growth rate analyzed by MANOVA with the whole model. Additionally, hDPSCs showed high expression for bone marrow mesenchymal stem cell markers (CD29, CD90 & CD105) and very low expression of specific hematopoietic cells markers (CD14, CD34 & CD45). All the 12 isolated hDPSCs for Group D showed hepatogenic differentiation and morphology changing from spindle into polygonal shape. The expression of differentiated hDPSCs for the hepatic metabolic function genes (αFP, Alb, CK-18, TO, G-6P) and liver-specific genes (C/EBPα, HNF1α and CYP1A1) were all positive for the differentiated hDPSCs. Glycogen storage in the differentiated hDPSCs cells was also demonstrated. Positive immunofluorescence staining of low density lipoprotein and albumin were observed commencing from the 14th day of differentiation medium culturing. Urea concentration in the medium was noted from the 6th week.

Conclusion: Th e isolated hDPSCs can be stored in LN2 for future potential usage and their hepatogenic differentiation properties would provide a potential source for autogenic transplantation.

Gender-based molecular pathogenesis of Sjögren’s syndrome
N. Ishimaru

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Sjögren’s syndrome (SS) is one of autoimmune diseases targeting lacrimal and salivary glands. Although SS is known to develop in postmenopausal women, the mechanisms by which estrogen deficiency influences autoimmunity remain unclear. It has been suggested that estrogenic action is responsible for the strong female preponderance of autoimmune diseases including SS. Recently, we found that tissue-specific apoptosis in the salivary and lacrimal glands spontaneously occurring in estrogen-deficient mice may contribute to the development of autoimmune exocrinopathy through one of cell cycle-related molecules, retinoblastoma associated protein (RbAp48). We reported that transgenic (TG) expression of RbAp48 resulted in the development of autoimmune exocrinopathy resembling SS, in which salivary and lacrimal epithelial cells produced IFN-γ and IL-18. In addition, RbAp48-expressing epithelial cells together with IFN-γ and IL-18 were observed in the salivary gland tissues of SS patients, but not controls. These results indicate a novel immunocompetent role of epithelial cells, resulting in loss of local tolerance before developing gender-based autoimmunity. Moreover, analyzing the expressions of these molecules may help diagnosing for SS or establishing a better therapeutic strategy for autoimmunity.
Toxoplasma encephalitis presenting clinically as a suspected relapse of malignant lymphoma involving the central nervous system: an autopsy case

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Case:
We present the case of a 66-year-old male scheduled for autopsy. In January 2004, the patient demonstrated symptoms of hemophagocytosis and was diagnosed with non-Hodgkin’s lymphoma following clinical examination. Temporary recovery ensued after chemotherapy with the CHOP and RICE regimens. However, in January 2006, following symptoms which pointed towards a relapse involving the central nervous system (CNS), auto-peripheral blood stem cell transfusion was performed. Despite maintenance therapy with methotrexate (MTX) and cytarabine, the CNS lesion recurred in April 2007; high-dose MTX therapy was administered from May 2007, but proved ineffective. Subsequently, breathing and consciousness levels deteriorated and the patient died in July 2007.

Autopsy findings:
Macroscopically, slight edema was observed in the brain and the sulci were not clearly demarcated. In the coronal section, a brown hemorrhagic lesion measuring 6 cm x 3 cm extended from the left basal nuclei to the radiation of the corpus callosum and the right basal nuclei. Moreover, multiple identical lesions (2 cm) were seen involving the frontal, parietal, temporal, and occipital cerebral lobes, cerebellar cortex, and dentate nucleus. Histologically, these lesions exhibited extensive necrosis with bleeding, fibrin deposition, edema, multiple macrophage aggregations, mild-moderate lymphoid infiltration, and mild neutrophilic infiltration. Mild gliosis was observed around the necrotic areas. In the midbrain, pons, medulla oblongata, and cervical spinal cord, small disseminated necrotic foci of up to 2 mm in size were observed with edema and mild lymphoid infiltration. Extensive invasion by Toxoplasma gondii was detected on the basis of cyst formation and dissemination of the lesions in the cerebrum, cerebellum, midbrain, pons, medulla oblongata, and cervical spinal cord. However, lymphoma cells were not observed in autopsy specimens. Immunohistochemically, anti-toxoplasma antibody positivity was identified, which indicated Toxoplasma gondii infection.

Comments:
Following chemotherapy for malignant lymphoma, relapse involving the CNS was clinically suspected from the results of MRI examination. Extensive encephalitis associated with toxoplasmosis was confirmed at autopsy. This infection presumably occurred due to an immunocompromised status following chemotherapy. The widespread presence of Toxoplasma gondii with extensive necrosis was observed in the cerebrum, cerebellum, midbrain, pons, medulla oblongata, and cervical spinal cord. Invasion of malignant lymphoma cells, which was clinically suspected, was not observed on autopsy. Moreover, although extensive MTX therapy was administered, disseminated necrotizing leukoencephalopathy was not observed. The cerebral lesions were localized, and since laminar cortical necrosis was not observed, it was difficult to envisage that septic shock was involved in the pathogenesis. Although examination of the chest and abdomen was not permitted at autopsy, on the basis of histological findings, it was presumed that toxoplasma encephalitis was the main cause of death.

The knowledge of pathological autopsy report.

T. Izumo
Department of Pathology, Saitama Cancer Center

Public Symposium: Our approach to early detection of oral cancer using smear cytology by general practitioners in collaboration with oral pathologists

Keynote lecture
Y. Tanaka
Tokyo Dental College Ichikawa General Hospital

Clinical application of cytology
H. Ishibashi, J. Sekine
Shimane University Faculty of Medicine

Mass screening of oral cancer
K. Kuyama
Nihon University School of Dentistry at Matsudo

Advantage and disadvantage of oral cytology
N. Yada
Faculty of Medicine, Oita University

Oral cancer detecting system Ichikawa network
K. Takakura
Ichikawa Dental Association

Case Discussion

DO-1. 1A-04. A case of adenoid cystic carcinoma partially-observed growth of myoepithelial cells
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²Department of Biology, Tokyo Dental College

We report a case of adenoid cystic carcinoma with
partially myoepithelial cells growth in the palate. The patient was a 69-years-old man who complained painless swelling of palate. CT and MRI showed salivary gland tumor-like lesion. The surgical resected specimen showed the cribriform pattern which was positive for PAS-Alcian blue and diagnosis was adenoid cystic carcinoma. The superficial part was observed the spindle-shaped myoepithelial cell which stained for α-SMA, HHF35 and p63. These staining characteristics suggest superficial part is different from adenoid cystic carcinoma or cartinoma ex pleomorphic adenoma.

D-1. 1A-05. A case of the tumor in the left inferolateral margin of the tongue
K. Yamanegi1, H. Ohyama1, M. Wato2, K. Tominaga2, M. Kokubu2,3, H. Kato2,3, K. Masuno2, T. Nishikawa2, A. Tanaka2
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3Graduate School of Dentistry, Osaka Dental University

The patient was a 63-year-old Japanese man who was treated with chemotherapy and radiation therapy for squamous cell carcinoma of the left tongue in 2009. One year later, he complained of a rapidly increasing mass without pain in the left inferolateral margin of the tongue. Histopathological examination of the biopsy showed a predominant edematous change with some spindle-shaped cells indicating atypia. The tumor excision with left radical neck dissection was performed. In addition, immunohistochemical staining revealed that tumor cells were positive for vimentin and α-SMA, but not for AE1/AE3.

D-2. 1A-06. Soft tissue tumour of the lower lip
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A 69-year-old woman had a painless solitary mass of the left lower lip for 4 years. The patient had neither trauma associated with the lesion nor medications for the lesion. She noticed an increase in the size of the mass. So she was referred to the Department of Oral and Maxillofacial Surgery, Kyushu University Hospital, for detailed examinations. The lesion showed a firm, non-ulcerated, and movable mass with 10 mm in size that is located in the submucosa of the left lower lip. No sensory or motor paralysis was observed in the lower lip.

D-3. 1A-07. Maxillary tumor
N. Katase1, Y. Takebe1, Y. Hirata1, S. Ito1, R. Tamamura1, T. Mano2, Y. Ueyama2, H. Nagatsu1
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2Department of Oral and Maxillofacial Surgery, Yamaguchi University Graduate School of Medicine

A 72-years-old female patient referred to the hospital, complaining of bleeding from tumor mass at the left upper gingiva. The lesion was 42 x 32 mm in size with ulcer. With the clinical diagnosis of malignant tumor, surgical resection of the tumor was performed. Histologically, the tumor cells consisted from spindle cells with round-edged nucleus. The tumor cells proliferate with bundle formation and exhibit bone invasion. However, the tumor cells lack in cellular atypia. Immunohistochemistry revealed that the tumor cells were focally positive for EMA, Vimentin and some CK markers, but negative for S-100, CD34, CD99 and myogenic markers.

D-4. 1A-08. Tumor of mandible
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2Oral Pathology Section, Department of Surgical Pathology, Niigata University Hospital
3Division of Oral and Maxillofacial Surgery, Niigata University Graduate School of Medical and Dental Sciences
4Department of Oral Surgery, Keinan General Hospital

A 31-year-old woman with familial adenomatous polyposis (FAP) was referred to our hospital for further examinations. Radiologically, a well-demarcated round tumor, measuring 10 mm in diameter, was found in her right molar region of the mandible. The resected tumor showed solid and cystic areas with tan color surrounded by thick capsule. Histopathologically, the tumor was composed of two different components which were connected from each other: one was anastomosing nests consisted of columnar cells at the periphery and spindle/stellate cells in the center, and the other was solid nests with whirled cellular arrangement with gland-like structures and hyaline droplets.

D-5. 2A-04. A case of parotid gland tumor
M. Urano, Y. Mizoguchi, M. Kuroda
Department of Diagnostic Pathology, Fujita Health University, School of Medicine

A 89-year old male noticed a swelling for several years on his left parotid region. He had no facial nerve paralysis. Adenoid cystic carcinoma was suspected on fine needle aspiration cytology.

On the gross finding, well demarcated gray whitish tumor, 2cm in diameter was observed. Histologically, the lesion mainly showed solid and trabecular pattern consisted of non-luminal clear cells which had oval to polygonal shape.
nuclei and small nucleoli. A few glands intermingled with solid tumor nests. On the periphery of the lesion, the tumor invaded surrounding tissue. A small focus of nodular epithelial-myoeipithelial carcinoma-like lesion was seen.

D-6. 2A-05. A case of palatal tumor
Department of Pathology, Nihon University School of Dentistry

The patient was 48-year-old Japanese male. He has had a palatal swelling for 2 years. In first visit, 5x 5mm, non-tender mass was noted on the palate. Histologically, the excised tumor was circumscribed with fibrous capsule and composed of papillary, ductal and solid epithelial structures. The papillary structures are continued with covering epithelium. The inner cells of double-layered ductal structures were positive for cytokeratin 8, cytokeratin 18 and epithelial membrane antigen. Outer cells and solid areas were positive for S-100 protein and vimentin. Alpha-smooth muscle actin was negative for any tumor cells. Few Ki-67 positive cells are dispersed.

D-7. 2A-06. Palatal tumor
I. Ogawa1, K. Kushitani2, T. Sakamoto3, M. Miyachi4, K. Inai2, T. Takata4
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2Department of Pathology, Graduate School of Biomedical Sciences, Hiroshima University
3Oral and Maxillofacial Surgery, Matsuda Hospital
4Department of Oral and Maxillofacial Pathobiology, Graduate School of Biomedical Sciences, Hiroshima University

A 73-year-old male had been pointed out a swelling in right side of the palate about half a year before. Physical examination revealed a solid tumor, 15mm in diameter, covered by normal mucosa. Histopathologically, the tumor is generally well-demarcated, but infiltrates into the salivary gland in some areas. The tumor consisted of solid or trabecular proliferation of polyhedral or short-spindle cells with myxoid substances. Small lumina were sparsely present and mucous cells distributed throughout the tumor. Nuclear atypia was absent and proliferative activity was low. The tumor cells expressed cytokeratin, p63, S-100 protein, GFAP and α-SMA with various degrees.

