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Cell-Specific Regulation of TGF-β1 Promoter in Dermal Papilla Cells from Androgenetic Alopecia

S. Inui, T. Nakao, S. Itami

Department of Regenarative Dermatology, School of Medicine, Osaka University, Osaka, Japan

Aims: We have already reported that TGF-β1 is up-regulated by androgen in dermal papilla cells (DPCs) from androgenetic alopecia (AGA) using our coculture system of DPCs transiently transfected with androgen receptor (AR) expression vector and keratinocytes (KCs). This finding suggested that the regulation of TGF-β1 by androgen is a key step in the pathogenesis of AGA. Our purpose is to examine the possible regulatory mechanism of TGF-β1 gene expression by androgen.

Methods and Results: We analysed TGF-β1 promoter activity of the pTGF-β1-Luc vector, which was constructed by connecting the 5' upstream region of the TGF-β1 gene (-1362 bp upstream from the transcription start site) to the luciferase reporter gene. When pTGF-β1-Luc and AR expression vector were cotransfected in DPCs from AGA, R1881 increased luciferase activity to around 10-fold. In contrast, this induction was not observed in CV-1 cells and transformed DPCs. Thus the regulation of promoter activity by androgen is cell-specific. From analysis of deleted pTGF-β1-Luc vector using DPCs from AGA, there are two possible regulatory regions (-1131~-731 and -459~-323) and two negative regulatory regions (-1326~-1127 and -735~-459). The region of -1131~-731 region contains the androgen-responsive consensus region (5'-GCC AGT TGG CGA GAA CAG TTG GCA CGG G).

Conclusions: We suggested the possibility that this region might function as a key regulatory site for androgenetic alopecia.

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Prevention of Amyloid β-Induced Memory Impairment by Fluvastatin, Associated with the Decrease in Amyloid β Accumulation and Oxidative Stress in Amyloid β Injection Mouse Model

H. Kurinami[1], *N. Sato*[1], *M. Shinohara*[1], *D. Takeuchi*[1], *S. Takeda*[1], *M. Shimamura*[2], *T. Ogihara*[3], *R. Morishita*[1]

[1]Department of Clinical Gene Therapy, Graduate School of Medicine, Osaka Universitsy, [2]Department of Advanced Clinical Science and Therapeutics, Graduate School of Medicine, The University of Tokyo, [3]Osaka General Medical Center, Osaka Prefectural Hospital Organization

Aims: Alzheimer disease (AD), the most common cause of dementia in the elderly, is characterized by Aβ-containing plaques and neurofibrillary tangles, synaptic and neuronal loss, along with progressive cognitive impairment. Although there are growing evidences suggesting the beneficial effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) on AD, this notion is still controversial. We investigated the efficacy of statins for Aβ-induced cognitive impairment.

Methods: We employed male ddy mice (6 weeks old), which were injected Aβ1-40 into the cerebral ventricle. Fluvastatin (5 mg/kg/day) was administered ollary for 2 weeks before Aβ injection, and mice were treated for a further 3 weeks. Then water-finding task were performed to evaluate cognitive function. Biochemical and immunohistochemical analysis were also performed.

Results: The present study demonstrated that pretreatment with fluvastatin, but not post-treatment just after Aβ exposure, prevented Aβ-induced memory impairment. We also observed that fluvastatin significantly decreased Aβ accumulation and oxidative stress after Aβ injection. Mice treated with simvastatin did not demonstrate prevention of Aβ-induced memory impairment, and showed no significant decrease in oxidative stress. More importantly, fluvastatin significantly prevented the loss of neurons in the basal forebrain induced by Aβ.

Conclusions: The present study demonstrated that fluvastatin significantly prevented memory impairment induced by Aβ. The beneficial effects of fluvastatin might be explained by the preservation of neurons through a significant decrease in Aβ accumulation and oxidative stress. In clinical practice, the timing of start of treatment with fluvastatin might be critical to acquire a beneficial effect on cognitive function.

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FGF2 Stimulates Preadipocyte Differentiation through a Reduction in Intracellular TAZ Protein

H. Eda[1,2], *K. Aoki*[1], *H. Ohtomo*[3], *K. Ohkawa*[1]

[1]Department of Biochemistry, The Jikei University School of Medicine, Tokyo, Japan, [2]Department of Orthopaedic Surgery, Chikuma Central Hospital, Nagano, Japan, [3]Department of Pain Clinic, The Jikei University School of Medicine, Tokyo, Japan

Aims: In recent years, excess visceral fat accumulation or obesity has reached epidemic proportions in the developed world, resulting in an increase in the prevalence of metabolic syndrome. Many studies have reported the importance of the rule of adipocytes as endocrine cells for energy metabolism and insulin sensitivity in metabolic syndrome [1][2][3]. Some of the mechanisms of adipocyte differentiation have been clarified, such as the role of PPARgamma in the transcription mechanism [4]. It is known that fibroblast growth factor 2 (FGF2) signaling induces the expression of PPARgamma [5] and the relation between FGF2 and the adipogenic differentiation [5] [6], however the detailed mechanism is still unclear.

