国際セッション口演

（A会場）

5月15日（金） A会場　 9：00～10：20

IO-01
2206

Intracellular cholesterol trafficking is involved in osteoblastic differentiation of MC3T3-E1 cells

Takashi Ode

Keywords: osteoblast, cholesterol, lysosome

Objective: It has been suggested that metabolic syndrome such as obesity is related to periodontal disease. Dysfunction of systemic cholesterol homeostasis has attracted much attention as the onset of the syndrome, mainly via the effects on adipose tissues or the accumulation in cardiovascular vessels. On the other hand, lysosomes, the digestive compartments within the cells, are crucial for the intracellular trafficking of cholesterol via receptor-mediated endocytosis of low-density lipoprotein. Since recent studies suggested that lysosomes are able to regulate cell growth and proliferation by sensing nutrients or stress conditions, we investigated a possible link between the intracellular trafficking of cholesterol and osteoblastic differentiation.

Materials and Methods: Mouse pre-osteoblastic MC3T3-E1 cells were cultured in differentiation medium supplemented with D, L-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), known to cause the accumulation of cholesterol in lysosomes. The cell differentiation was confirmed by ALP and von Kossa staining. The cells were stained with filipin for cholesterol and anti-lysobisphosphatidic acid (LBPA) antibody for lysosomes and observed by confocal microscopy.

Results: The PDMP treatment attenuated the osteoblastic differentiation based on the ALP staining and von Kossa staining. Accumulation of cholesterol in lysosomes was observed in the PDMP-treated cells.

Conclusion: The results suggest that intracellular cholesterol trafficking is involved in the osteoblastic differentiation in MC3T3-E1 cells.

IO-02
2205

Generation of odontogenic cells from human induced pluripotent stem cells

Takehito Ouchi

Keywords: human iPS cells, odontogenic epithelial cells, odontogenic mesenchymal cells

Aim: Periodontitis, dental caries, trauma, and malignant diseases can cause tooth loss, which is generally repaired with artificial materials. However, current artificial materials sometimes cause diseases such as peri-implantitis. Natural tissues are derived from original cells, not from artificial materials. Teeth develop through interactions between odontogenic epithelial cells and mesenchymal cells. Here, we show generation of odontogenic epithelial cells and mesenchymal cells using induced pluripotent stem cell (iPS cell) technology.

Materials and Methods: To generate odontogenic epithelial cells, human iPS cells were induced to differentiate into epithelial cells with odontogenic stimulation. For generation of odontogenic mesenchymal cells, human iPS cells were induced to differentiate into mesenchymal cells with neural crest and odontogenic stimulation. These newly generated cells were analyzed in vitro.

Results: Odontogenic epithelial cells showed a cobblestone appearance, and odontogenic mesenchymal cells formed spindle-shaped colonies. Each cell type expressed appropriate, specific markers.

Conclusion: Here, we show generation of odontogenic cells that may be useful for regenerative medicine such as bioengineered teeth. In addition, we sometimes see edentulous patients with congenital diseases that are caused by developmental arrest of the odontogenic epithelium and mesenchyme. Thus, odontogenic cells generated using iPS cell technology may also be useful for treating specific diseases.

IO-03
2499

Relationship between oxidative Stress and oral disease in a rural area, Lao PDR

Vorasack Phounsiri

Keywords: oxidative stress, epidemiological study, oral disease, rural area, Lao PDR

Objective: In rural areas of developing countries, rapid-aging and reduced life expectancy have been observed, and it has been hypothesized that excess oxidative stress is one cause of the rapid-aging. Oral disease may contribute to one of risk factors in production of oxidative stress. The purpose of this study was to examine the influence of oral disease on oxidative stress in rural area, Lao PDR.

Material and Methods: 50 subjects who are living in rural area in Phone Hong district, Lao PDR were examined as target group, and data were obtained regarding caries experience, probing depth (PD), and bleeding on probing (BOP). The subjects' oxidative stress was evaluated by the diacron Reactive Oxygen Metabolites (d-ROMs) test.

Results: The results revealed that the level of d-ROMs was correlated with age, number of healthy teeth (HT), number of missing teeth (MT), decayed, missing and filled teeth (DMFT) index, number of present teeth (PT), rate of 4 mm PD, and BOP rate. In stepwise multiple regression analysis, BOP rate was the most influential parameter linked to the production of oxidative stress as an independent variable. HT was secondary, followed by number of filled teeth (FT). A control group, 30 residents of Vientiane City with urban was compared with target group. The target group had significantly higher levels of oxidative stress, caries experience, periodontal status, and diastolic blood pressure (BP).

