and POSTN expression were performed by using quantitative real-time polymerase chain reaction (qRT-PCR).
Results: These findings demonstrated that GPC-3 expression in musculoskeletal tumors was significantly lower when compared to controls ($p=0.02$). However, POSTN expression in musculoskeletal tumor was not significantly different when compared to that in controls.
Discussion and Conclusion: This study concluded that GPC-3 expression was significantly lower musculoskeletal tumor patients. Therefore, it can suggest that neoplastic tissue had down regulated in cell division that causing GPC-3 had lower expression when compared to that in non-neoplastic adjacent tissue. On the other hand, POSTN expression in neoplastic tissue seemed to be higher than that in non-neoplastic adjacent tissues, indicating that neoplastic tissues had abnormal development. For further research, larger sample size will be necessary for validating the results.
http://dx.doi.org/10.1016/j.jot.2016.06.181

Session: Disease & Treatment — Ligament, Tendon and Meniscus

299
RELATIVE TELOMERE LENGTH AND MITOCHONDRIA DNA COPY NUMBER IN LIGAMENTUM FLAVUM OF LUMBAR STENOSIS PATIENTS

Sinsuda Dechsupa [a], Worawat Limthongkul [b], Wicharn Yingsakmongkol [b], Weerasak Singhatanadgige [b], Sittisak Honsawek [a]
[a]*Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Thailand*
[b]*Department of Orthopaedics, Faculty of Medicine, Chulalongkorn University, Thailand*

Background: Degenerative lumbar disease is the common cause of low back pain. In elderly patients, lumbar spinal stenosis is characterized by hypertrophic ligamentum flavum (LF), a decrease of elastin-to-collagen ratio, and age-related fibrosis. Several studies have been reported that the telomere shortening was associated with age and degenerative diseases. However, the association between the telomere length and mitochondria DNA (mtDNA) copy number, and LF of lumbar spinal stenosis is unknown. Therefore, the purposes of this study were to compare the relative telomere length (RTL) and mtDNA copy number between non-pathologic and pathologic LF from lumbar spinal stenosis patients.
Subjects and Methods: A total of 33 patients with lumbar spinal stenosis were recruited in the present study. RTL and mtDNA copy number of non-pathologic and pathologic LF were performed by real-time polymerase chain reaction (qRT-PCR).
Results: The results showed that pathologic LF tissue had significantly shorter telomeres than non-pathologic LF tissue ($p=0.004$). A significantly shortened telomere length could be found in pathologic LF tissue within more than 60 years old group as compared to non-pathologic LF tissue ($p=0.023$). RTL in pathologic LF tissue of female and male groups had significantly shorter telomere than non-pathologic LF tissue ($p=0.038$). Furthermore, there was significant difference in RTL between female and male within pathologic LF group ($p=0.27$) between two groups, and there was no association in non-pathologic and pathologic LF tissue between the RTL and mtDNA copy number from lumbar stenosis patients.
Discussion and Conclusion: This study is the first to examine RTL in ligamentum flavum of lumbar spinal stenosis patients. Our results showed that patients with lumbar spinal stenosis displayed an accelerated telomere shortening in pathologic LF tissue, as compared to non-pathologic LF tissues. The explanation for lower RTL in pathologic LF tissue may be due to the high turnover rate of cell proliferation. In accord with this study, Le Maitre et al. reported the mean telomere length was significantly lower in degenerative disc than non-degenerative disc.
http://dx.doi.org/10.1016/j.jot.2016.06.182