Previously it has been reported that the transcriptional coactivator with PDZ-binding motif (TAZ) protein acts as a transcriptional regulator for the differentiation of mesenchymal stem cells into osteoblasts and adipocytes [7]. Additionally we reported that FGF2 reduces the TAZ protein in osteoblastic cells and that the expression of TAZ is involved in osteoblast proliferation and differentiation [8]. Because preadipocytes and osteoblasts derive from mesenchymal stem cells, this study investigates the detailed mechanism of FGF2 stimulation on the adipogenic differentiation.

Methods: Preadipocyte-like cells, MC3T3-G2/PA6, were cultured with various concentrations of FGF2 or without. Two days later, total mRNA was extracted from these cells and aP2 mRNA and osteocalcin mRNA, which are the markers for adipocyte and osteoblast differentiation, respectively, were detected by RT-PCR. Additionally, TAZ protein levels in these cells were detected using western blotting.

Results: FGF2 increased aP2 mRNA level, dose dependently and decreased osteocalcin mRNA level in preadipocyte-like cells. Furthermore, FGF2 significantly decreased intracellular TAZ protein levels which act as a corepressor of PPARgamma, an aP2 transcriptional factor, and a coactivator of Runx2, an osteocalcin transcriptional factor.

Conclusions: Our previous report demonstrated that FGF2 stimulated preosteoblast proliferation but inhibited differentiation and calcification through a decrease in the TAZ protein level [6]. The present study indicates that FGF2 signaling decreases the intracellular TAZ protein, and the reduction of TAZ increases aP2 mRNA through activation of PPARgamma. The effects of FGF2 signaling may be a key cause of adipogenic differentiation. These results contribute to understanding of the mechanism of adipogenic related deseases.

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4

Lifespan Extension of Caenorhabditis Elegans by an Antioxidant, Platinum Nanoparticle

J. Kim[1], *M. Takahashi*[2], *T. Shimizu*[2], *T. Shirasawa*[3], *M. Kajita*[4], *A. Kanayama*[5], *Y. Miyamoto*[1]

[1]Department of Integrated Biosciences, University of Tokyo, Chiba, [2]Research Team for Molecular Biomarkers, Tokyo Metropolitan Institute of Gerontology, Tokyo, [3]Department of Aging Control, Graduate School of Medicine, Juntendo University, Tokyo, [4]APt Co., Ltd, Tokyo, [5]Department of Global Infectious Diseases and Tropical Medicine, National Defense Medical College, Saitama, Japan

Aims: Recently, we have shown that platinum nanoparticles (nano-Pt) are a superoxide dismutase (SOD)/catalase mimetic. Accumulated data indicate that antioxidants are helpful materials to extend the *Caenorhabditis elegans (C. elegans)* lifespan. However, the effect of well-known SOD/catalase mimetic EUK-8 on the lifespan is controversial. Thus, further clarification of the effects of SOD/catalase mimetics on lifespan is needed. The present study was designed to elucidate the survival benefit conferred by nano-Pt, as compared to EUK-8.

Methods: We cultured age-synchronized wild-type N2 and *mev-1(kn1)* mutant in liquid culture medium with nano-Pt or EUK-8 at 20°C. The *mev-1(kn1)* mutant has the shortened lifespan due to excessive oxidative stress. We counted survivals and transferred them into fresh medium every other day. In the paraquat assay, we measured the survival of nano-Pt or EUK-8-pretreated worms with 0.4 M paraquat incubation for 12 hrs. Fluorescent intensity of lipofuscin was estimated with 10-day-old worms and detection of ROS with fluorescent probe DCF was performed with nano-Pt or EUK-8-pretreated worms for 2 days from day 5. Thermotolerance assays were performed with 5-day-old N2 at 35°C for 16 hrs. Moreover, we monitored the effects of nano-Pt on dietary restriction.

