Session: Disease & Treatment — Osteoporosis

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EPIGENETIC LANDSCAPE IN PPARγ2 IN THE ENHANCEMENT OF ADIPOGENESIS OF MOUSE OSTEOPOROTIC BONE MARROW STROMAL CELL

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Introduction: Osteoporosis is one of the most prevalent skeletal system diseases; yet, its pathophysiological mechanisms remain elusive. Adipocytes accumulate remarkably in bone marrow of osteoporotic patients. The potential processes and molecular mechanisms underlying adipogenesis in osteoporotic BMSCs have attracted significant attention as adipocytes and osteoblasts share common precursor cells. Some environmental factors influence bone mass through epigenetic mechanisms; however, the role of epigenetic modifications in osteoporosis is just beginning to be investigated, and there is still little data regarding their involvement. In the current study, we investigated how epigenetic modifications, including DNA methylation and histone modifications, lead to adipogenesis in bone marrow during osteoporosis.
Subjects and Methods: A glucocorticoid-induced osteoporosis (GIO) mouse model was established, and BMSCs were isolated from bone marrow. Osteoporotic BMSC osteogenic and adipogenic potentials were evaluated through ALP staining, Alizarin red staining, oil red O staining and quantitative RT-PCR. In osteoporotic BMSC, PPARγ2 regulatory region DNA methylation status was studied through bisulfite sequencing PCR and the status of histone acetylation and H3K9 di-methylation in PPARγ2 regulatory region was studied through Chromatin immunoprecipitation (ChIP). Western blot, lentivirus, luciferase reporter assay and in vitro methylation assay were employed to inhibit adipogenic potential of osteoporotic BMSC through altering epigenetic modification status in PPARγ2 regulatory region.
Results: Compared with normal BMSCs, osteoporotic BMSCs had significantly increased adipogenesis potential and decreased osteogenesis potential. In osteoporotic BMSCs, PPARγ2 regulatory region DNA hypo-methylation, histone 3 and 4 hyper-acetylation and H3K9 hypo-di-methylation were observed. These epigenetic modifications were involved not only in PPARγ2 expression but also in osteoporotic BMSC adipogenic differentiation potential. We also found that Wnt/β-catenin signal played an important role in the establishment and maintenance of epigenetic modifications at PPARγ2 promoter in osteoporotic BMSCs. Finally, we inhibited adipogenesis and rescued osteogenesis of osteoporotic BMSCs by modulating those epigenetic modifications.
Discussion and Conclusion: In the current study, a significant enhancement of adipogenic differentiation potential was observed for osteoporotic BMSCs compared with normal BMSCs, and these results are consistent with a number of previous reports. The current study aimed to elucidate the molecular mechanisms underlying osteoporotic BMSC adipogenesis. The environment significantly affects bone mass, even during gestation: the intra-uterine environment was shown to play an essential role in fetal skeleton development not only at birth but also later in life. The current study provides direct evidence that the epigenetic landscape of the PPARγ2 regulatory region is altered in osteoporotic BMSCs, with changes including DNA hypo-methylation, histone hyper-acetylation and H3K9 hypo-di-methylation, as well as evidence regarding how these epigenetic modifications regulate PPARγ2 expression and osteoporotic BMSC adipogenic differentiation. In summary, the results of the current study suggest that DNA is de-methylated, H3 and H4 tails are acetylated, and H3K9 di-methylation is reduced in PPARγ2 regulatory regions in osteoporotic BMSCs compared with normal BMSCs. These epigenetic modifications lead to an active chromatin structure that activates PPARγ2 transcription in response to adipogenic induction. Consistent with this idea, osteoporotic BMSCs showed enhanced potential for adipogenic differentiation in response to PPARγ2 epigenetic modifications.
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Session: Disease & Treatment — Ligament, Tendon and Meniscus

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A NEW ANIMAL MODEL OF TENDINOPATHY—FAILED TENDON HEALING BY HYDROGEN PEROXIDE