Conclusion: The findings of this study indicate that periodontal disease may increase the production of oxidative stress, and the data obtained suggest that HT and FT contribute to reduction of the level of oxidative stress.

IO-04
2206

Lysosome-associated membrane proteins (LAMPs) regulate bidirectional transport of lysosomes in MC3T3-E1 cells

Anupama Rajapakshe

Keywords: lysosomes, intracellular trafficking, osteoblastic cells

Objectives: In eukaryotic cells, organelles move along microtubules to the minus-ends by cytoplasmic dynein, and to the plus-ends by various members of the kinesin family. Recently, we reported that lysosome-associated membrane proteins-1 and -2 (LAMP-1 and LAMP-2), which are major protein components of the lysosomal membrane, may regulate the intracellular positioning of mitochondria in the pre-osteoblastic cell line MC3T3-E1 [Exp. Cell Res. (in press)]. In the present study, to investigate the regulatory role of LAMPs in the positioning of the intracellular organelle further, we evaluated the role of LAMPs in the bidirectional transport of lysosomes.

Materials and Methods: MC3T3-E1 cells were treated with siRNA against either LAMP-1 or LAMP-2. Then, the plus- or minus-end directed transport of lysosomes was promoted by changing intracellular pH. The localization of lysosomes was observed by immunocytochemistry using antibodies for LAMP-1, LAMP-2 or lysobisphosphatic acid and analyzed using the "Field Particle Vector Analysis" program.

Results: Intracellular acidification caused lysosome dispersion likely related to the increase in the plus-end directed transport, whereas intracellular alkalization caused lysosome accumulation in the perinuclear region due to the increase in the minus-end directed transport. Both transports of lysosomes were impaired when LAMP-1 or LAMP-2 was downregulated in MC3T3-E1 cells.

Conclusion: The result suggests that LAMPs are involved in the bidirectional transport of lysosomes in MC3T3-E 1 cells.

(This work was done in collaboration with Shion Orikasa at TMDU)

IO-05
2402

Serum level of inducible nitric oxide synthase in rat model of preeclampsia-like syndrome induced periodontal disease

Banun Kusumawardani

Keywords: inducible nitric oxide synthase, preeclampsia, pregnancy, periodontal disease

Introduction: Periodontal disease may serve primarily as a vascular stressor and bring an additional infectious/inflammatory burden to the placental-fetal unit, thereby the increasing risk of preterm delivery in preeclamptic women. The tissue injury in inflammation involves the induction of iNOS by certain cytokine or endotoxin, which leads to the production of large quantities of nitric oxide. Objective: This study attempted to evaluate the serum level of iNOS in the rat model of preeclampsia-like syndrome induced periodontal disease.

Methods: Female rats were infected with live-*Porphyromonas gingivalis* at concentration of 108 cells/ml into subgingival sulcus of the maxillary first molar before and/or during pregnancy. The serum level of iNOS was observed in two groups of healthy pregnant and preeclampsia on gestational day (GD)-14 and GD20. The level of iNOS was measured in maternal serum samples using ELISA kits.

Results: On GD14, the serum level of iNOS demonstrated a similar increased trend in healthy pregnant group (4.38 ± 1.31) and preeclampsia group (4.77 ± 2.29); however, the increase was not statistically significant (P=0.75). On GD20, the sera from preeclampsia group (4.96 ± 2.46) had significantly lower iNOS level compared with healthy pregnant group (9.88 ± 3.01). Conclusions: The serum level of iNOS increases with advancing gestation during normal pregnancy; however, the reducing serum level of iNOS may have an adverse effect on placental hemodynamic function in preeclampsia. Periodontal disease could be an important trigger of the chronic inflammatory response that characterizes preeclampsia.