Results: At 0.5 mM, nano-Pt significantly extended the lifespan of N2 nematodes and at 0.25 and 0.5 mM, nano-Pt recovered the shortened lifespan of the *mev-1(kn1)* mutant. In both instances, EUK-8 at 0.05, 0.5, and 5 mM did not extend *C. elegans* lifespan. When 0.4 M paraquat was loaded exogenously, nano-Pt (0.1 and 0.5 mM) and EUK-8 (0.5 and 5 mM) were effective in rescuing worms. Moreover, 0.5 mM nano-Pt significantly reduced the accumulation of lipofuscin and ROS induced by paraquat. We measured the in vitro dose-dependent quenching of O_2 and H_2O_2, indicating that nano-Pt is a more potent SOD/catalase mimetic than EUK-8. Nano-Pt neither increased thermotolerance nor interacted with dietary restriction signal for lifespan extention.

Conclusions: These results suggest that exogenously-treated nano-Pt can extend lifespan of *C. elegans* by reducing oxidative

stress, regardless of thermotolerance or dietary restriction. Both of nano-Pt and EUK-8 counteract acute oxidative stress such as paraquat-induced. Taken together, nano-Pt has interesting anti-aging properties.

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Lifestyle Factors, and the Radial Artery Augmentation Index in Post-Menopausal Women

Kaoru Fujii[1], *Ayako Higo*[1], *Hiroshi Hirose*[1,2], *Hirokazu Yokoyama*[1,2], *Takanori Moriki*[1,2], *Hirotaka Shibata*[2], *Minako Tsujioka*[1,2], *Hiroshi Kawabe*[1,2], *Ikuo Saito*[1,2]

[1]Health Center, [2]Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan

Aims: Augmentation Index (AI) is a parameter that reflects including aortic compliance, central blood pressure, and cardiac load. The radial artery augmentation index (rAI) is significantly correlated with the aortic augmentation index (Aortic AI), and has been tentatively used as an alternative to the aortic AI clinically. We report here the relationship between rAI measurements and lifestyle factors in pre- and post-menopausal women.

Methods: The study subjects were 327 women (age: 50 ± 7 years). We categorized the subjects into pre-menopausal and post-menopausal groups, and then according to smoking status, alcohol consumption level, exercise level, and salt intake. We analyzed the relationships between these factors and rAI. We used the HEM-9000 AI Omron Digital automated sphygmomanometer for rAI measurements and rAI values were adjusted for a pulse rate of 75 beats/min. Although a well-trained nurse conducted the rAI measurements, an error pertaining to the sensor position occurred at a frequency of one in three people. Both the distance to the reflection point and the reflection efficiency affect rAI measurement; thus, before using measured values for analysis, variation should be monitored and reproducibility assured.

A self-administered questionnaire was used to obtain details about the subject's lifestyle, and Excel Eiyo-kun FFQg software (Kenpakusha) was used to calculate salt intake. The Mann-Whitney U-test was used to compare the two groups, and a difference of $P<0.05$ was considered statistically significant.

Results: Age and pulse pressure were both positively correlated with rAI, and the post-menopausal group (N = 167) had higher rAI values than the pre-menopausal group (N = 160). Both total cholesterol and low-density lipoprotein cholesterol were positively correlated with rAI in the pre-menopausal group, but not in the post-menopausal group.

In the post-menopausal group, rAI values were higher in smokers than in non-smokers ($P=0.0160$), but there was no difference between smokers and non-smokers in the pre-menopausal group, which suggests that women become more sensitive to tobacco after menopause. In the pre-menopausal group, subjects who engaged in regular exercise had significantly lower rAI values ($P=0.0317$) than the subjects who did not exercise regularly. For alcohol consumption and salt intake, there was no difference in rAI values between the pre-menopausal and post-menopausal groups.

Conclusions: In the post-menopausal women, rAI values were influenced by smoking. In the pre-menopausal women, the subjects who engaged in regular exercise had significantly lower rAI values than the subjects who did not exercise regularly.

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Multiple Cytokine/Chemokine Analysis Related to the Development of Impaired Glucose Metabolism and DM in Human Plasma

Kazuko Uno[1], *Masumi Tominaga*[2], *Goji Hasegawa*[2], *Michiaki Fukui*[2], *Katsumi Yagi*[1], *Mari Tanigawa*[1], *Setusya Fujita*[1], *Toshikazu Yoshikawa*[3], *Naoto Nakamura*[2]

[1]Louis Pasteur Center for Medical Reserch, [2]Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, [3]Department of Gastoroenterological Medicine, Kyoto Prefectural University of Medicine, Graduate School of Medical Science

Aim: Cytokines and chemokaines are suspected to have a role in the development of impaired glucose tolerance (IGT) and diabetes mellitus (DM). We aim to characterize the pattern for the status of 3 groups (healthy subjects, IGT patients, and DM patients) through an analysis of multiple cytokine and chemokine levels in their blood plasma

Methods: We determined 27 cytokines/chemokines (IL-1 β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, TNF-α, Eotaxin, FGFbasic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-bb, RANTES, VEGF) in human plasma using the beads array. For this study, we included healthy subjects and subjects with IGT, and DM. They were classified into 3 groups based on the data from complete medical checkups.