D-8. 2A-07. A case of odontogenic tumor
M. Nagayama, Y. Sakano, M. Ehara, J. Tanuma
Department of Oral Pathology, Asahi University School of Dentistry

An 11-year-old girl was referred to our hospital for evaluation of a delay of right mandible canine tooth. Radiographically, the lesion sized 10mmx5mm was located in the alveolus showing irregular radiopaque findings and connected to the impacted canine tooth through the radiolucent material. Histologically, the removal specimen revealed the mass growth showing columnar to cuboidal and reticular shaped odontogenic epithelium with keratinized ghost cells. Beneath these tumor cells, immature enamel, dentin with dentinal tube and dysplastic dentin without dentinal tube were also seen. Impacted canine tooth was surrounded with normal dental follicle connecting to the above lesion.

Case Report

C-1. P-05. Ameloblastic carcinoma of the maxilla.
A. Erkhembaatari1,2, K. Kubo1,2, Y. Sugita1, E. Sato1, W. Yoshida1, M. Takayama1, M. Jinno1, Y. Honda1, H. Maeda1,3
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2Maternal And Child Health Research Center
3Aichi Gakuin University Dental Hospital

Ameloblastic carcinoma (AC) is a rare lesion of odontogenic origin. It is defined as a malignant epithelial odontogenic tumor that histologically has retained the features of ameloblastic differentiation and also exhibits cytologic features of malignancy.

A 45-year-old woman presented at Aichi Gakuin University Dental Hospital, complaining of a persistent swelling in her upper right jaw for approximately 1 month. Histopathologically, it included basal cells that were columnar, hyperchromatic, and arranged in a palisaded fashion. In some areas, however, peripheral palisading and polarization were not always evident. Classic stellate reticulum-like areas were almost completely absent, or were present in only small amounts. In the central part of islands, squamous metaplasia were observed. In some areas, the epithelial component exhibited a cytologic malignancy characterized by an increased nucleus-to-cytoplasm ratio and nuclear pleomorphism. In addition, perineural invasion was clearly visible. The immunohistochemical profile of the present case was positive for CK5, 14, 18, 19, Ki67, PCNA, P-63, P-53, vimentin, syndecan-1, calretinin, EMA, GFAP and CB1, but negative for CK13, αSMA, podoplanin and HPV. These features were consistent with a diagnosis of ameloblastic carcinoma.

C-2. P-06. Ameloblastoma, - a case report and treatment conditions in Laos.
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There are three major hospitals in Vientiane, Laos. Generally, the Mahosot Hospital focuses on tumor of the digestive system, the Setthathilath Hospital focuses on tumor of the breast and uterus. The Mittaphap Hospital focuses on the cases of Oro-facial tumors. All of them are cooperating each other about the patient care. In 2008,
Minister of Ministry of Health developed the Lao National Cancer Center at Mittaphap hospital. The Head & Neck Surgery division collected 237 cases of Oro-facial tumors from 2004-2010, including 36 cases (15.18%) of Ameloblastoma, 16 females and 20 males with an age range of 11-60 years, with a mean age at presentation of 31(±3.8) years, the patients received following treatment; Tumor Enucleation, Resection interrup-trice or Hemi-mandibu-loectomy.

Case report;
A 20-years-old male visited to the local doctor with complain of mandible swelling in right angle of the chin. The patient was referred to the Head & Neck Surgery Division of Mittaphap Hospital for further examination. The patient did not complain of pain. On the X-ray examination, a cyst-like radiolucent lesion was recognized in the right mandible. The marked root absorption was identified on the 31-33 and 41-48. The patient received a hemimandibularectomy. The patient revealed good prognosis with no symptoms.

Conclusion:
Most patients with Ameloblastoma admitted to the hospital in the advanced stage of disease. They were received hemimandibularectomy, and then they have lost a chewing function after the surgery. Thus, it is important that patients receive a diagnosis and treatment in early stage of ameloblastoma.

Y. Sato
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Adenoid cystic carcinoma (AdCC) is the second common malignant tumor involving both the major and minor salivary glands. High-grade transformation (HGT) in AdCC is a recently recognized entity. We report three cases of AdCC with HGT arising in the parotid glands and hard palate. The patients’ ages (3 male) ranged from 31 to 74 years (median, 57 y). The tumor was composed predominantly of high grade component with various amount of conventional AdCC. Immunohistochemical study of the high grade component as compared to conventional AdCC showed greater over-expression of p53.

C-4. P-32. A case of metachronous carcinoma ex pleomorphic adenoma arising from the buccal minor salivary gland and the contralateral parotid gland
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We report a case of rare metachronous carcinoma ex pleomorphic adenoma (Ca-ex-PA). In 1997, a 71-year-old woman was referred to Nagasaki University Hospital because of a painless swelling of the right buccal mucosa. Biopsy indicated PA, but the removed tumor included PA as well as myoepithelial carcinoma. In 2003, a slowly growing mass of the left parotid gland was detected. The lesion was composed of myoepithelial carcinoma. Histopathological diagnosis of Ca-ex-PA was made because PA was intermingled. Recurrent tumor in the parotid gland was found in 2006, and histologically determined as recurrent Ca-ex-PA including myoepithelial carcinoma and squamous cell carcinoma.

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Mucoepidermoid carcinoma is the most common malignant tumor of the salivary glands and has a wide histomorphologic spectrum. Three examples were selected from presenter’s own consultation files. The first case was dedifferentiated mucoepidermoid carcinoma mainly consisted of bizarre pleomorphic round cells with clear cell foci scattering intermingled. The second was a high-grade tumor with sarcomatoid foci, which presented solid nests with comedo-necrosis and cellular “stroma” including rhabdoid cells. The last was a tumor histologically consistent with sclerosing mucoepidermoid carcinoma with eosinophilia rarely found in the thyroid gland. The detail of each case would be described and diagnostic problems discussed.

C-06. P-34. Mucoepidermoid carcinoma of the sublingual gland with mandibular invasion: a case report
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Mucoepidermoid carcinoma (MEC) of the sublingual...
glands is a rare entity. We report the case of a 48-year-old woman with MEC of the left sublingual gland, accompanied by invasion into the mandible. Histopathological examination of the resected specimens revealed nests of squamous epithelial cells with peripheral palisading that had proliferated in a solid cord pattern. In addition, mucin staining revealed a few ductal structures and mucous cells. Most tumor cells demonstrated p63 positivity on immunohistochemical examination; however, the samples were negative for myoepithelial markers. A good response to treatment was observed in the five-month postoperative follow-up period.

C-07. P-36. A case of leiomyoma arising in the lingual edge
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Leiomyoma is the benign neoplasm derived from smooth muscle tissue and usually affects uterus, gastrointestinal tracts and skin, whereas oral leiomyoma is unusual entity with less than 150 reported cases among the English literature. In addition to the histological study, the diagnosis of leiomyoma requires immunohistochemical examination to show smooth muscle diff entention. We describe a rare case of leiomyoma arising in the lingual edge of 25-year-old female. The tumor was encapsulated mass measuring 18x15x7mm and consisted of bland-looking spindle cells with myxoid stroma. In immunohistochemical study, tumor cells were positive for α-SMA and desmin, confi rming the diagnosis of leiomyoma.

C-08. P-37. A case of myxoid tumor of the lower lip
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A 68-year-old female patient had noticed swelling at the lower lip. Clinical examination showed a soft mass located in the lower lip, measuring 10mm×12mm. The lesion was well demarcated, tender to palpation, and covered by normal tissue. The clinical diagnosis of a tumor, the mass was surgically removed. Histologically, the lesion showed to be composed of spindle and stellate cells in a myxomatous substance. Some area of the lesion at the periphery was composed of some adipocytes. Tumor cells were positively stained for S-100 protein, vimentin, and CD34, and negatively for pankeratin, CD68, desmin, NSE and α-SMA.

C-09. P-39. A case of large cell neuroendocrine carcinoma of the tongue
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Neuroendocrine carcinoma (NEC) is a rare neoplasm that may occur in various organs. Large cell neuroendocrine carcinoma (LCNEC) is a subtype of NEC. In oral cavity, LCNEC is extremely rare. We report a case of LCNEC of the tongue. A man of 60’s admitted to our hospital with the tumor of the tongue. The patient underwent surgical resection after neoadjuvant chemotherapy. Histological examination revealed the tumor was composed of atypical cells forming nests and cords with necrosis, rosette formation, and peripheral palisading pattern. Immunohistochemically, tumor cells were positive for AE1/AE3, chromograninA and synaptophysin. The tumor was diagnosed as LCNEC.

C-10. P-40. Pilomatricoma with extensive ossifi cation mimicking a salivary stone in a panoramic photograph: a case report
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Although calcification is common in pilomatricoma, it is rare that all tumors show ossification. We report a case of pilomatricoma with extensive ossification in a 56-year-old woman. The tumor was observed in the subcutaneous part of her right cheek with skin pigmentation. In a panoramic photograph, the tumor appeared similar to a salivary stone. Histopathologically, the excised specimen showed that the tumor consisted of cancellous bone with fi bro-fatty bone marrow. Some tumor cells, such as shadow cells with a few basaloid cells, were observed over the underlying trabecular bone. The one-month post-operative follow-up showed good response to treatment.

C-11. P-42. A case of maxillary gingival ulcer in relation to the Antirheumatic drug (MTX)
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Although methotrexate (MTX) is used in several medical specialties including dermatology, rheumatology, and oncology, drug-induced mucocutaneous ulcerations rarely are reported. We present oral event related to dose methotrexate in RA patient. A case is 80-year-old male with RA who take MTX and prednisolone. He revealed ulcer of
induces impairments in oral mucosa. Symptoms of bone-marrow suppression. MTX therapy ulceration of oral mucosa may began with prodromal ltrate in subepithelial area. In this case, we speculated that lymphocyte, plasma cell and eosinocyte become highly infi
ulcerations on his disappearanced oral epithelium area and the left maxillary gingiva. Histopathologically, erosions and ulcerations on his disappeared oral epithelium area and lymphocyte, plasma cell and eosinocyte become highly infiltrate in subepithelial area. In this case, we speculated that ulceration of oral mucosa may began with prodromal symptoms of bone-marrow suppression. MTX therapy induces impairments in oral mucosa.

C-12. P-43. A case of primary oral sporadic-Burkitt’s lymphoma in an adult female
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We report a case of sporadic-Burkitt’s lymphoma of the oral cavity in a 61-year-old Japanese female. She was aware of paresthesia and dull pain in the lower lip and mandibular posterior tooth. Rapid swelling of left face with trismus was noted. CT showed a mass lesion in masseter and medial pterygoid muscle area. Histopathologically, tumor cells were medium-sized lymphoid cells and showed a diff use monotonous pattern with “starry sky” appearance. Immuno-

C-13. P-50. Strawberry gingivitis as the fi rst presenting sign of Wegener’s granulomatosis: Report of a case
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7Hard Tissue Pathology Unit, Matsumoto Dental University Graduate School of Oral Medicine

Case report: A 50 year-old male was referred to the Oral Medicine Clinic, University of Malaya for management of unusual gingival lesions. Accordingly, the patient first attended the Primary Dental Care Unit here for a routine dental check-up. During the course of intraoral examination, large lobulated purplish-red swellings were found affecting the labial gingival mucosa extending from the distal of the right maxillary first premolar to the distal of the left maxillary central incisor. Histopathology: Histopathology showed covering parakeratinized stratified squamous epithelium with an irregular lobular surface and exhibiting pseudoepitheliomatous hyperplasia. In the underlying connective tissues, a granulomatous inflammatory response was observed. A diffuse mixed inflammatory cell infiltrate comprising mostly neutrophils which formed subepithelial abscesses and smaller numbers of eosinophils, plasma cells and lymphocytes was present. Scattered multinucleated giant cells, some resembling Langhans-type giant cells with horse-shoe arrangement of their nuclei, were occasionally seen. However necrotizing vasculitis was not evident. Special stains were negative for fungi and mycobacterial bacilli. Based on the fi ndings, a diagnosis of Wegener’s granulomatosis was made. Management: The patient was referred to the Medical Department for a systemic work-out. Investigations yielded normal indices for haemoglobin, complete blood count, erythrocyte sedimentation rate and urine analysis. Chest X-rays were also normal. ANCA test was not available. A decision was taken to treat the patient with prednisolone 60 mg daily and cyclophosphamide 100mg daily. At one and three weeks post-treatment, a dental evaluation showed that the gingival swellings had completely resolved. Subsequent reviews were uneventful.