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Introduction: Tendinopathy includes cases with chronic tendon pain as well as cases with spontaneous tendon ruptures. There is no consensus on representative animal model of tendinopathy, which affects development of new treatment strategies. Animal models by overuse training could induce tendon degeneration, but painful response and mechanical weakness were not recorded. Collagenase injection is another common method to induce tendinopathic changes with painful responses, but this aetiopathological factors may not be applicable to humans. As overuse can impose both mechanical stress and oxidative stress to tendons, we propose to develop a new tendinopathy animal model by imposing oxidative stress to result in failed tendon healing found in tendinopathy.
Subjects and Methods: A total of 24 male Sprague Dawley rats (8—10 weeks old, 200—300 g) were used in the study. A patellar tendon window injury was created on the right knee according to our previous protocol. Postoperatively the rats were randomly assigned to three groups (n = 8), which received three weekly subcutaneous injections over the patellar tendon (from the 3rd to 5th week post operation) of either saline, 50 μM or 500 μM H_2O_2 solution (100 μl per injection). Animal gait data (CatWalk XT, Noldus) for pain assessment and 3D ultrasound imaging data (Vevo770, Visualsonics) for tendinopathic changes were collected non-invasively at pre-injury and 6-week post operation (n = 8). At day 42, the rats were euthanized to harvest knee specimens for either histology (n = 2) or tensile mechanical test (n = 6). Repeated measures ANOVA and the non-parametric Kruskal Wallis test were used to compare the treatment effects. Statistical significance was accepted at $\alpha = 0.05$.
Results: The mechanical properties (ultimate stress, elastic modulus) of the healing patellar tendons were significantly impaired by 50 μM H_2O_2 treatment ($p = 0.021$). Treatments with 50 μM H_2O_2 also led to pain-associated gait asymmetry ($p = 0.038$) and significant tendon swelling at distal patella region as compared to the saline control. Histological examination showed hypercellularity and matrix disturbance in the 50 μM H_2O_2 treatment group.
Discussion and Conclusion: The results demonstrated that H_2O_2 impaired tendon healing and elicited tendinopathic changes with respect to pain, structural abnormalities and mechanical weakening. Failed tendon healing in this animal model resembled most tendinopathic features in humans, and the pathological changes were attributed to oxidative stress which is presumably involved in overuse. This animal model is thus useful for investigation of pathogenesis of tendinopathy as well as development of new treatment strategies.
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Session: Traumatology — Fracture Healing

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ESTROGEN RECEPTOR EXPRESSION IN OSTEOBLASTS IS NOT ENHANCED *IN VITRO* BY LOW-MAGNITUDE HIGH-FREQUENCY VIBRATIONAL STIMULATION IN THE ABSENCE OF ESTROGEN

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Background: Low-magnitude high-frequency vibration (LMHFV) enhances ovariectomy-induced osteoporotic fracture healings in rats [1,2] in terms of increased callus formation as shown in callus morphometry, gene expression, and angiogenesis [1—3]. Mechanical stimulation can be transmitted by estrogen receptors (ER), and that LMHFV was shown to enhance ER expression and callus formation capacity at early to mid-stage of the healing process *in vivo* [4]. We hypothesized that the LMHFV may enhance ER expression in osteoblasts that contributes to enhanced osteogenic activities.
Methods: Primary cultures of 24 SD rats (n=4 per group) calvaria osteoblasts were treated with or without LMHFV stimulation. Different groups (CTRL+E2, VT+E2, CTRL, VT, and VT+ICI) were substituted with or without estrogen at 1.0×10^{-8} M to mimic estrogen (E2) and estrogen deficiency (CTRL) in osteoblasts; and with or without the ER antagonist ICI 182, 780 at 1.0×10^{-8} M to investigate the role of ER in osteogenic effect in response to LMHFV at 20 min/day, 5days/week, 35Hz and 0.3g. ER expression was assessed 4 hours after mechanical stimulation. Osteoblast differentiation (ALP activity) and osteogenic activity (calcium nodule formation) were assessed at week 2 after LMHFV treatments.
Results: ER-α expression was up-regulated in the VT+E2 group compared to the CTRL+E2 group without difference (p=0.229), but higher than the CTRL and VT+ICI groups (p=0.045, p=0.020, respectively). No difference was detected among the CTRL, VT and VT+ICI groups. ALP activity was found to be increased in the VT+E2 group compared to the CTRL+E2 group, however without difference (p=0.067); and increased in the VT group compared to the CTRL group with significance (p=0.012). The ALP activity was detected to be lowered in the VT+ICI group compared to the VT group with significance (p=0.016). No difference was detected between the VT+E2 and CTRL+E2 groups (p=0.800); and between the VT and CTRL groups (p=0.956). Difference was detected between the CTRL+E2 and CTRL groups (p=0.018). No difference was detected between the VT+ICI and the CTRL groups (p=1.000) and between the VT+ICI and VT groups (p=0.456).