Results: In comparison to healthy subjects, subjects with IGT showed elevated levels of MIP-1α, IL-2, TNF-α, IL-12p70, IL-13, IL-6, MIP-1β, Eotaxin, IL-1ra. Where as subjects with DM showed elevated levels of IL-2, IL-12p70, MCP-1, Eotaxin, IL-1ra, but reduced levels of IL-4 and IL-17.

Discussion: These results indicate that impaired glucose homeostasis leads to changes of cytokine and chemokine networks, thus indicating that the production of cytokines/chemokines by immune cells, adipose tissues, and endothelial cells play important roles in the development of IGT and DM. This suggests a kind of feedback loop of causality where the artherogenesis induces inflammation and cytokine/chemokine production, which in turn advances the artherogenesis. This process significantly contributes to the development of IGT in to DM. As the multi-cytokine/chemokine analysis revealed that many kinds of cytokines/chemokines are related to the development of IGT and DM, these results will be useful for further research into preventative intervention. Additionally, analysis of the cytokines/chemokines identified in this paper will be useful as bio-parameters to know the risk of DM/artherogenesis development. Additionally, these data will be useful in determineing the risk of the development of DM or artherogenesis.

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Early-Onset of Aging-Related Diseases in Type III Na-Dependent Phosphate Transporter Overexpressing Rat

Shogo Asano, Atsushi Suzuki, Sahoko Sekiguchi, Megumi Shibata, Mitsuyasu Itoh

Division of Endocrinology and Metabolism, Department of Internal Medicine, Fujita Health University, Toyoake, Aichi, Japan

Aims: Suitable mammalian models for aging are desirable to study human age-associated pathology. Among these model animals, klotho-deficient mouse could generate multiple premature aging-like features with hyperphosphatemia. Type III sodium-dependent inorganic phosphate (Pi) transporter, Pit-1, is a ubiquitous protein. Pit-1 is essential to maintain the cell activity, because Pi should be supplied for ATP synthesis. However, accumulating evidences suggest that Pi overload from the extracellular millieu would be stress for the cells, because Pi uptake itself might induce the formation of apoptosome, resulting in the apoptosis of the cells. The aim of the present study was to investigate the effect of Pit-1 overexpression on the development of different organs.

Method: Mouse Pit-1 gene was ubiquitously expressed under the control of CAG promoter in transgenic (Tg) rats, and pronuclear DNA microinjection into the rat zygotes. All rats were allowed free access to tap water and a normal rodent chow. Proteinuria was measured by the biurent method on a 24 h urine collection at each time points. Routine serum chemistries were measured by an automatic analyzer.

Results: An early onset of cataract development was observed after 2 to 8 weeks of age. Progressive degeneration of core protein of lens occurred in accordance with aging. Skeletal development and height were normal in Tg compared with WT animals. However, later in their life, Tg rats had lower bone mass, analyzed by DXA and μCT, suggesting the development of early-onset osteoporosis. Body weight was significantly decreased in Tg compared with WT rats, a difference that was more pronounced in male compared with female animals. Tg rats showed progressive proteinuria associated with hypoalbuminemia and dyslipidemia, suggesting the development of nephrotic syndrome. Proteinuria was detected at 3 months of age and glomerular abnormality assessed by transmission electronmicroscopy already observed in kidneys of 8 weeks old Tg rats indicating the development of nephrotic syndrome. Survival was much shorter in Tg rats. Tg rats died because of malnutrition and cachexia between 7 and 9 month of age.

In conclusion, results presented in this study suggest that Pit-1 overexpression induces cellular stress on several organs, resulting in the early-onset of aging-related diseases such as cataract and osteoporosis. In addition, phosphate overload causes alteration in the integrity of the glomerular system leading to nephrotic syndrome.

8

The Relationship between a Habit and Functional Age, Oxidative Damage Degree and Anti-Oxidative Ability

T. Kusano, T. Higuchi

Department of Ant-Aging Dock, Aeba Surgical Hospital, Japan

Aims: This study is aimed to examine the relationship between a habit and functional age and the oxidative stress markers.