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Background: Aberrant Notch activity has been implicated in numerous human diseases including cancers; however its role in chondrogenic tumors has not been clarified. Materials and methods: Tissue samples from a case of primary chondrosarcoma of the maxilla and its recurrent tumor were examined immunohistochemically for Notch1-4 and their ligands (Jagged1, Jagged2 and Delta1) expression. Immunoreactions were performed using the Dako Envision Kit. Results: Both primary and recurrent tumors were histopathologically diagnosed as conventional hyaline chondrosarcoma (WHO Grade I). Hypercellular tumor areas strongly expressed Notch3 and Jagged1 in spindle and pleomorphic cells suggesting up-regulation of these protein molecules at sites of tumor proliferation. Expression patterns were distinct with some overlap. Differentiated malignant and atypical chondrocytes demonstrated variable expression levels of Jagged1, and weak to absent staining for Notch1, 4 and Delta1. Protein immunolocalization was largely membranous and cytoplasmic, sometimes outlining the
lacunae of malignant chondrocytes. Hyaline cartilage demonstrated a diffuse or granular precipitation of Jagged1 suggesting presence of soluble Jagged1 activity at sites of abnormal chondrogenesis. No immunoreactivity for the other Notch members was observed. Calcified cartilage was consistently Notch-negative indicating down-regulation of Notch with cartilage maturation. Stromal components namely endothelial cells and fibroblasts variably expressed Notch1, 3 and Jagged1 but were mildly or non-reactive for the other members. Conclusion: The results indicate that Notch signaling pathway may participate in cellular differentiation and proliferation in chondrosarcoma. Findings implicate Notch3 and Jagged1 as key molecules that influence the differentiation and maturation of cells of chondrogenic lineage.

C-15. P-56. Oral exfoliative cytology is useful in diagnosing early cancer; report of two cases
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Oral exfoliative cytology, has been recognized as a useful and less-invasive examination in diagnosing squamous cell carcinoma.

We present two cases of carcinoma that Oral exfoliative cytology was effective in detecting early cancer. Both cases had only slight erosion and appeared nonmalignant clinically when first diagnosed. We diagnosed them as Class V, Papanicolaou classification. Because their superficial and parabasal cells had atypical findings, such as nucleo enlargement and deeply-stained chromatins. Finally, they were diagnosed as well-diff erentiated squamous cell carcinoma by excisional biopsy.

C-16. P-62. A case of acinic cell carcinoma arising in the lower lip
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We present a case of acinic cell carcinoma with papillary-cystic pattern arising in the lower lip of a 62-years-old female. Histopathologically, tumor had numerous, variably sized cystic structures, which grew papillary toward the cystic lumen with single-layered lining epithelium. Immunohistochemically, the tumor cells were positive for S-100 protein, keratin, and a few tumor cells were positive for amylase, and lactoferrin. Histochmically, a few tumor cells were positive for PAS but were negative for mucicarmine. Therefore, this case was diagnosed as acinic cell carcinoma with papillary-cystic pattern.
GO-03. 1A-03. Hemophagocytosis-induced Par-2 expression regulates cytoskeletal rearrangement and cell growth of oral squamous cell carcinoma
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Background and Purpose: Protease-activated receptor-2 (PAR-2), a G-protein-coupled receptor, that is cleaved and activated by trypsin, tryptase, or synthetic peptides mimicking their cleavage part, has been shown to be involved in cellular proliferation of many kinds of cancer. We have found the expression of PAR-2 in association with hemophagocytic syndrome and oral carcinoma in-situ and squamous cell carcinoma (SCC), and these findings motivated us to study roles of PAR-2 in oral SCC cell proliferation.

Materials and Methods: To approve this hypothesis, the PAR-2 expression was immunohistochemically investigated in formalin-fixed paraffin sections of oral SCC surgical specimens. ZK1 cells, a cell system established from tongue SCC, were examined for their expressions of PAR-2 and related molecules by immunofluorescence and RT-PCR to elucidate how hemophagocytic signals affect oral SCC cell proliferation.

Results: The expression of PAR-2 was confirmed in cell proliferating zones, which were shown by Ki-67 immunofluorescence and RT-PCR to be related to PAR-2 and related molecules by immunohistochemistry. Hemophagocytosis-induced Par-2 expression was also related with heme oxygenase-1 and keratin 17 expression levels in dose-dependent manners. The PAR-2 enhancement was also related with heme oxygenase-1 and keratin 17 expression levels as well as fillopodia formation, and eventually with cell proliferation. Conclusions: The results suggest that PAR-2, induced by hemoglobin endocytosis, plays an important role in cell proliferation of oral SCC.

GO-04. 2A-01. Genomic profile of oral squamous cell carcinoma by oligonucleotide-array comparative genomic hybridization
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Using array CGH, we analyzed the difference in genomic copy number aberration (CNA) in order to investigate lymph node metastasis-related genes in oral squamous cell carcinomas (OSCC). The comparison among CNAs of each primary tumor with every metastatic ones revealed that the primary and metastatic tumors from same patients showed the highest similarity but not completely same. Moreover, no significant difference in the CNAs between primary and metastatic tumors was found. Taken together, our data suggest that the divergent clonal evolution rather than sequential acquisition of CNAs is possibly involved in the process of lymph node metastasis.

GO-05. 2A-02. The role of thrombospondin-1 (TSP1) in invasion of oral cancer
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Background: Thrombospondin-1 (TSP1) is a secretory glycoprotein that binds to many proteins and mediates diverse cellular functions. Its significance in oral carcinogenesis has been suggested, but is yet poorly understood.

Materials and methods: Thirty formalin fixed, paraffin embedded specimens of surgically excised oral squamous cell carcinoma (OSCC) were used for immunohistochemical analysis. For cells culture experiments, we used human oral cancer cell lines (BHY, HSC3 and Ca9-22) and a mouse stromal cell line (ST-2) was used to examine the proliferation, movement and invasion of cancer cells.

Results: In normal oral mucosa, TSP1 expression was observed preferentially in the capillary endothelial cells. In OSCC, TSP1 expression became to be dominant at fibroblastic cells adjacent to cancer cells as well as capillary endothelial cells, and TSP1 also accumulated in the extracellular matrix. Cell culture experiments revealed that TSP1 induced no effects on cancer cell proliferation, but it promoted movement of both cancer cells and stromal cells. TSP1 also stimulated invasion of cancer cells.

Conclusion: TSP1 expressed in fibroblastic cells around cancer cells may play an important role in cell movement and invasion of OSCC.

GO-06. 2A-03. The differential diagnosis and histopathogenesis of thyroglossal duct cyst: a comparative immunohistochemical study
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Background: Thyroglossal duct cyst (TDC) is believed to develop from vestigial elements of the thyroglossal tract, though this pathogenetic concept is not always based on scientific evidence. It remains unknown whether the cyst develops from ectopic thyroid gland follicles or from the thyroglossal duct itself. Our aim was to compare immunohistochemical profiles between main and daughter cysts and associated thyroid tissues of TDC and normal thyroid tissues for discussing its pathogenesis.

Materials and methods: Five surgical specimens of TDC and 4 autopsy specimens of normal thyroid gland were selected. Formalin-fixed paraffin sections were used for
immunohistochemistry for keratins 19 (K19), 7 (K7), and 13 (K13), p63 protein (P63), thyroglobulin, MUC-1, and Ki-67.

Results: Normal thyroid follicular epithelial cells were immunopositive for K7, MUC-1, and thyroglobulin. Intrafollicular colloid was positive for MUC-1 and thyroglobulin. TDC Lining epithelia were classified into four types: i) ciliated pseudostratified columnar, ii) two-cell-layered, iii) simple squamous, and iv) stratified squamous. Thyroid was positive in basal cells of the cystic lining, while MUC-1 was localized in both basal and luminal cells. Thyroglobulin was present in the cystic lining in cystic contents. Ki-67-positive cells were occasionally found.

Discussion: This is the first immunohistochemical investigation on TDC. The differential expression profiles for these 7 molecules could serve as an aid for the diagnosis of TDC. From the immunohistochemical discrepancy between TDC epithelia and normal thyroid, TDC’s thyroid follicular epithelial origin is unlikely.

GO-07. 2A-04. The Basic Oral Pathology Atlas
K. Komiyama

The Board of Education of the Japanese Society of Oral Pathologists

The database subcommittee of “The Basic Oral Pathology Atlas”

Oral cavity plays an important role in food intake, articulations, and respiratory infections. There is numerous disease recognized in this area such as the malformation, infectious disease, various odontogenic and non-odontogenic tumors, including cysts. Although oral mucosal surface is covered with the epithelium, which forms different appearance in each anatomical site, it is reflected into characterize the onset of disease. In addition, upper and lower jaw and teeth, salivary glands and tongue develop each tissue-specific disease. In order to study the oral lesions that appear in these areas, the basic histopathology can see images of Atlas. The Japanese Society of Oral Pathologists (JSOP) set up the database subcommittee under the Board of Education at the 2009 annual meeting. The committee began collecting pathological images of oral disease, including the clinical images with the help of the Japanese Society of Oral Surgery and dental educational institutions nationwide. Students, not only dental students but also intern students, residents and hygienists, are able to learn oral disease through “The Basic Oral Pathology Atlas” on the Web. We have summarized the key points for study of histopathology and clinical appearance with using posted images. We would like to deepen their understanding of lesions of the oral area with the Web system. The Atlas is working toward the publication of this fall, and we expect additional feedback from members of the JSOP.

Members of the database subcommittee
Kazuo Komiyama (Nihon University School of Dentistry)
Kunihsa Taniguchi (Fukuoka Dental College)
Akio Tanaka (Osaka Dental University)
Kayo Kuyama (Nihon University School of Dentistry at Matsudo)
Tomoyuki Ohuchi (formerly Health Sciences University of Hokkaido)
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G-01. P-01. Immunohistochemical localization of Notch signaling molecules in ameloblastomas

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Background: We examined Notch signaling molecules in large cases of solid/multicystic ameloblastomas. Materials and methods: A total of 40 cases of typical solid/multicystic ameloblastoma were selected. We examined on the distribution of transcription factors of Notch1 and Jagged1 by immunohistochemical (IHC) techniques. The number of positive cells was totaled, and the ratio of the number of positive cells to the total of the object cells of the strong enlarged image by a light microscope was assumed to be the CSIndex. Results: Histopathologically, the peripheral layered cells of these cell nests were columnar or cuboidal, hyperchromatic, and lined up in a palisade fashion. The peripheral layers resembled internal dental epithelium or preameloblasts. The central cells were loosely arranged, showing stellate reticulum. Furthermore, the central cells sometimes showed squamous metaplasia. Through IHC, we described using the following criteria. 1) Peripheral cuboidal cells of nests: Notch-positive products were detected in the cytoplasms of the cells in frequently. In the same cells, Jagged-positive cells were occasionally positive, but less than Notch-positive cells. 2) Peripheral columnar cells of nests: Notch-positive cells were observed in the cytoplasm of most columnar cells, but Jagged was frequently detected in the cells. 3) Central reticular cells of nests: The central reticular cells showed frequent positivity to both of Notch and Jagged. 4) Central squamous cells of nests: Both Notch and Jagged-positive cells were frequently detected in the squamous cells. Conclusion: The results showed the Notch and related factors are closely related to cytological differentiation in ameloblastomas.
G-02. P-02. Immunohistochemical observation of Notch signaling in a case of calcifying cystic odontogenic tumor
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Purpose: Calcifying cystic odontogenic tumor (CCOT) is characterized by odontogenic epithelial islands having ghost cells and calcified foci within the neoplastic epithelial cell nests and sometimes in the ectomesenchymal and/or stromal tissues. Furthermore, neoplasm dysplastic dentin may be observed. We examined Notch and Jagged and their genes in a CCOT. Materials and Methods: A CCOT was the examined specimen, and an ameloblastic fibroma (AF) and odontogenic myxoma (OM) served as control specimens. The materials were examined by IHC and ISH techniques for Notch and Jagged. Results: Regarding Notch and Jagged, IHC staining showed positive both of the epithelial components and ectomesenchymal components. In the dentinoid area, the Notch- and Jagged-positive products were present in the cytoplasms of just adjacent cells of the dentinoid tissues and/or the embedded cells. In ISH, the mRNA of the Notch and Jagged were in the cytoplasms of both the epithelial and mesenchymal cells. In the dentinoid formation area, the Jagged gene signals were observed in the cytoplasms of the IHCpositive cells. Regarding AF, IHC examination showed Notch positive products existed in both mesenchymal cells and epithelial nest cells. Jagged peptide was observed in the same cells. In ISH, they were detected as the mRNAs of both of Notch and Jagged. OM was no epithelial islands. In IHC and ISH, no Notch related positive products were observed all over the specimens. Conclusion: The results showed that these Notch and related factors are closely related to cytological diff erentiation in neoplastic cells especially in relation between epithelial-mesenchymal interactions.