Session: Disease & Treatment — Tumors

301
EZRIN PROMOTES METASTASIS AND INVASION OF OSTEOSARCOMA BY UPREGULATING lncRNA HOTAIR VIA PI3K/Akt/IRF1 PATHWAY

Jiaming Zhang, Junming Huang, Zhengtao Lv, Fengjing Guo
Tongji Hospital, China

Background: Osteosarcomas are sarcomas of the bone with a high propensity to metastasize. Ezrin is a member of the ezrin-radixin-moesin (ERM) family of proteins that functions as a cross-linker between the actin cytoskeleton and the plasma membrane, and plays a vital role in maintaining cell shape and polarity and facilitates membrane-trafficking pathways, cell migration, cell signaling, growth regulation, and differentiation. Extensively previous studies had elucidated that ezrin promotes metastasis of osteosarcoma by activating PI3K/AKT pathway. Furthermore, it has been reported that AKT promotes HOTAIR, which is commonly overexpressed in osteosarcoma and correlated with poor prognosis, by inhibiting IRF1 expression in cancer cells. In this manuscript, we try to evaluate the mechanisms of ezrin in promoting the metastasis and invasion of osteosarcoma, and the relationship between ezrin and HOTAIR.
Subjects and methods: Transwell assays and Wound healing assay were conducted to detect the influences of ezrin by using inhibitor small interfering RNA(siRNA). The expression of MMP2/MMP9, AKT, p-AKT, IRF1 and HOTAIR were tested by real-time PCR (RT-PCR) assays and Western blot. Furthermore, IRFI was knockdowned and overexpressed to demonstrating the mechanisms of ezrin in regulations of HOTAIR.
Results: The metastasis and invasion of osteosarcoma were suppressed by knockdown of ezrin. The level of p-AKT and HOTAIR were reduced and IRF1 was upregulated when silencing ezrin. Furthermore, the overexpression of IRF1 downregulated HOTAIR levels in MG63 cells; whereas, the knockdown of IRF1 expression had no obvious effect on HOTAIR expression.
Discussion and Conclusion: Human osteosarcoma usually presented a high tendency to metastatic spread and caused poor outcomes, however, the underlying mechanism was still largely unknown. Previous efforts had emerged that Ezrin and HOTAIR are related with these procedures. In this study, we identified the ezrin promotes metastasis and invasion of osteosarcoma by activating PI3K/AKT pathway and downregulating IRF1, which binds to the HOTAIR promoter region, resulting in promoting HOTAIR. Ezrin thus represents a promising target for the development of novel and effective strategies aimed at preventing the progression of osteosarcoma.
http://dx.doi.org/10.1016/j.jot.2016.06.183

Session: Disease & Treatment — Osteoporosis

302
DIHYDROMYRICETIN INDUCES WHITE ADIPOSE TISSUE BROWNING AND INHIBITS OVX AND LPS-INDUCED BONE LOSS

Xuejun Zhang, Yuting Wang, Yonghui Dong, Xin Huang, Anmin Chen, Shilong Huang
Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, China

Background: Dihydromyricetin (DMY), an active small molecule recently discovered from natural medicines, can provide a wide range of health benefits. It was demonstrated to have anti-inflammatory, antioxidant and anti-tumor effects, also to improve insulin sensitivity of liver and skeletal muscle in nonalcoholic fatty liver disease and diabetes. Inflammation and reactive oxygen species (ROS) has an important role in postmenopausal osteoporosis, so we suppose that DMY also could reduce inflammatory factors and ROS and inhibit ovariectomized (OVX)-mediated bone loss.
Subjects and Methods: We used two animal models to test our hypothesis: OVX and lipopolysaccharide (LPS, 5mg/kg)-induced bone loss models. We injected DMY (200mg/kg) in intraperitoneally in OVX model for 6 weeks and in calvarial surface in LPS model for 8 days. Bone structure and volume was evaluated by μ-CT. H&E, TRAP and immunohistochemical staining to assess related markers and cell numbers.
Results: In OVX model, we scanning femur with μ-CT at 3 weeks in vivo and 6 weeks in vitro, after 3D reconstruction, BV/TV (8.6%), Tb.Th (13%) and Tb.N. (11.6%) significantly increased but Tb.Sp (11.6%) decreased compared with sham group, it indicated that DMY could significantly inhibit OVX induced bone loss. H&E and TRAP staining also show that DMY treat group had fewer osteoclasts number compared with sham group, and the adipose tissue volume decreased in DMY treat group. Then we analyst peripheral subcutaneous adipose tissue (SAT, scapular region and inguinal region), bone marrow adipose tissue (MAT) and visceral adipose tissue (VAT) by immunohistochemical staining UCP-1, PPARα/γ, C/EBPβ, both SAT and VAT has more UCP-1 expressing level than sham group, but PPAR-γ, C/EBPβ were down-regulated; In LPS model, the whole calvarial stained with TRAP solution indicated DMY also prevented LPS-induced bone destruction. Serum IL-1, IL-6, TNFα, and ROS also decreased in both model of DMY group. In vitro study, DMY inhibit LPS-stimulated RAW264.7 cell proliferation with a dose dependent manner without influencing the viability of cells by CCK8. DMY also suppressed actin ring formation and bone resorption and prevents RANKL-induced osteoclasts formation in vitro. Western-blot and PCR results indicated that DMY down-regulated osteoclast differentiation marker genes c-Fos, NFATc1 in RANKL-induced RAW264.7 cells.
Discussion and Conclusion: Our results indicated that DMY treatment significantly reduced OVX and LPS-induced bone loss, and also induced WAT browning. We hypothesised that DMY might induce adipose tissue white-to-brown to inhibit osteoclasts differentiation and function, and DMY also might promote osteoblasts differentiation and bone formation in vivo. The crosstalk between bone and adipose tissue and involved signaling pathway and regulatory factors need further research.
http://dx.doi.org/10.1016/j.jot.2016.06.184