Methods: The subjects were 104 (52 men, 29-85 years old, 52 women, 30-84 years old) who underwent Anti-Aging Dock in this hospital from June 2006 to April 2008. For diagnosis of functional aging, the muscle age was evaluated by body composition analysis measuring weight bearing index (WBI), blood vessel age by pulse velocity analysis, neurological age by Wisconsin Card Sorting test (WCST), hormone age (serum IGF-I, DHEA-s), and bone age by DEXA measuring bone mineral density (BMD). For the risk factors, oxidative stress (urine 8-OHdG, isoprostane, STAS: serum total anti-oxidative score, OSPPI: oxidative stress prospective index), mental stress (serum cortisol, DHEA-s/cortisol ratio), and metabolic function. In addition, the anti-aging QOL common questionnaire data were collected from all subjects. They were divided into 2 groups according to good or bad habits in eating, exercise, sleep, smoking, drinking, and mental symptoms. The statistical analysis was performed for these parameters.

Results: "Good eating" group tended to be younger in muscle age and hormone age. WBI was 7.7% higher (0.821±0.120, P=0.031) and cortisol was 25.0% higher (13.20±5.74μg/dl, P=0.034). STAS was 13.1% higher only in men (1261±70, P=0.000). "Good exercise" was significantly associated with muscle age (P=0.010) and hormone age (P=0.038). BMD was 10.9% higher in men (1.141±0.178g/cm 2, P=0.043). High sensitive CRP was −50.7% lower (52.2±45.6μg/dl, P=0.021) and urine 8-OHdG production was 40.2% higher in women (8.30±4.64ng/kg/hr, P=0.042). "Bad sleep" (below 6 hours) was significantly associated with hormone age, decreasing DHEA-s (766±472ng/ml, by −34.0%, P=0.030,) and DHEA-s/cortisol ratio (7.05±4.41, by −39.0%, P=0.013). "Smoking" was significantly associated with blood vessel age (P=0.015), increasing serum homocystein (12.51±5.89nmol/ml, by 36.9%, P=0.006). It significantly affected hormone age (P=0.003) and bone age (P=0.019) in men, decreasing IGF-I (151.1±41.5ng/ml, by −24.6%, P=0.045), BMD (YAM 86.53±14.71%, by −9.7%, P=0.044) and OSPPI (5.9±21.1, by −72.3%, P=0.034). Non-smoking habits positively affect muscle age in women, increasing WBI by 12.9% (0.746±0.069, P=0.011). "Bad drinking" (over 30 mg/day alcohol) deteriorated hormone age in men (P=0.007), decreasing IGF-I (161.7±48.2ng/ml, by −24.8%, P=0.029), however in women, increasing IGF-I (196.3±47.4ng/ml, by 34.3%, P=0.051). In both sex, isoprostane generation rate was increased (3.49±3.94 ng/kg/hr, by 96.0%, P=0.007). "Bad mental symptoms" were significanly associated with muscle age (P=0.004) and hormone age(P=0.004), decreasing DHEA-s/cortisol ratio (8.84±5.57, by −32.6%, P=0.003) and STAS (1166±95, by −4.7%, P=0.013).

Conclusion: Our data indicate that bad habits can affect the functional aging partially mediated by oxidative stress. It will be important that practicants repeat examinations of the statistics of clinical inspection data and establish a scientific evidence for an anti-aging medicine.

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Effects of Cultured Autologus Fibroblast Grafting on the Rejuvenation of Facial Skin

Y. Hori[1, 4], *H. Kagami*[2], *S. Oshima*[1], *K. Fushimi*[1], *A. Hayashi*[1], *S. Nakada*[3], *Y. Watanabe*[4]

[1]TES Holdings Co. Ltd, [2]The Institute of Medical science, University of Tokyo, [3]CIINTEXE Clinic, [4]Department of Pharmacology and Pharmacotherapy, Nihon Pharmaceutical University

Aim: Although xenogenic collagen and hyaluronic acid have been widely used as soft tissue fillers, they might cause adverse effects such as allergic reaction and infection. Accordingly, novel fillers that are less allergic and longer lasting have been awaited. Recently, we have introduced a novel skin rejuvenating treatment using autologous fibroblasts, which would be able to overcome those shortcomings of xenogenic fillers. To date, the effect of autologous fibroblast grafting has been evaluated mostly by subjective manner and there is limited information for the objective findings. In this study, we evaluated the clinical effects of cultured autologus fibroblast grafting on skin surface topography using 3D-replica analyzing system.

Methods: From January 2007 to April 2008, 30 subjects including 8 males and 22 females (between 30 and 66 years old, 50±12 in average of age) have been treated. Before the treatment, both the general health condition and the presence of infectious diseases were screened. Autologous fibroblasts were obtained from skin biopsies. After enzymatic digestion, the primary culture of fibroblasts was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% autoserum at 37°C under the presence of 5% CO_2 for 6-8 weeks. On the treatment day, the cells were detached from the dishes, washed and resuspended in saline. For the injection, $3x10^7$cells were used and injection was repeated twice every two weeks. At one and six months after the injection, the condition of skin was analyzed using 3D-replica analyzing system. Statistical differences were determined by paired-*t* test. A P value less than 0.05 was considered statistically significant.