G-03. P-03. Differential expression of Notch receptors and their ligands in calcifying epithelial odontogenic tumors
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Purpose: Dysregulation of Notch has been implicated in the tumorigenesis of some odontogenic neoplasms but its role in the calcifying epithelial odontogenic tumor (CEOT) remains unclarified. The aim here was to investigate Notch expression in CEOT and to speculate on its significance. Materials and methods: Receptors Notch1-4 and their ligands (Jagged 1, Jagged 2 and Delta 1) were examined immunohistochemically in six CEOT cases. Expression levels were quantified according to the percentage of positive tumor cells, amyloid-like proteins and calcifications: (-), negative staining; (+), mild and focal positivity <25%; (++) , moderate positivity in significant areas 25-50%; (+++), strong positivity in predominant areas >50%. Results: CEOT epithelium demonstrated variable expression levels for Notch1, 3, 4, Jagged1 and Delta1 suggesting upregulation of these molecules at sites of tumor differentiation. Distribution patterns were distinct with some overlap. Their localizations were largely membranous and/or cytoplasmic. Notch2 and Jagged2 were absent. Amyloid-like materials strongly expressed Jagged1 but variably Notch1, 3 and Delta1 implicating that these signaling proteins maybe competitive substrates with CEOT amyloid-like proteins for proteolysis. Notch2, 4 and Jagged2 were absent. Mineralized substances including Liesegang rings were negative for Notch receptors and ligands suggesting that calcification process is associated with downregulation of these molecules. Stromal endothelium and fibroblasts were stained variably positive. Conclusion: The current data suggest that Notch receptors and their ligands may play differing roles in the acquisition of cell fates in CEOT. Notch. Notch accumulations within amyloid-like protein suggest impaired proteolysis.
G-04. P-04. Co-expression of BMP-2 and -7 in the epithelium of calcifying epithelial odontogenic tumor with selective BMP-7 expression in amyloid materials
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Purpose: The calcifying epithelial odontogenic tumor (CEOT) has a distinguishing feature of presence of extracellular eosinophilic amyloid material. We examined the BMP expression in this neoplasms by IHC. Materials and methods: The test sample consisted of six CEOT cases. Examination was carried out by histopathology with HE and Congo red, by IHC for BMP-2 and -7. BMP distribution pattern and levels of staining intensity in all six CEOT cases were evaluated. Results: Histological examination revealed interspersed extracellularly among these epithelial sheets are amyloid-like substances occurring as eosinophilic amorphous globules or as diffuse stromal deposits. These amyloid globules were Congo-red positive and gave an apple-green birefringence under polarized light. Scattered foci of basophilic acellular calcified materials, some exhibiting concentric laminations (Leisegang rings) were present. BMP-2 protein distribution was detected in all six CEOT cases. Positive staining was observed heterogeneously in the sheets, strands and islands of polyhedral epithelial cells. Expression levels ranged from mildly focal to moderately strong and extensive. Amyloid-like protein products were consistently negative for BMP-2. Calcified materials and Liesegang rings were non-reactive for BMP-2. Regarding BMP-7, all six CEOT cases demonstrated overexpression in both the tumoral epithelium as well as the amyloid protein globules. Protein localization was membranous and/or cytoplasmic. In the stromal endothelium and fibroblasts, BMP-7 was weak to barely detectable. Conclusion: The results suggests that these protein molecules most likely participate in the process of cellular proliferation and differentiation in this neoplasm. The strong BMP-7 expression in CEOT-associated amyloid-like substances is a differentiation in this neoplasm. The strong BMP-7 participate in the process of cellular proliferation and differentiation.

G-05. P-07. Clinicopathological study in kerato-cystic odontogenic tumors
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In the 2005 WHO classification, odontogenic keratocyst (OKC) was categorized as a benign odontogenic tumor under the name of keratocystic odontogenic tumor (KCOT).

Parakeratosis of the lining epithelium KCOT, the orthokeratosis were classified as orthokeratinized odontogenic cyst (OOC).

However, there is no statement concerning combination, in this classification.

In this retrospective study, we examined 174 KCOTs treated at the Osaka Dental University Hospital from 1995 to 2009. In particular, we examined what has become ambiguous with respect to diagnostic criteria. Those with combined lining epithelium was considered reasonable to classify the KCOT.

G-06. P-08. Fate of epithelial cell rests of Malassez on MNU-induced rat molar regions
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Epithelial cell rests of Malassez becomes the epithelial lining of an odontogenic cyst due to the active inflammation, and it later forms the odontogenic tumor. However, the detailed mechanisms are unknown. Female Lewis rats were treated i.p. with 50mg/kg N-methyl-N-nitrosourea (MNU) at 4 weeks of age. At the age of 12, 18 and 30 weeks, rats were sacrificed to sample maxilla and mandible areas with H&E staining. The MNU-treated group showed greater number of epithelial cells in each nest compared with the controls. We report histological result of the epithelial cell nests and bibliographic consideration.

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To establish an experimental model of oral carcinogenesis, we made cancerous and precancerous lesions in tongue mucosa with 4-nitroquinoline-1-oxide (4NQO)-induced rat tongue carcinogenesis experimental model. 4NQO susceptible Dark-agouti rats given 10 ppm 4NQO containing were sacrificed every week to evaluate histological and immunohistochemical examinations. At the sixth week after dose, neoplastic white patches and protruded lesions were observed, and at the eighth week early invasive cancer was observed in the posterior tongue mucosa. The 4NQO-induced carcinogenesis model was useful for analyses of fi eld cancerization and precancerous lesion.

G-08. P-10. Cytokeratin expression in oral epithelial hyperplasia
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Cytokeratin (CK) analyzes are useful for diagnosing oral borderline malignancies such as CK13, CK17 and CK19. Therefore, we evaluated CK distribution in epithelial hyperplasia. We performed immunohistochemical examination of
epithelial hyperplasia arising in mucous cysts. In the controls, CK13 was expressed in parabasal to superficial layers, and CK19 localized in basal layers, while CK17 was not present. In the epithelial hyperplasia, CK13 and CK19 were not shown, although CK17 was localized in parabasal to superficial layers. Ki-67 was irregularly distributed in basal and parabasal cells. Abnormal CK13, CK17 and CK19 expression signify not only borderline malignancy but also epithelial hyperplasia.

G-09. P-11. Laser light-mediated topical photodynamic therapy for oral precancers
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Background: Oral leukoplakia (OL), oral erythroplakia (OEL), and oral verrucous hyperplasia (OVH) are three common oral precancerous lesions. One of the best oral cancer prevention strategies is to eliminate oral cancers at their precancerous stage to stop their further malignant transformation.

Methods: In this study, a topical 5-aminolevulinic acid-mediated photodynamic therapy (topical ALAPDT) with the 635-nm laser light was used to treat 40 OVH, 40 OEL and 40 OL lesions once a week and another 40 OL lesions twice a week.

Results: We found that all the 40 OVH lesions exhibited complete response (CR) after an average of 3.6 PDT treatments. Of the 40 OEL lesions, 38 showed CR after an average of 3.4 PDT treatments and 2 showed partial response (PR). The 40 OL lesions treated once a week demonstrated CR in 7, PR in 20 and no response in 13. Furthermore, the 40 OL lesions treated twice a week showed CR in 17 and PR in 23. Chi-square test showed that the twice-a-week treatment modality is better than the once-a-week treatment modality for OL lesions. In general, better PDT outcomes were significantly associated with OVH, OEL and OL lesions with the smaller size, pink to red color, epithelial dysplasia, or thinner surface keratin layer.

Conclusion: The laser light-mediated topical ALA-PDT is very effective for treatment of OVH and OEL lesions. Therefore, topical ALA-PDT using either the laser or light emitting diode (LED) light may serve as the first-line treatment of choice for OVH and OEL lesions.

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Member of the tropomyosin receptor kinase (Trk) contains TrkA, TrkB, and TrkC and activation of Trks is observed in many tumors. We examined the role of Trk family in oral squamous cell carcinoma (OSCC). TrkB or TrkC expression were significantly related with microvessel density (MVD), lymphovessel density (LVD), and poor prognosis. A strongly relationship was found between expression levels of TrkB and VEGF-A, VEGF-C, or VEGF-D, however, no significantly association was found between expression of TrkC and VEGF family. These results suggested that Trks might be a useful therapeutic target of OSCC.

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Anti-cancer drug resistance genes are divided into the intrinsic resistance gene that highly express in resistant cell properly, and the acquired resistance gene that is induced by anti-cancer drug exposure. The resistant cell lines have advanced most of the researches identifying anti-cancer drug resistant gene; however, candidate genes from the methods contain many acquired resistance gene.

Therefore we established the new method to extract only intrinsic resistance gene, and osteopontin was identified as the intrinsic resistant gene. The reduction of sensitivity for cisplatin was observed in osteopontin tranfected cells, and knock down of osteopontin induced the sensitivity for cisplatin.

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Introduction: Involvement of Notch signaling in several malignant tumors is well known, but its role in oral squamous cell carcinoma (OSCC) remains poorly characterized. The purpose of this study was to investigate the role of Notch signaling in the development and progression of OSCC.

Materials and Methods: Immunohistochemical analysis for Notch-1, Jagged1 (Jag1, one of the important ligands for Notch-1 receptor) and Laminin5γ2 chain (L5γ2, a marker of invasiveness) was performed on formalin-fixed, paraffin-embedded oral tissues, which included various pathological tissues. An OSCC cell line, SAS, harboring high Notch-1 expression was used for examining the Notch-1 dependent proliferation activity and invasiveness in vitro.

Results: The rate of Notch-1, Jag1 and L5γ2 positive specimens tended to increase in relation to progression of the mucosal neoplasm. A statistically signifi cant c (p < 0.001)
strong correlation (r = 0.738) was observed between Immunostaining scores of Notch-1 and that of L5γ2. In OSCC specimens, Notch-1 positive cells mainly distributed at the invasive fronts of tumor and co-localized with L5γ2. In vitro studies, growth activity was significantly suppressed under the Notch-1 knocked down or γ-secretase inhibitor treated conditions. In addition, the invasiveness of SAS was activated by TNF-α and that was inhibited by γ-secretase inhibitor. Moreover, these TNF-α dependent activation of cell invasiveness was not observed in the Notch-1 knocked down condition.

Conclusions: Our results suggested that Notch-1 probably play an important role in development and progression of OSCC. Furthermore, Notch-1 may contribute to invasion activity in cancer cells via TNF-α dependent manner.

G-13. P-15. MUC1 expression in squamous cell carcinoma of the tongue
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Mucins are high-molecular-weight glycoproteins with oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by o-glycosidic linkages. In the recent reports, MUC1 mucin is associated with invasive growth of the tumors and poor outcome of the patients.

We performed immunohistochemical staining of MUC1 and analyzed the relation with the clinicopathologic characteristics in human tongue cancers.

Oral biopsy specimens of 30 cases of tongue cancer diagnosed and treated at Jichi Medical University Hospital were used. Clinical information for the subjects was reviewed and statistical analysis was performed using the chi-square test.