IO-06
2504

Use of BMP-2 and GDF-5 in periodontal tissue engineering

Jung-Seok Lee

Keywords: BMP-2, GDF-5, growth factor, periodontal tissue engineering, regeneration

During several past decades, many researchers and clinicians have been interested in tissue engineering using growth factors; bone morphogenetic protein, platelet-derived growth factor, growth and differentiation factor, and etc. Among these, bone morphogenetic protein-2 (BMP-2) was spotlighted for its inductive effects of mineralized tissue, and approved for clinical application by FDA. However, several recent studies demonstrated not only mineralized tissue formation but also adipose tissue formation. Because of complex of many cascades involving growth factors, in vivo or clinical results could be produced differently from our expectation, or our misunderstanding on growth factors might induce these differences. Recently, growth and differentiation factor-5 (GDF-5) was introduced in periodontal regeneration, and evaluated in vivo using standardized periodontal defect model. This presentation will show researcher's expectations on BMP-2 and GDF-5, and in vivo results showing failure of our expectations in several experimental and clinical studies.

ACKNOWLEDGEMENT: This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant no. NRF-2013R1A1A2060464).

IO-07
2206

The effect of rHAm on the osteogenic differentiation of human PDLCs under inflammatory microenviroment

Zhongchen Song

Keywords: periodontitis, periodontal regeneration, inflammatory microenvironment, recombinant amelogenin, periodontal ligament cells

Objectives: This study was to investigate the effects of recombinant human amelogenin (rHAm) on the osteogenic differentiation of human periodontal ligament cells (PDLCs) under inflammatory microenvironment in vitro, and to observe the role of wnt/beta-catenin signaling.

Material and Methods: Human PDLCs were isolated in vitro. The proliferation rates of human PDLCs were tested by MTT assay. The PDLCs' osteogenic differentiation was investigated by real-time PCR, western-blot, and ALP activity under different concentration of *P.gingivalis* LPS, rHAm, and rHAm with *P.gingivalis* LPS. The expression of genes (LRP5, LRP6, wnt1, β-catenin) were detected by real-time PCR.

Results: The proliferation of human PDLCs was inhibited by 10 μg/ml LPS, and the gene expression of osteogenic differentiation including ALP, RUNX2, Collagen I, BMP2 siginificantly decreased. When PDLCs treated with 10 μg/ml LPS plus 20 μg/ml rHAm, LPS-induced inhibition of PDLCs was attenuated and genes of osteogenic differentiation were upregulated. Furthermore, ALP staining assay showed that the expression of ALP increased by rHAm. In addition, the gene expression of wnt signaling (Wnt1, LRP5, LRP6, β-catenin) involved in the rHAm osteogenic role.

Conclusion: 10 μg/ml *P.gingivalis* LPS inhibits the osteogenic differentiation of human PDLCs. However, 20 μg/ml rHAm up-regulates LPS-mediated the reduction of osteogenic differentiation of PDLCs under inflammatory microenvironment. All these effects could be modulated through wnt/β-catenin signaling.

IO-08
2504

Gelam (*Melaleuca Cajuputi*) Honey : Potential Malaysian Honey in reducing inflammatory mediator and preventing bone loss in induced periodontitis rats

Badiah Baharin

Keywords: honey, gelam honey, periodontitis, periodontal disease, animal studies

Honey is a natural product which have been proven to have antimicrobial and anti-inflammatory action against periodontal disease. Malaysian, Gelam (Melaleuca Cajaputi) honey which is cheaper and readily available in the country was also proven to have antibacterial, antioxidant and antibacterial by local reseachers. However this honey has not been studied on periodontal tissues. The aim of this study was to evaluate its effect to reduce the level of inflammatory marker interleukin-1β (IL-1β) and prevent alveolar bone loss in induced periodontitis Sprague–Dawley rats. Thirty male rats were divided into 4 groups: NLS (control periodontitis treated with saline), NLH (control treated with 3g/kg Gelam honey), LS (periodontitis treated with saline) and LH (periodontitis treated with 3g/kg Gelam honey). On day 15, rats were sacrificed and plasma was collected and analysed using ELISA while the tissue sample was analysed histologically and immunohistochemically. Alveolar bone level was also determined by radiography and histomophometry. The LS group exhibited significantly higher levels of IL-1β locally than any other groups ($p<0.05$). No significant difference of the level was found in plasma IL-1β ($p>0.05$). There was no significant difference in alveolar bone level and number of osteoclasts between groups found. In conclusion, Gelam honey was able to reduce the level of IL-1β locally in periodontitis rats treated with honey (LH) in tissue samples compared to the ligature induced periodontitis rats treated with saline (LS). However Gelam honey was unable to prevent alveolar bone resorption between control and test groups.