Results and Discussion: Inflammation seen in the injected region was disappeared within a few days after the treatment. The results from silicon 3D-replica analyses showed that our newly developed skin rejuvenating treatment increased skin surface fineness for 63.3% and reduced the depth of wrinkles by 6.2% at 6 months after treatment. Moreover, these improvements were getting to be better and better day by day. And such improvements between age of the subjects were not detected any significant differences. In addition, the improvements of female subjects were about 1.4 times much more than that seen in male subjects. These results indicate the importance of daily skin care such as maintenance of skin moisture. Thus this newly developed skin rejuvenating treatment might be highly potent for skin anti-ageing. Since the improvement was observed even at 6 month after treatment, the effects of this new treatment will be maintained over years. Through this treatment, no serious adverse effect has been observed. Although the underlying mechanisms of the efficacy are not fully understood, the results from basic study showed ultrastructural changes consistent with new collagen formation at the end of the 3-month treatment period.

Conclusion: This study documents that the efficacy of our newly developed skin rejuvenating treatment was clearly proved, which showed greater longevity, fewer side effects and more natural appearance.

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Oxidative Stress Marker 8-Hydroxy-2'-Deoxyguanosine (8-OHdG) and Periodontitis in Saliva

T. Komatsu[1], *A. Sasahara*[1], *Y. Maehata*[2], *Y. Inoue*[3], *A. Miyagi*[1], *MC-il. Lee*[2], *M. Ikeda*[4]

Department of Clinical Care Medicine, [1]Division of Dentistry for Special Patients, [2]Pharmacology and ESR Laboratories, Kanagawa Dental College, [3]Kanagawa Children Hospital, [4]Department of Comprehensive Dentistry, KDC Yokohama Clinical Training Center, Yokohama, Japan

Aims: The 8-hydroxy-2'-deoxyguanosine (8-OHdG) is well known as a biomarker to evaluate oxidative stress-induced disease such as Alzheimer disease or early aging. Recently, 8-OHdG has also been a useful biomarker for assessing status of periodontitis. It is well known that early aging including rapidly progressive periodontitis occurs in Down syndrome (DS) patients. We had reported that the generation of reactive oxygen species (ROS) increased in cultured gingival fibroblasts (GF) from DS patients. Thus, the aim of this study was to evaluate oxidative stress and periodontitis from DS using salivary 8-OHdG.

Methods: The study group consisted of DS patients aged from 1year to 39 years and systemically healthy subjects (control) aged from 4 years to 45 years. Informed consent was obtained and care was taken to ensure that none of these individuals were under any medication. The levels of periodontal status were judged from standard measurements of probing depth (PD), gingival index (GI). The salivary levels of 8-OHdG were determined using an enzyme-linked immunosorbent assay.

Results: The mean values of clinical indices, PD and GI, were not significantly different between young (≦12 years) patients with DS (DS-1) and controls (C-1; young), adults (from 30 years to 45 years) patients with DS (DS-2) and controls (C-2; adults). The salivary levels of 8-OHdG in DS-1 and DS-2 were significantly higher than those in C-l and C-2.

Conclusion: These results suggest that the progressive oxidative stress in DS due to increased the value of 8-OHdG in saliva. These redox imbalances may occur to storage ROS in DS. The high oxidative stress of DS in saliva may lead to clinical features of DS, especially early aging including rapidly progressive periodontitis.

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Therapeutic Effect of Parthenolide on Androgenic Alopecia

Tetsuya Tomita, Daiki Morimoto, Hideki Yoshikawa

Department of Orthopaedics, Osaka University Graduate School of Medicine

Introduction: There are many patients with androgenic alopecia (AGA). Dihydrotestosterone (DHT) that is one of androgenic hormones was considered to be a major cause of AGA. There are many drugs for AGA, which inhibit transforming testosterone into DHT, but the etiology of AGA remained unclear.

It has been well known that feverfew have the potent role of anti-inflammatory and used as folk remedy for attack of fever. Recently, the component of feverfew was analyzed and the main component is clarified to be parthenolide (PTH). It was demonstrated that parthenolide inhibited the nuclear transition of NF-kB, which was important transcription factor and promoted osteoclast formation.

In previous study, we demonstrated that parthenolide was effective for rheumatoid arthritis (RA). Recently patients with RA and AGA who has been administered PTH orally for RA treatment has shown the effect of restoring hair. In this study, we evaluated the effect of PTH for AGA *in vivo* and *in vitro* experiments.