The overall MUC1 positivity was 60%. The percentage of MUC1-positive specimens in the T3+T4 group (86%) was higher than that in the T1+T2 group (52%). The percentage of MUC1-positive specimens in the N+ group (67%) was slightly higher than that in the N0 group (58%). A higher percentage of MUC1 expression was shown in the advanced mode of invasion (4C) group (71%) compared with that of the mode of invasion (2+3) group (56%). The percentage of MUC1-positive specimens in the postoperative neck lymph node metastasis group (75%) was higher than that in the no metastasis group (54%). However, statistical significance was not found.

MUC1 was preferentially expressed in advanced and metastatic squamous cell carcinoma of the tongue and appears to be one of predictive marker for lymph node metastasis.

G-14. P-16. CXCR-4 overexpression increases the cell proliferation and invasion via MAPK/ERK signaling in oral cancer cells.
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Chemokines are small molecules that regulate leukocyte trafficking and homing. Their receptors are seven-transmembrane, G-coupled proteins. Recent data suggest that the interactions between chemokines and their receptors are also critical components in the regulation of tumor progression and metastasis in breast cancer and other tumors. However, there are few reports about the role of chemokine receptors in oral cancer behavior. In this study, we induced CXCR-4 overexpression in oral cancer cells, and examined the effect of CXCR-4 in tumourigenesis. CXCR-4 overexpression induced morphological change to spindle shape and increased cell proliferation in oral cancer cells. Also, in vivo study, CXCR-4 overexpression in oral cancer cells increase tumor size. In addition, CXCR-4 overexpression increased migration and invasion in vitro study. The effects of CXCR-4 overexpression were correlated with SDF-1 mediated activation of downstream signaling via p-ERK1/2. Together, these results demonstrate that enhanced CXCR4 signaling is via increased MAPK signaling. These findings suggest that CXCR-4 could be a useful molecular target for the treatment of oral cancer and mitogen-activated protein kinase/ERK signal transduction pathway may be involved in SDF-1 mediated proliferation and migration of oral cancer cells.

G-15. P-17. Intraductal papilloma with peri-luminal extension of the minor salivary gland: a new disease entity by clinicopathological analysis of 82 cases
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Background: Intraductal papilloma (IDP) has been considered to be a rare salivary gland tumor characterized by its papillary growth within a unicystic ductal space. However, it is rare to encounter such a typical histology when IDP arises in minor salivary gland within the oral cavity. To prove our hypothesis that IDP does not limit its growth to the luminal space only but involve the outside of the primary duct wall and that “extra-luminal IDP” involving the peri-luminal space is often misdiagnosed as pleomorphic adenoma (PA), we examined our archives of introral PA cases retrospectively.

Materials and methods: Reviewing oral pleomorphic adenoma cases filed at Niigata University and Sichuan
University in the last thirty-year period, we found 82 typical cases of extra-luminal IDP. They were examined for common clinicopathological characteristics as IDP. In addition, immunohistochemical profiles for the constituent tumor cells were investigated.

Results: Histopathologically, IDP had dilated cystic spaces, which were basically lined by two-cell layered duct epithelium-like cells, in the periphery of the tumor but had no complete intraluminal growth. There was definite papillary proliferation of two-cell layered cells with hyaline stroma containing blood vessels. The luminal cells were immunohistochemically keratins 7/19 positive, while basal cells were positive for vimentin, calponin, and occasionally S-100 protein.

Discussion: The present result indicates that the extra-luminal IDP is not so rare but accounts for about 10% of PA, which may be nearly the same as the frequency of myoepithelioma. There was no apparent difference in prognoses between IDP and PA.

G-16. P-18. miR-146a expression is associated with the oncogenicity of oral carcinoma
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MicroRNAs are short, non-coding RNAs that regulate gene expression and crucial for tumorigenesis. Oral squamous cell carcinoma (OSCC) is a prevalent malignancy worldwide. miR-146a has been reported to regulate Toll-like receptor and cytokine signaling. Up-regulation of miR-146 was identified in OSCC tissues. However, it roles in carcinogenesis appear controversial as it was shown suppressive to breast, colon and gastric carcinomas. The present study investigated the pathogenic implications of miR-146a in oral carcinogenesis. Microdissected OSCC exhibited higher levels of miR-146a expression than matched adjacent matched mucosal cells. The plasma miR-146a level in patients was significantly higher than control subjects, and it decreased drastically after tumor resection. Infection of a lentivirus carrying pre-miR-146a sequence increased the miR-146a expression. The activity of ectopic miR-146a was demonstrated by a reporter assay, the down-regulation of both IRAK1 and TRAF6, and the decreased NF-κB activities. Exogenous miR-146a expression significantly increased the in vitro oncogenic phenotypes, and the xenografts growth and metastasis of OSCC cells. Administration of miR-146a antagonist also significantly inhibited the growth of xenographic OSCC tumors. Interestingly, knockdown of both IRAK1 and TRAF6 drastically increased the oncogenic phenotypes and tumorigenesis of OSCC cells. Evidences from this study substantiate that miR-146a expression might be contributive to oral carcinogenesis by targeting IRAK1 and TRAF6.

G-17. P-19. Nuclear Survivin expression is well correlated with tumor progression of head and neck cancer
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Survivin is a member of the inhibitors of apoptosis (IAP) family. Besides its role as IAP, Survivin recently appears to function as a subunit of the chromosomal passenger complex (CPC) for regulating cell division with other CPC proteins including Aurora-B and INCENP. Nuclear Survivin is suspected to control cell division, whereas cytoplasmic Survivin is considered cytoprotective. Here we examined cytoplasmic and nuclear Survivin expression and its functional correlation with Aurora-B in head and neck squamous cell carcinoma (HNSCC). In HNSCC, high expression of nuclear and cytoplasmic Survivin was well correlated with malignant behaviors. Nuclear Survivin expression was significantly correlated with Ki-67 and Aurora-B expression. Notably, HNSCC cases with nuclear Survivin and Aurora-B expression exhibited marked malignant behaviors. Interestingly, Aurora-B expression was decreased by Survivin knockdown, and Survivin expression was decreased by Aurora-B knockdown. Moreover, accumulation of Survivin or Aurora-B protein was induced by proteasome inhibitor treatment in siRNA treated cells, suggesting that interaction between Survivin and Aurora-B may regulate their protein stability. Importantly, both Survivin and Aurora-B knockdown inhibited cell growth and tumorsphere formation. Overall suggest that nuclear Survivin may be involved in tumor progression, and that Survivin can be useful diagnostic markers and therapeutic targets of HNSCC.

G-18. P-20. Proteomic profile of VX2 induced rabbit OSCC (early- and late- stage) - preliminary report
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Oral squamous cell carcinoma (OSCC) is the most common malignant form of oral cancer. It seems not many reliable and predictable serum biomarkers can be found in the literature. The purpose of study is try to use a proteomic analysis in VX2 induced rabbit OSCC in order to observe the difference between early and late stage of tumors, ultimately to identify novel-invasive serum biomarkers of OSCC.

Ten adult white New Zealand male rabbits, each normal and experimental rabbit were anesthetized intramuscularly by ketamine. Venous blood were drawn from each rabbit. All serum samples were separated from whole blood by centrifugation at 800 g for 10 minutes and aliquoted for storage at -80°C.
Before 2D gel electrophoresis analysis, the sera were treated with Affi -Gel Blue Gel to remove albumin. The sera were separated using 18 cm, pH 3-10 IPG strips for the first dimension and 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis for second dimension. Protein spots were visualized by silver staining and identified by liquid chromatography/tandem mass spectrometry.

Nineteen protein-spots of sera samples show significantly changed during the development of VX2-induced rabbit submandibular carcinomas. Transthyretin, paraoxonase 1, paraoxonase 1B, alpha-2-glycoprotein 1 and alpha 1B-glycoprotein-like, were identified by mass spectrometry. Among them, transthyretin and paraoxonase 1 was strongly decreased after tumor inoculation.

The application of proteomic technologies in VX2-induced rabbit submandibular carcinomas animal model would be helpful to evaluate reliable cancer-associated biomarkers. The relationship between these biomarkers and OSCC development remains to be further tested in cancer patients.

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It is known that several percent of leukoplakia that develop in the oral mucosa become cancerous. Leukoplakia is a clinical diagnostic term, and histopathologically most cases show hyperkeratosis with epithelial dysplasia or hyperkeratosis without epithelial dysplasia. On the other hand, difficulties in histopathological diagnosis of severe epithelial dysplasia and carcinoma in situ are often encountered. In the present study, with the objective to achieve a higher precision in histopathological diagnosis, we conducted and compared the immunohistochemical stainings of CK13, CK17 and p53 in 10 cases each of hyperkeratosis without epithelial dysplasia, hyperkeratosis with epithelial dysplasia, carcinoma in situ, and SCC in the tongue. The following results were obtained. CK13 was positive in all 10 cases of hyperkeratosis without epithelial dysplasia, and negative in all cases of hyperkeratosis with epithelial dysplasia, carcinoma in situ, and SCC. CK17 was negative in 9 of 10 cases of hyperkeratosis without epithelial dysplasia, and was positive in all 10 cases of hyperkeratosis with epithelial dysplasia, carcinoma in situ, and SCC. For p53, positive reaction was observed in some cells of the basal cell layer in 2 of 10 cases of hyperkeratosis without epithelial dysplasia, while high positive rates were found in hyperkeratosis with epithelial dysplasia (9 of 10 cases) and SCC (9 of 10 cases). On the other hand, only 5 of 10 cases of carcinoma in situ were positive for p53.

G-20. P-22. Roles of N-cadherin in head and neck squamous cell carcinoma
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The loss of E-cadherin and the gain of N-cadherin expression are known as the “cadherin switching”. Cadherin switching is a major hallmark of epithelial-mesenchymal transition (EMT). EMT is a crucial process in cancer progression providing cancer cells with the ability to escape from the primary focus, to invade stromal tissues and to migrate to distant regions. We found that high expression of N-cadherin was observed in 52 of 80 head and neck squamous cell carcinoma (HNSCC) cases and was significantly correlated with malignant behaviors. Cadherin switching was observed in 30 of 80 HNSCC cases and was well correlated with histological differentiation, pattern of invasion and lymph node metastasis. Moreover, N-cadherin expression was observed in 7 of 16 HNSCC cell lines, and cadherin switching was observed in 2 of 4 HNSCC cells with EMT features.

To know the utility of N-cadherin as a therapeutic target for HNSCC, I treated N-cadherin antibody in HNSCC cells with EMT features (HOC313 cells). We found that N-cadherin antibody suppressed the proliferation and the invasion of HOC313 cells. N-cadherin antibody treated HOC313 cells showed morphologic changes from a spindle shape to a cobblestone-like shape. We also found the reduced expression of Snail1, vitronectin and MMPs including MMP3, MMP7, MMP9, MMP10, MM12 and MMP13 in HOC313 cells with N-cadherin antibody treatment.

In conclusion, we suggest that i) N-cadherin may play an important role in malignant behaviors of HNSCC, and ii) blocking function of N-cadherin can be a therapeutic target for HNSCC.

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Objective: Oral squamous cell carcinoma (OSCC) has highest incidence among malignant tumor of head and neck. Matrix metalloproteinases (MMPs) and tumor associated macrophages (TAMs) play important roles in the tumor metastasis. In this study, we attempted to compare the intensities of MMP2 and MMP9 between primary and metastatic lesions in relation to the distribution of TAMs.

Materials and Methods: Total of 29 OSCC cases were examined. The subjects contained 13 cases of lingual, 14 cases of gingival, and 2 cases of buccal OSCCs. Formalin fixed, paraffin-embedded sections were stained with anti-MMP2, MMP9 and CD68 antibodies as primary antibodies,
followed by HRP-conjugated IgG Ab. Result: Both MMP2 and MMP9 tend to be expressed stronger in metastatic lesion than primary lesion. In MMP2, most of primary lesion showed negative or weak staining, but metastatic lesion showed higher staining intensity.

While, MMP9 was detected positive staining in the primary lesion, and showed much stronger positive staining in the metastatic lesion.

Next, we compared the MMPs staining intensity of metastatic lesion between the gingival and lingual cancer. MMP2 staining intensities were stronger in gingival cancer than lingual cancer, however MMP9 expression showed no difference.