Material and Methods: *In vivo* experiment, the hair of C3H/He male mouse on their back was shaved at day 0. In control group, 0.5%methyl cellulose solution at 10 ml/kg as vehicle was administered p.o and in PTH group, 40mg/kg parthenolide solution was administered p.o from day 1 to day 19. The photograph of growing area of hair on the back was taken every other day and measured the ratio of restore hair area on the back. At day 19, all mice were sacrificed.

Human follicle dermal papilla cells (HFDPCs) from white male were purchased and used for *in vitro* experiments. In control group, HFDPCs were cultured with medium and in DHT group HFDPCs were cultured with 50 nM DHT. In PTH group, HFDPCs were cultured with 2 μg/ml PTH. Cell proliferation was evaluated with BrdU assay. In control, DHT and PTH group, HFDPCs were cultured and nuclear protein was extracted. The amount of NF-kB p65 protein from nuclear protein of cultured cells was measured with the optical density at the wavelength of 450 nm and calibrated by standard protein.

Results: *In vivo* experiment, the hair on the back showed growing faster in PTH group than that in control group and mean ratio of restore hair area on the back showed significant differences between control and PTH group at day 15,17 and 19.

In vitro experiments, there were no significant differences in BrdU assay between control and DHT and PTH group. The amount of NF-kB p65 protein from nuclear protein was 2.54 ng in control group, 2.28 ng in PTH group and 4.33 ng in DHT group respectively. Although there were no significant differences between three groups, these results suggested that the amount of NF-kB p65 protein from nuclear protein was higher tendency in DHT group than that in control group.

Conclusion: In this study, it was suggested that parthenolide have potent role of growing hair *in vivo* experiment and the amount of NF-kB p65 protein in nuclear protein increased in DHT group. These results suggested that in the etiology of AGA there were novel pathway in which NF-kB was involved.

Although the effect of parthenolide in the etiology AGA remained unclearly, it was demonstrated that parthenolide was promising for AGA treatment in this study.

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Novel SNP Detection as a Genetic Marker for Spinal Osteoarthritis in Japanese Postmenopausal Women

Tomohiko Urano[*†], *Ken'ichiro Narusawa*[‡], *Masataka Shiraki*[§], *Takahiko Usui*[*†], *Yasuyoshi Ouchi**, *Toshitaka Nakamura*[‡], *Satoshi Inoue*[*†]

From the *Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, †Department of Anti-Aging Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, ‡Department of Orthopedic Surgery, University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Japan, §Research Institute and Practice for Involutional Diseases, Nagano, Japan

Spinal Osteoarthritis is a very common condition in the axial skeletons of aged people and a major cause of back symptoms. Vertebral osteophytes, endplate sclerosis and intervertebral disc narrowing are recognized as characteristic features of spinal disc degeneration. Recent studies indicate that the appearance of these radiographical features is influenced by genetic factors, physical loading and other environmental factors. Moreover, spinal osteoarthritis has been shown to have a familial component and in some studies to be influenced by specific genetic risk factors. In the present study, to identify common genetic variants associated with spinal osteoarthritis,we examine an association between polymorphisms in bone and cartilage metabolism-related genes and radiographic feautures of spinal osteoarthritis including osteophyte formation, endplate sclerosis and disc space narrowing number.

For this purpose, we evaluated the presence of osteophytes, endplate sclerosis, and narrowing of disk spaces in Japanese postmenopausal women. The severities of spinal degeneration including osteophyte formation, endplate sclerosis and disc space narrowing were assessed semi-quantitatively from Th4/5 to L4/5 disc level or from Th4 to L4 vertebrae by using the grading scale of Genant. Then we assessed radiographical spinal osteoarthritis using scoring system. Briefly, osteophyte formation at a given disc was graded 0-3 degrees, endplate sclerosis at given vertebra was graded 0-2 degrees, and disc space narrowing was graded 0-1 degrees. Then we defined sum of each degree from Th4/5 to L4/5 disc level for osteophyte formation on anteroposterior radiographs as a score of osteophyte formation. We also defined sum of each degree from Th4 to L4 vertebra for endplate sclerosis and that from Th4/5 to L4/5 disc level for disc space narrowing on lateral radiographs as a score of endplate sclerosis and disc narrowing, respectively. The selected SNPs in bone and cartilage metabolism-related genes were extracted from the Assays-on-Demand SNP Genotyping Products database (Applied Biosystems, Foster City, CA) and genotyped using the TaqMan (Applied Biosystems) polymerase chain reaction (PCR) method according to the manufacture's protocol.