In interestingly, the number of TAMs showed much higher in the primary lesion than that of the metastatic lesion.

Conclusion: These results indicated that MMPs staining intensities and the number of TAMs in the lesion were in negative correlations. Moreover, MMP2 might play relevant roles for the metastasis of the gingival cancer.


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Human podoplanin is a type-1 transmembrane sialomucin-like glycoprotein consisting of 162 amino acids. The protein was originally detected in puromycin-induced nephrosis on the surface of rat podocytes as a 38-kDa mucoprotein and is now utilized as a specific marker for recognizing lymphatic vessels. Recent studies have shown that podoplanin is expressed in not only normal tissues but also abnormal tissues with dysplastic changes, or in various malignant neoplasms. It has been implicated that podoplanin can be involved in tumor cell invasion and metastasis. However, the role of the protein in physiological and pathological conditions has been retained unclear. In this study immunohistochemical and molecular biological analyses were performed to examine the importance of podoplanin expression in oral precancerous and cancerous lesions. One hundred and three samples of epithelial precancerous and cancerous lesions were classified according to the degree of differentiation of carcinoma. PD-L1 expression on OSCC cells and their densities were correlated with clinicopathological factors. We examined the expression of PKM1 and PKM2 and the ratio of PKM2/PKM1 by IHC in OSCC, dysplasia/leukoplakia (DPLP) and inflammation tissue (ID). The ratio of PKM1/PKM2 may be useful for the distinguishing OSCC from benign lesions.

G-23. P-25. Expression of pyruvate kinase isozymes in oral squamous cell carcinoma

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Unlike normal cells, which metabolize glucose by oxidative phosphorylation, tumor cells preferentially metabolize glucose by anaerobic glycolysis. The metabolic shift is partly achieved by a switch in the splice isoforms of the pyruvate kinase. Although normal cells express the pyruvate kinase M1 isoform (PKM1), tumor cells express the M2 isoform (PKM2). We prompted to verify the PKM1-PKM2 switching as a marker of malignancy.

We examined the expression of PKM1 and PKM2 and the ratio of PKM2/PKM1 by IHC in OSCC, dysplasia/leukoplakia (DPLP) and inflammation tissue (ID). The ratio of PKM1/PKM2 may be useful for the distinguishing OSCC from benign lesions.


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Objectives: Tumor-infiltrating lymphocytes (TILs) are considered to represent immune reactions of the host to a malignant tumor. Tumor cells defend against the actions of TILs by expressing molecules that hinder host immunity, such as programmed death receptor ligand-1 (PD-L1). In the present study, we investigated the densities of CD4+/CD8+ TILs and PD-L1 expression of tumor cells in oral squamous cell carcinoma (OSCC).

Methods: Forty-five cases of OSCC were selected. We evaluated PD-L1 expression and the infiltration degree of each lymphocyte by immunohistochemical examination. TILs were separately assessed in tumor cell nests and in the stroma near the invasive front of the tumor. These data were analyzed in connection with clinicopathological factors.

Results: Peritumoral CD8+ TILs were observed in every patient with OSCC, and their densities were correlated with lymph node metastasis (P<.001), tumor size (P=.003), and clinical stage (P<.001). PD-L1 expression on OSCC cells
was observed in 39 cases and was associated with the density of intratumoral CD8+TILs (P=.047). PD-L1 expression of tumors <4cm in size was correlated with the histological grade of the tumor (P=.022). Patient survival and immunohistochemical results were not associated.

Conclusion: Some clinical parameters such as the size of tumor, lymph node metastasis and tumor stage, were significantly associated with the densities of peritumoral TILs. PD-L1-positive tumor cells were found in most OSCC patients, and which were associated with the densities of intratumoral TILs. The development of a strategy to block the interactions of PD-L1 with TIL would be a useful tool for inhibiting tumor growth.

G-25. P-27. Ni2+ ion inhibited the secretion of IL-8 by oral squamous cell carcinoma cells (OSCCs).
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Objective: OSCCs express TLR4 and respond to LPS by producing proinfl ammatory cytokines. Recently, nickel (Ni2+) ion was shown to interact with TLR4 and induce the inflammatory reactions. In the present study, we attempted to examine the eff ect of Ni2+ ion on the IL-8 secretion by OSCCs.

Materials and methods: Three different OSCCs, HSC2, HSC3 and Ca9-22, were used. The cells were cultured with or without various concentrations of Ni2+ ion for indicated length of times. The culture supernatants were harvested and IL-8 concentrations were measured by ELISA. Contribution of TLR4 to Ni2+ ion-mediated IL-8 secretion was examined by anti-TLR4 antibody pre-incubation. Luciferase assay was performed with pNF-kappaB-Luc plasmid, which contains 6 NF-kappaB-binding sites.

Results: All three OSCCs secrete IL-8 spontaneously. The most active production was observed in HSC3 cells. Secretion of IL-8 was partially inhibited by NF-kappaB specific inhibitors indicating that spontaneous IL-8 production was attributed to aberrant NF-kappaB activity. The eff ect of Ni2+ ion was observed. Surprisingly, Ni2+ ion inhibited the secretion of IL-8 in a dose- and time-dependent fashion. Pre-incubation of HSC3 cells with anti-TLR4 specific antibody recovered the secretion of IL-8. The eff ect of Ni2+ ion on the activity of NF-kappaB was further examined by luciferase assay. Ni2+ ion reduced NF-kappaB activity after 1 h of stimulation. All these results indicated that Ni2+ ion has an inhibitory eff ect on the secretion of IL-8 by OSCCs.

Conclusions: Ni2+ ion inhibited spontaneous IL-8 secretion of OSCCs by inhibiting the activity of NFkappaB through TLR4.

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Comparative characterization of tumor endothelial cells isolated from highly and low metastatic tumors It has been an important concept of tumor angiogenesis that tumor endothelial cells (TECs) are genetically normal and homogenous.

However, we have reported that the TECs are diff erent from normal endothelial cells (NECs). Whether there were differences in tumor endothelial cells derived from many type of tumors remains to be unclear. In this study, to investigate this question, we isolated two types of murine TECs from human tumor xenografts: highly metastatic melanoma (HMM) and low metastatic melanoma (LMM) in nude mice and investigated whether there were phenotypic and genetic diff erences between these TECs.

G-27. P-29. Expression of PTHrP in mucoepidermoid carcinoma
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PTHrP is known to induce bone resorption by activating RANKL as well as PTH. We have reported that PTHrP is expressed in oral squamous cell carcinoma cell lines, and PTHrP expression is correlated with malignant phenotype of oral cancer cells. Immunohistochemical detection of PTHrP was performed in 21 cases of mucoepidermoid carcinoma in head and neck region. PTHrP was highly detectable in intermediate cells and it may concern lymph node metastasis or recurrence of tumor.

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We examined the histological pattern of salivary gland tumors using triple-immunostaining method. Formalin-fixed, paraffin-embedded sections of pleomorphic adenoma, Warthin tumor, adenoid cystic carcinoma and mucoepidermoid carcinoma were stained by the triple-immunostaining method using DAB, PermaBlue, PermaGreen and Permanent Red as chromogens. DAB, PermaBlue and Permanent Red of chromogens make high contrast to observe. The triple-immunostaining was a useful tool to examine the histological
pattern of salivary gland tumors.

G-29. P-35. Endoglin expression in oral hemangioma - A comparative study with expression of endothelial markers and angiogenic factors - M. Tsuchiya¹, N. Matsumoto¹, S. Vongosa¹, D. Omagari¹, M. Asano¹, K. Komiyama¹
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Both of capillary and cavernous hemangioma is relatively common benign oral tumor conditions. However, the precise etiology of hemangioma remains unclarified. We examined the characteristics of capillary and cavernous hemangioma with endoglin expression. It is a component of TGF-β receptor, which is synthesized in activated endothelial cells (ECs). We also examined for the proliferation activity and angiogenic factors.

Immunohistochemistry was performed for 32 capillary hemangiomas, 33 cavernous hemangiomas, and 5 non-pathological oral mucosa with using following antibodies; CD34, endoglin, von Willebrand factor (vWF), podoplanin, alpha-SMA, VEGF, COX-2 and Ki-67. The results assessed by the staining grade system and Ki-67 labeling index (LI).

All of capillary hemangiomas showed the endoglin in high expressions, whereas low in the cavernous hemangioma. An intense expression of CD34 found in non-pathological conditions, while low expression in the cavernous hemangioma. Ki-67 LI revealed higher in capillary hemangioma than that of cavernous hemangioma.

These results suggest the ECs of capillary hemangioma show more proliferative nature, which are regulated by VEGF. Whereas, the ECs of cavernous hemangioma have weak proliferative activity. One possible explanation for the etiology of cavernous hemangioma is not angiogenesis but the dilation of blood vessels because the vessel is lining discretely with alpha-SMA positive cells. Moreover, the biological profiles of both ECs in capillary and cavernous hemangiomas are obviously different.

G-30. P-38. Immunohistochemical study of carcinoma cuniculatum-subtype of oral squamous cell carcinoma
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Purpose: Carcinoma cuniculatum (CC) is a distinct variant of oral squamous cell carcinoma characterized with keratin filled crypts and cuniculatum architecture which are unique and different from other subtypes. The immunohistochemical analysis of CC was rare. Methods: The present series included 15 patients. The immunohistochemical study of Ki-67, p53, p63, and cytokeratins were performed. Results: CC was low expressed in p53 and Ki-67. High expression of p63 in the basal and suprabasal cells consisted with unique architecture of CC. CC was high positive in CK10 while low expressed or negative in CK13 and CK17. Conclusion: CC is a well-diff erentiated variant.

G-31. P-41. Mast cell and myofibroblast in denture-related fi brous hyperplasia
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Denture-related fibrous hyperplasia (DRFH, so-called denture fibroma) is a non-tumor condition, is often found in the denture edge. This lesion is relatively common disorder in varied size, the lesion composed of fibrous tissue over growth. However, precise mechanism of the exfolic lesion development is poorly understood. Recent contemporary finding showed that the presence of mast cells and myofibroblasts are probably contributes to wound healing and organ fibrosis. We have examined the presence of two types of mast cells (MT and MT/C) and myofibroblasts in the denture-related fibrous hyperplasia to investigate its role in progression of the lesion.

The serial sections from surgically excised 33 cases of DRFH were stained immunohistochemically for tryptase and chymase to determine subpopulation density and distributions. In addition, alpha smooth muscle action is also stained to identify the myofibroblast differentiation.

The samples revealed that the female-mail ratio was 5:3, and the age distribution at age 58 to 82 years, the average was 69.7. A 68% of patients wear the full denture. The lesion size varied from 5mm to 85cm in diameter.

The lesion split into two parts such as granular layer and fibrotic layer, the presence of both MT and MT/C were clearly higher in the granular layers. Moreover, clearly high numbers of MT presented than MT/C. Myofibroblast differentiation was identified 40% of the lesion, where located close to granular layers.

The results suggest that mast cells subpopulations may contribute to DRFH development. The differentiation of myofibroblast was recognized DRFH, indicates remodeling of extracellular matrix.

G-32. P-44. Areca nut extract reduces the phagocytic function of human peripheral blood mononuclear cells
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Areca chewing, a popular oral habit in Taiwan and some Asia countries, is highly associated with several oral diseases. Inflammation is known to modulate and enhance the progression of diseases. Extract of areca nut (ANE) enhances the production of inflammatory mediators by immune cells, but reduces the activation of T cells. The study was to determine the possible effects of ANE on the phagocytic activity of human immune cells, peripheral blood mononuclear cells (PBMC). Flow cytometry and
confocal laser scanning microscopy were the methods used in this research. The results revealed that the uptake of both opsonized (C-Beads) and non-opsonized (Beads) fluorescent microspheres by PBMC, especially the monocytes gate, was dose-dependently reduced in the cell group with 24-h ANE treatment, but not for that with 4-h ANE treatment. The levels of internalized fluorescent microspheres in monocytes decreased significantly when 20 ug/ml of ANE was used. Furthermore, the inhibitory effects of ANE on the phagocytic function of PBMC could be reversed by PI3K inhibitor (LY294002) and GSK-3 inhibitor X, but not by the antioxidant DPI. Taken together, ANE impaired the phagocytic function of human PBMC might be via the signaling pathways such as PI3K and GSK, but not via the generation of reactive oxygen species. Alterations in the defensive properties of PBMC by ANE might promote colonization of microorganisms and reduce phagocytic clearance during inflammation, and this could result in oral infection and prolonged inflammation.

G-33. P-45. Expression of hBD-2 and infiltration of T lymphocytes in chronic hyperplastic candidiasis
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Objectives: Chronic hyperplastic candidiasis (CHC) is characterized by epithelial hyperplasia of the oral mucosa associated with candidal hyphae. The immune status of host is one of the factors that induce clinically evident candidal infection. Host defense mechanisms include inflammatory cells, epithelial barrier, and antimicrobial peptides such as human beta-defensin-2 (hBD-2). In the present study, we investigated the densities of CD4+/CD8+ T lymphocytes and hBD-2 expression of epithelial cells in CHC.

Methods: Immunohistochemical staining was performed on 10 cases of CHC using CD4, CD8 and hBD-2. Ten specimens of chronic mucositis were selected for comparison, and went through the same examination.

Results: hBD-2 was expressed in the stratum granulosum and stratum spinosum of 7 CHC patients, while the epithelium of chronic mucositis did not demonstrate the hBD-2 expression except for one case. Also, hBD-2 expression was stronger when the hyphae invaded the upper stratum spinosum (P=0.019). Both CHC and chronic mucositis showed the infiltration of CD4+ and CD8+ T lymphocytes. There was no difference in the densities of lymphocytes recruited under the epithelium between the two conditions. However, the densities of intraepithelial CD8+ T lymphocytes were lower in the patients expressing hBD-2 (P=0.018), suggesting that the ability of CD8+ T cells to enter the epithelium and target the pathogenic hyphae was decreased in CHC.

Conclusion: Increased hBD-2 expression was significantly associated with the candidal infection, while not promoting the cell-mediated immune reaction in CHC.

G-34. P-46. miR-214 acts as a repressor of adenovirus proliferation by inactivating E1A.
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Adenovirus early-transcribed gene E1A plays the most important role in virus infection and duplication. We have identified miR-214 that might assume hAd5 E1A by in silico experiment. hAd5 was introduced in HeLa and Saos-2 cells, and adenovirus productivity was lower in Saos-2 cells although the CAR, an adenovirus receptor, was expressed in both cells. miR-214 was highly expressed in Saos-2, and expression of luciferase reporter gene fused with E1A 3’ UTR was decreased in Saos-2. These results indicate that cellular miR-214 is capable of inhibiting adenovirus replication by degrading E1A mRNA.

G-35. P-47. Adenovirus E4orf6/7 activates ASPP1 that induces apoptosis
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E4orf6/7 is one of the early gene products of human adenovirus. E4orf6/7 is known to form a stable complex with E2F transcription factor to transactivate both viral and cellular genes. It is suggested that this function is partly responsible for the induction of p53-dependent apoptosis. However, none of the target gene for E4orf6/7 to induce apoptosis has been identified to date.

ASPP1, apoptosis-stimulating protein of p53, is one of the ASPP family recently identified to induce apoptosis in p53-dependent manner. Promoter analysis revealed that E2F binding sites exist in ASPP1 promoter region, and E2F was shown to increase ASPP1 expression.

We investigated the effects of adenovirus E4orf6/7 on E2F and ASPP1 mediated cellular apoptosis. Wild-type E4orf6/7 transfection into 293T cells induced PARP cleavage. When the wild-type or C-terminus conserved E4orf6/7 expression plasmids were transfected, increased expression of ASPP1, and its nuclear localization were observed. However, little upregulation and nuclear localization of ASPP1 were seen in C-terminus deleted E4orf6/7 mutant transfectants. Our results suggested the significance of E4orf6/7 in ASPP1 mediated apoptosis.
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Purpose: Immunohistochemical (IHC) expression of HSP27 and p-HSP27 were simultaneously examined after subjecting the periodontal tissues to mechanical stress by orthodontic movement. Materials and method: The Waldo method of inducing mechanical stress-load in mouse periodontal tissues was followed. The maxillary molar with the periodontal tissues were removed were examined by IHC. Results: In the 10 minutes group, HSP expression localized in the PDL fibroblasts in the tension side was slightly stronger compared to the control group. In the 20 minutes group, cytoplasmic IHC expression of HSP27 on the PDL fibroblasts seen in the tension and pressure sides was stronger than the 10 minutes group. In the 3 hours group, the PDL fibroblasts and cementoblasts in the tension side strongly expressed HSP27. At this point, p-HSP27 expression showed no difference in strength. In the 9 hours group, HSP27 was strongly expressed in the cytoplasm of PDL fibroblasts and cementoblasts as well as in osteoblasts. In this period, p-HSP27 started to become strong in the “pressure” side. In the 24 hours group, HSP27 was strongly expressed in the “tension” side by the entire PDL fibroblasts and cementoblasts as well as by osteoblasts.

Conclusion: The study suggests that HSP27 has been closely involved in the repair of tissue to maintain homeostasis of the periodontal tissues. Also, the data adds to the existing trend in role of Msx2 and Runx2 in the activation of osteoblasts during bone formation suggesting the role of HSP27 as a molecular chaperone during orthodontic treatment induced by mechanical stress.

G-37. P-49. Promoting effect of mechanical stress to transplanted bone marrow-derived cell migration into periodontal tissues
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Background: Bone marrow-derived cells have abilities of cell migration and differentiation into teeth and related tissues/organs, especially into periodontal ligament fibroblast cells. We examined the effect of orthodontic mechanical stress to the transplanted bone marrow-derived cell migration into periodontal tissues. Materials and methods: Bone marrow-derived cells from green fluorescence protein (GFP) transgenic mice were transplanted into C57BL/6 immuno-compromised recipient mice, which had undergone 10Gy of lethal whole-body irradiation. After successful transplantation, the mice received orthodontic mechanical stress using the Waldo method in 5 weeks. After that, the regional tissues were immunohistochemically analyzed using a Dako Envision+Kit-K4006 (Dako, Glostrup, Denmark) and a primary anti-GFP-polyclonal rabbit antibody (#598: 1/500; MBL, Nagoya, Japan). Results: The immunohistochemistry revealed that GFP-positive cells were detected in the periodontal tissues, both in the experimental and control specimens. In the experimental group, there were numerous GFP-positive cells appearing in the experimental periodontal tissues which received intermittent stimulation of orthodontic mechanical stress, but there were few GFP-positive cells in the control specimens. Thus, these data indicated that orthodontic mechanical stress acts as a possible promoting factor of transplanted bone marrow-derived cell migration into periodontal tissues, and of differentiation to fibroblasts and capillary endothelial cells. Although the clinical implications of adult bone-marrow cell plasticity are still unclear, focusing on the mechanisms in which cell ascertains fate in human is likely to benefit the areas of cell therapy and regenerative medicine. Conclusion: This phenomenon suggests that the possibility of short-term orthodontic treatment with of transplantation of bone marrow cells.

G-38. P-52. Low level laser therapy at acupuncture points in temporomandibular joint disorder
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Introduction: Low level laser therapy (LLLT) has been used to treat temporomandibular joint disorder (TMD) for years. In this study, we reported the clinical outcomes of 6 patients with TMD treated with LLLT at acupuncture points.

Materials & Methods: Six (6) TMD patients were treated with diode K-Laser (wavelength 800 nm) at 4 acupuncture points once a week. The selected acupuncture points were ipsilateral points at ST6 (Jiache), ST7 (Xiaoguan), one ashi point, and a contralateral point at LI4 (Hegu). The treatment outcome was evaluated with a visual analog scale (VAS) at each appointment.

Results: All 6 patients exhibited free of pain after an average of 4 (range 2-7) visits. In addition, VAS evaluation showed an average of 38% (range 9-67%) pain reduction after each LLLT treatment. There is no recurrence of pain of the 6 patients after a follow up period of 2-4 months.

Conclusion: LLLT at acupuncture points is an effective alternative treatment for TMD.
A comparative study of seven devices used for oral squamous cell sampling
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It has been recently reported that oral brush cytologic examination in private dental clinics is quite effective to detect oral early cancers. There are many brushes available for the examination which we can select on the market and under development, but it has not been reported which brush is the most useful to gather enough amount of cells for the examination. A purpose of this study is to propose a brush which any dentist can easily collect enough amounts of cells for diagnosis, in order to spread the examination in our country.

The oral cavity is amenable to both direct visualization and direct palpation by clinicians. Therefore, malignant tumors in the oral cavity can be detected earlier than other organ’s tumors. We have started an oral cancer detecting system in routine practice in a private dental clinic since 2007, which we reported at annual meeting of JSOP in 2008. The oral cavity is the most useful to gather enough amount of cells for the examination which we can select on the market and under development, but it has not been reported which brush is the most useful to gather enough amount of cells for the examination. A purpose of this study is to propose a brush which any dentist can easily collect enough amounts of cells for diagnosis, in order to spread the examination in our country.

The oral cavity is amenable to both direct visualization and direct palpation by clinicians. Therefore, malignant tumors in the oral cavity can be detected earlier than other organ’s tumors. We have started an oral cancer detecting system in routine practice in a private dental clinic since 2007, which we reported at annual meeting of JSOP in 2008. And liquid based cytology is used in this system. In this study we investigated the cases of oral cancer had been detected using the liquid based cytology, and evaluated this study we investigated the cases of oral cancer had been detected using the liquid based cytology, and evaluated this study.

Usefulness of Oral Exfoliative Cytology as a Follow-up Examination for Leukoplakia
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Development of medical science makes it possible to cure many diseases, but problems which we cannot solve remain in super aging societies. It is difficult to deal with aged patients suffering from serious underlying diseases. Such diseases occasionally prevent the patients from undergoing appropriate medical procedures, especially operations. We report a case that we had to postpone an operation for Proliferative verrucous leukoplakia because of patient’s atrial fibrillation and bradycardia-tachycardia syndrome, from the point of view of cytopathology and histopathology.

Histone deacetylases (HDACs) inhibitor, Trichostatin A, induces G2/M phase arrest and apoptosis in oral squamous carcinoma YD-10B cells
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Histone acetylation is one of the key chromatin modifications that control gene transcription during development and tumorigenesis. Recently, it was reported that histone deacetylase inhibitor, Trichostatin A (TSA), is a well-known antitumor agent that induces tumor growth arrest and apoptosis. However, the molecular mechanisms of their eff ects are not clear. Th e purpose of this study was to investigate the effects of TSA on human oral squamous carcinoma cells and its underlying mechanisms. MTT assay showed that TSA inhibited cell proliferation in YD-10B cells. TSA arrested eff ectively cycle cell progression at G2/M phase through up-regulation of p21waf expression, down-regulation of Cyclin B1 as well as inhibitory dephophorylation of Cdc2. In addition, mitochondrial membrane destruction was induced by TSA treatment for 48h. TSA also induced cytochrome c release and proteolytic activation of caspase 3 and caspase 7 in YD-10B cells. Taken together, these observations suggest the potential value of TSA in oral cancer YD-10B cells.

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Inhibition of autophagy enhances apicidin-induced apoptosis in human oral squamous carcinoma cells
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Objectives: Apicidin acts as a potent histone deacetylases (HDAC) inhibitor and the precise mechanism for its anti-tumor activity in human oral squamous cell carcinoma (OSCC) cells has not been examined. The aim of this study was to evaluate the anti-tumor efficacy of apicidin through apoptosis and autophagy in OSCC cells.

Materials and Methods: Cells were treated with apicidin and cell death was quantified. Cell cycle and apoptosis were measured using flow cytometry assay, immunoblot. Autophagy was characterized by the increase of LC3B-II and the formation of acidic vesicular organelles (AVOs).

Result: Apicidin significantly inhibited the proliferation of OSCC cells in a dose-dependent manner. Apicidin markedly up-regulated p21WAF1 led to G2/M phase arrest. Apicidin significantly increased the number of apoptotic cells compared to untreated control. Apicidin induced not
only apoptosis but also autophagy in OSCC cells. Apicidin dramatically increased the levels of LC3 type II expression, ATG5 protein expression and the accumulation of AVOs. Inhibition of autophagy enhanced apicidin-mediated cytotoxicity through an increase in apoptosis.