In these search, we found the association of single-nucleotide polymorphism (SNP) in the insulin-like growth factor-1 receptor (IGF1R) with spinal disc narrowing. We compared those who carried the G allele (GG or GC) with those who did not (CC) in the IGF1R gene at intron 1 (rs11247361). We found that the subjects with the G allele (GG or GC) were significantly over-represented in the subjects having higher disc narrowing score. We also found that a synonymous SNP (Q89R) in the LRP5 gene (rs41494349) is significantly associated with spinal osteophyte formation score and a SNP at 3' UTR region in the Wnt-1-induced secreted protein 1 (WISP1) gene (rs2929970) is significantly associated with spinal endplate sclerosis score. LRP5 and WISP1 are Wnt/LRP5 signaling molecules.

These data suggest that IGF1R, LRP5 and WISP1 are genetic determinants of bone and cartilage metabolism. SNPs of IGF-I/IGF1R and Wnt/LRP5 signaling genes will serve to facilitate early diagnosis, treatment and prevention of spinal osteoarthritis.

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Oxidative Stress in Skeletal Muscle Causes Severe Disturbance of Physical Activities without Muscle Atrophy

Tetsuro Horie[1], *Chizuru Tsuda*[1], *Shin Ishikawa*[1], *Yoshihiro Noda*[1], *Hirotomo Kuwahara*[1,2], *Satoru Kawakami*[1,4,5], *Tomoe Yoshida*[1], *Yusuke Ozawa*[1], *Takuji Shirasawa*[1,3,5], *Takahiko Shimizu*[1,4,5]

Research Team for Molecular Biomarkers, [1]Tokyo Metropolitan Institute of Gerontology, [2]Department of Orthopedics and [3]Department of Ageing Control Medicine, Juntendo University Graduate School of Medicine, Applied Biological Chemistry, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology4, Anti-Aging Science, Co., Ltd 5., Minato-ku, Tokyo, 108-0021, Japan

Aims: The age-related muscle weakness and loss of muscle mass are major contributors to frailty in the elderly. These changes cause the loss of independence and impair the quality of life. One of the factors triggering these age-related pathological conditions is the accumulation of the cellular damages due to reactive oxygen species. Muscle contraction requires a large amount of ATP in skeletal muscles. The vast majority of such ATP is generated by OXPHOS. The high rate of energy consumption in skeletal muscles can cause an electron leakage from the electron transfer chain and results in increased oxidative damages. Accordingly, oxidative stress and damages observed in mitochondria of skeletal muscles may be attributable to phenotypic changes associated with aging, such as the loss of skeletal muscle mass and functions. In order to investigate the pathological significance of oxidative stress in the skeletal muscle, we generated skeletal muscle-specific manganese superoxide dismutase (Mn-SOD)-deficient (muscle-Sod2-/-) mice.

Methods and Results: Muscle-Sod2-/- mice showed severe disturbance of physical activities, but no atrophic changes in skeletal muscle. In histological and histochemical analyses, the mutant mice showed centralized nuclei in muscle fibers. In addition, we found that NADH dehydrogenase (Complex I) and SDH (Complex II) activities in mitochondrial respiratory chain complexes were selectively reduced in muscle-Sod2-/- mice. Particularly, the loss of Complex II activity was remarkable and was hardly detected in skeletal muscle of muscle-Sod2-/- mice while a strong enzymatic activity was detected in those of control mice. Furthermore we showed that the selective loss of enzymatic activity led the reduced ATP synthesis in skeletal muscle. Subsequently, we performed the rescue experiment using EUK-8. EUK-8 is one of the synthetic salen-manganese compounds with the activities of superoxide dismutase, catalase and oxyradical scavengers. These enzymatic activities confer these compounds the ability to inactivate superoxide anions and their dismutation products hydrogen peroxide, thereby preventing hydroxyl radical formation. We observed that the single intraperitoneally injection of EUK-8 significantly extended the endurance time of muscle-Sod2-/- mice in treadmill task even 96 hours after a injection and increase cellular ATP contents. Therefore, muscle-Sod2-/- mice is a good model to evaluate the muscle dysfunction and the declined physical activities without muscle atrophy of the elderly.

Conclusion: We for the first time showed that muscle-Sod2-/- mice exhibited drastic physical disturbance without muscle atrophy and the impairment due to oxidative stress generated in mitochondria. Furthermore, we demonstrated the efficacy and limitations of antioxidant salen-manganese compounds. Subsequent studies with our mice should help to develop novel drugs or new dietary supplements, which would contribute to the improvement of physical disturbance as well as the quality of senescent life.

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