Conclusion: These results suggest that apicidin exerts anti-tumor effects by inducing apoptosis and autophagy and provide novel evidence of apicidin-induced autophagy and autophagy inhibition enhances apicidin-mediated apoptosis in OSCC cells. This study was supported by a grant of the NRF funded by the MEST (No.R13-2008-010-00000-0).

G-44. P-59. In vitro and in vivo photodynamic therapy using phophorhobide A induces apoptosis in mouse oral cancer AT-84 cells

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This study was examined the therapeutic effect of photodynamic therapy using the photosensitizer Phophorhobide a (Pa-PDT) in mouse oral squamous cell carcinoma AT-84 cells. Pa-PDT treatment significantly induced cell growth inhibition and the intracellular ROS generation, which is critical for cell death induced by PDT. Cell cycle analysis showed the increased sub-G1 proportion of cells in Pa-PDT treated cells. Induction of apoptotic cell death was confirmed by DAPI staining and the reduction of mitochondrial membrane potential (ΔΨ (m)). The changes in apoptosis-related molecules were next examined using Western blot after Pa-PDT treatment. The release of cytochrom C and the cleavage of caspase-3, PAPR were exhibited, whereas Bcl-2 protein level was decreased. To determine the therapeutic effect of Pa-PDT in vivo, mouse oral squamous cell carcinoma animal model was established. Treatment of mice with Pa-PDT significantly inhibited tumor growth. Immunohistochemistry and TUNEL assay showed decreased PCNA expression and increased apoptotic cells compared to vehicle-treated controls. Our findings suggested the therapeutic potential of Pa-PDT in the oral squamous cell carcinoma both in vitro and in vivo. This study was supported by a grant of the NRF funded by the MEST (No.R13-2008-010-00000-0).

G-45. P-60. Effect of Dentin Sialoprotein on Proliferation, Migration and Differentiation in Human Dental Pulp Cells

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Background and Objectives: Dentin sialophosphoprotein (DSP) is an extracellular matrix, typically dentin-and bone-specific gene, which plays an important role in dentin mineralization and tooth development. DSP is cleaved into dentin sialoprotein (DSP) and dentin phosphoprotein (DPP). In developing dentin, DSP is expressed in odontoblasts and in preameloblasts. However, little is known about the direct role of DSP in human dental pulp cells (HDPCs). This study was conducted to investigate the effects of DSP on proliferation, migration and odontoblastic differentiation in HDPCs.

Methods: Cell proliferation and migration were determined by MTT assay and chemotaxis cell migration assay, respectively. Differentiation were measured by alkaline phosphatase (ALP) activity, calcified nodule formation by alizarin red staining, and odontoblastic/osteoblastic marker mRNA expression by reverse transcription polymerase chain reaction (RT-PCR). MAPK, AKT, NF-κB, smad 1/5/8 and beta-catenin expression evaluated by western blotting.

Result: Cell proliferation and migration were increased in dose- and time dependent manner in HDPCs. ALP activity, the mineralization and mRNA expression for odontoblastic/osteoblastic markers were enhanced by DSP in HDPCs. DSP showed degradation and phosphorylation of IxB-α, activation of p65 in dose dependent manner stimulated phosphorylation of Akt, ERK, p38, JNK with a maximal response within 10 min in HDPCs. DSP induced IxB-α phosphorylation, subsequent degradation of IxB-α, and nuclear translocation of NF-κB p65 subunit. Also, phosphorylation of Smad1/5/8 and active β-Catenin protein levels were up-modulated by DSP treatment

Conclusion: Our studies demonstrated that DSP-induced growth promotion, migration and odontoblastic differentiation of HDPCs are mediated by p38, ERK, JNK MAPK, Akt, NF-κB and β-Catenin signaling pathways. Th us, DSP can be crucial in the repair and regeneration of human dental pulp.

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G-46. P-61. The Role of SIRT1 on Angiogenic and Odontogenic Potential in Human Dental Pulp Cells

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Background and Objectives: Sirt1, a NAD+-dependent histone deacetylase, controls critical functions of mammalian cell physiology including differentiation in skeletal muscle cells and adipocytes. However, the role of Sirt1 in human dental pulp cells (HDPCs) is unknown at present. Therefore, the aim of this study was to evaluate expression of Sirt1 and its effect on the odontoblastic and vascular differentiation in HDPCs.

Materials and Methods: Odontoblastic and angiogenic differentiation ability was evaluated by Alizarin Red S stain, reverse transcription polymerase chain reaction (RT-PCR).

Results: Treatment with resvaratrol, a SIRT1 activator, increased odontoblastic genes such as alkaline phosphatase (ALP), osteopontin (OPN), dentin matrix protein (DMP-1), and dentin sialophosphoprotein (DSP) as well as gene expression of agngiogenic markers such as vascular endothelial
growth factor (VEGF), endothelial cell adhesion molecules (VE-Cadherin, pECAM), and basic fibroblast growth factor (FGF-2), and enhanced calcification of the extracellular matrix in a time and dose dependent manner in HDPCs. In contrast, downregulation of SIRT1 expression by SIRT1 inhibitor, and SIRT1 siRNA which inhibit odontoblastic differentiation and mRNA expression for angiogenic markers. Furthermore, adnovirusmediated overexpression of SIRT1 induced odontoblastic and vascular differentiation markers.

Conclusions: These results imply that SIRT1 may have a pivotal role in odontogenesis and angiogenesis of HDPCs. Furthermore, this study suggests that the administration of SIRT1 donors or the modulation of the endogenous production of SIRT1 may be therapeutic benefit in the pulp regeneration and revascularization.

Key Words; SIRT-1; Differentiation; Human Dental Pulp Cells

ACKNOWLEDGEMENTS: This study was supported by a grant from the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea. (A084458)

G-47. P-63. Effi cacy of fi ne needle biopsy in the diagnosis of oral and maxillofacial lesions

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This study was planned to reveal the efficacy of histopathological examination using fine needle biopsy (FNB) with the new Monopty biopsy instrument (MBI; Bard Urologic Division; Covington, GA) and FINCORE (Dr. JAPAN Co., Ltd., Tokyo, Japan) in the diagnosis of oral and maxillofacial lesions. We applied FNB to 8 lesions using MBI and FINCORE in oral and maxillofacial lesions. We applied FNB to 8 lesions using MBI and FINCORE (Dr. JAPAN Co., Ltd., Tokyo, Japan) in the diagnosis of oral and maxillofacial lesions. We applied FNB to 8 lesions using MBI and FINCORE (Dr. JAPAN Co., Ltd., Tokyo, Japan) in the diagnosis of oral and maxillofacial lesions.

The histopathological aspect by FNB was compared with the final diagnosis from the surgically resected tissue. The target lesions were accurately biopsied by FNB in all cases, and the accuracy rate of FNB diagnosis was 87.5%.

No complication such as abnormal hemorrhage or nerve damage was seen in all cases. This technique would be a safe, effective means of obtaining adequate tissue for histopathological examination of oral and maxillofacial lesions.

G-48. P-64. Effect of surgical instruments on cytological features

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In the oral cancer surgery, composite resection is indicated. Enough surgical margin is needed to achieve the complete resection. We maxillofacial surgeons are always worrying over how to examine that the surface of surgical margin is cancer free or not. Histopathological examination using frozen-section during surgery is the most common technique to examine the surgical margin. However, it is nothing but checking a part of the lesion.

Then we have applied cytological examination using ultrafast Papanicolaou staining. In this technique, as surgical margin is stamped on the slides, overall surface would be able to be examined. However, as cytological features were degenerated by heating using electric scalpel, accurate cytological evaluation was difficult in some cases. This study was then planned to reveal the effect of surgical instruments on cytological feature.

Five-week-old, 20 - 25 g, male C3H/HeNCrj mice (n = 6) were used. NR-S1 mouse squamous cell carcinoma was transplanted to the middle of the tongue. Tumors that grew to about 7 x 7 mm in size by about 10 days after transplantation were resected by conventional surgical scalpel (No. 11), electric scalpel and Harmonic ScalpelTM. Resected surface was stamped on the slides and stained by routine Papanicolaou and Giemsa. Following the preparation of cytological sample, tissues were processed for paraffin embedded sections.

Thermal degenerative changes were seen especially in the nuclei of tumor cells resected by electric scalpel. Morphological changes were not so specific in the specimens by Harmonic ScalpelTM. Detailed cytological aspect is discussed comparing with histological findings.

G-49. P-65. Our cytological approach to oral lesions

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In the field of oral and maxillofacial surgery, various lesions are manifested including tumor, cyst and inflammation. As most of the oral lesions are visible, biopsy is easily applied. Regarding the history of oral cytology, first case of oral malignancy was reported in 1950’s. Detailed diagnostic standard has not been established. In our hospital, cytology is widely applied for screening of oral lesions.
This study presents our cytological approach to oral lesions. A total number of 754 cytological samples (male: 324, female 430, mean age 64.6-yr-old) were examined from June 2007 to March 2011. Samples were taken from tongue (237 cases), buccal mucosa (138 cases), maxillary gingival (129 cases), mandibular gingival (113 cases), palate (33 cases), lower lips (22 cases), oral floor (16 cases), and upper lips (8 cases), others (58 cases). Papanicolaou classification was Class I (51 cases), Class II (311 cases), Class II or III (57 cases), Class III (98 cases), Class III or IV (12 cases), Class IV (17 cases), and Class V (21 cases).

Biopsy was done in 13 of 57 Class II to III cases, 18 of 98 Class III cases and 10 of 10 Class III to IV cases. Histopathologically, malignancy was detected in 15.4 % in Class II to III cases, 77.8 % in Class II cases, and 100 % in Class III or IV cases with the diagnosis of squamous cell carcinoma, verrucous carcinoma, carcinoma in situ. In this presentation, detailed cytopathological findings are discussed.

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Cytological examination of oral and maxillofacial lesions is minimum invasive and feasible method for benign and malignant lesions, and plays an important role for the making the clinical diagnosis in many cases. In our department, the number of the cases examined by cytology has been increased steadily in every year. And we depend thoroughly on the cytological examination for the making the clinical diagnosis and follow up study of benign and malignant cases.

For making the correct diagnosis of cytological examination, an accurate knowledges for histopathological features of the lesions are important for the cytologist and pathologist. In the present study, we show the relationship between the histopathological, cytological and clinical features in the precancerous lesions, precancerous conditions and malignant lesions. In addition, clinical findings for oral and maxillofacial lesions are also important for the cytological diagnosis.

Thus, we also present and discuss the point aimed at early detection for oral and maxillofacial region for the cytologist and pathologist with special attention to the abnormality of color and shape as compared with normal region in the oral cavity.

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We have established oral cancer detecting system using smear cytology in collaboration with general practitioners and Shimane University Hospital. From December 2008 to February 2011, we have examined 151 cytological specimens, and 5 cases of squamous cell carcinoma were detected histopathologically. Incidence of oral cancer detected in our system was 3.3 percent, suggesting the feasibility of individual screening in oral cancer detection.

However, mass screening is also important for cancer detection, as reported in the other field of malignancies. This time, we had examined about 1,000 people to detect oral cancer following regular general and dental checkup in Iinan-cho, Shimane Prefecture from May to July, 2011.

Following the dental checkup by one dental surgeon, accredited maxillofacial surgeons examined extraoral conditions including cervical lymph nodes and oral cavity bimanually. Cytology was applied to the ulcer-like or swelled lesions with the color of red, white and black while the subjects were seated in the chair under ideal light. Cytological samples taken by cotton swab from tongue, gingival, oral floor, buccal mucosa, palate and other soft tissue in the oral cavity, which were sent to our laboratory and diagnosed following Papanicolaou Classification by specialists in our department. Before and after the screening, questionnaire regarding oral symptom and general knowledge of oral cancer was done to all examinees.

As of 20 June, 510 subjects were examined, and 71 cytological samples were taken. Though no cancer was detected, 16 cases of epithelial dysplasia were pointed out. This paper describes the detailed data at the end of this mass screening scheduled to be completed at the end of this July.