vital to maintain the balance of bone homeostasis. Our early article elucidated myricitrin could protect from osteoporotic bone mass reduction via reducing reactive oxygen species (ROS) in osteoblastic bone formation; however, the influence of osteoclastogenesis by myricitrin is still unclear. Here, we exhibited that myricitrin could decrease the TRAP positive cell number significantly in a dose-dependent manner during osteoclast maturation, but it had no effect to pre-osteoclast proliferation. Consistently, osteoclast maturation markers, and the bone resorptive pits number and area were decreased. Taken together, myricitrin could inhibit osteoclastogenesis.

http://dx.doi.org/10.1016/j.jot.2016.06.087

297 ASSOCIATION OF TELOMERE LENGTH AND MITOCHONDRIAL DNA COPY NUMBER IN MUSCULOSKELETAL TUMOURS

*Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Bangkok, Thailand
**Department of Orthopaedics, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Bangkok, Thailand

Background: Telomere length plays a vital role in genomic stability and shortened telomeres may cause genomic instability and carcinogenesis. Mitochondria exert a potential role in energy metabolism, free radical production, apoptosis, and may be involved in cancer progression. Both telomere length and alteration of mitochondrial DNA (mitDNA) copy number have been proposed as biomarkers for several cancers. Nevertheless, few studies have examined the association of telomere length and mitDNA copy number in musculoskeletal tumours. This study aimed to examine telomere length and mitDNA copy number in peripheral blood leukocytes, neoplastic tissues, and non-neoplastic adjacent tissues of patients with musculoskeletal tumours. The second objective of this study was to investigate the relationship of telomere length and mitDNA copy number in musculoskeletal tumours.

Subjects and Methods: Peripheral blood leukocytes (n=41), neoplastic tissues (n=46), and non-neoplastic adjacent tissues (n=32) were obtained from patients with musculoskeletal tumours. Relative telomere length and relative mitDNA copy number were evaluated by quantitative real-time polymerase chain reaction.

Results: Relative telomere length in neoplastic tissues was significantly shorter than that in non-neoplastic adjacent tissues (p<0.001). Shorter relative telomere length in neoplastic tissues compared to non-neoplastic tissues was observed in female and male (p=0.018 and p=0.019, respectively), and in relative telomere length in malignant tissues was significantly decreased as compared to that in adjacent non-neoplastic tissues (p=0.016). The relative telomere length in malignant tissues seemed to be lower than that in benign tissues, there was no significant difference. The relative telomere length in peripheral blood leukocytes was not correlated with that in neoplastic tissues. In contrast, relative mitDNA copy number in neoplastic tissues was not different compared to that in non-neoplastic adjacent tissues. The relative mitDNA copy number in peripheral blood leukocytes was not associated with that in neoplastic tissues. Interestingly, relative mitDNA copy number in neoplastic tissues was significantly higher than that in peripheral blood leukocytes (p<0.001). Further analysis showed that there was a negative association between relative telomere length and mitDNA copy number in patients with musculoskeletal tumours (r=-0.306, p=0.104).

Discussion and Conclusion: Relative telomere length in neoplastic tissues was significantly shorter than that in non-neoplastic adjacent tissues, suggesting that neoplastic tissues (especially malignant tissues) may undergo several cell divisions which could lead to progressively shorter telomeres. This finding demonstrated that relative mitDNA copy number in tissues was significantly higher than that in peripheral blood leukocytes. The explanation of this finding could be due to specificity of mitDNA copy number in different types of tissues. The relative telomere length is inversely associated with the relative mitDNA copy number, indicating that telomere length attrition and mitDNA alteration are necessary events in musculoskeletal tumour progression.

http://dx.doi.org/10.1016/j.jot.2016.06.088

340 DOWN-REGULATION OF μ-OPIOID RECEPTOR MEDIATED EPIDEMIOGENICALLY BY NEURON-RESTRICTIVE SILENCER FACTOR IS INVOLVED IN THE REDUCED MORPHINE ANALGESIA IN A BONE CANCER PAIN ANIMAL MODEL

Chao Zhu, Tan Ding, Liu Yang, Zhe Wang, Zhuo-Jing Luo
Kijing Hospital, The Fourth Military Medical University, China

Background: Primary and metastatic cancers that affect bone are frequently associated with severe and intractable pain. Bone cancer pain has been reported with musculoskeletal tumours (there was a negative association between relative telomere length and mitDNA copy number in different types of tissues). The relative telomere length is inversely associated with the relative mitDNA copy number, indicating that in non-neoplastic adjacent tissues. Bone cancer pain has been reported with musculoskeletal tumours. The relative telomere length and relative mitDNA copy number in peripheral blood leukocytes, neoplastic tissues, and non-neoplastic adjacent tissues of patients with musculoskeletal tumours. The second objective of this study was to investigate the relationship of telomere length and mitDNA copy number in musculoskeletal tumours.

Method: Using a sarcoma inoculated murine model, pain behaviours that represent continuous or breakthrough pain were evaluated. Immunofluorescent staining was used to check the expression of NRSF in the dorsal root ganglion (DRG). Reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis were used to quantify expression of NRSF at the transcriptional and translational levels, respectively. Additionally, chromatin immunoprecipitation assays were used to detect NRSF binding to the promoter of MOR. Furthermore, NRSF was genetically knocked out by antisense oligodeoxynucleotide (AS-ODN), and the expression of MOR and the effect of morphine were subsequently analysed.

Results: Our results indicated that in a sarcoma murine model, expression of NRSF is upregulated in the DRG neurons and the expression of NRSF mRNA is significantly negatively correlated with expression of MOR mRNA. Additionally, chromatin immunoprecipitation analysis revealed that NRSF binding to the neuron-restrictive silencer element within the promoter area of the MOR gene is significantly promoted with a hypo-acetylation state of histone H3. Furthermore, genetically knocking down of NRSF with AS-ODN rescued the expression of MOR, with potentiality of system morphine analgesia.

Discussion and Conclusion: The present results suggest that in sarcoma induced bone cancer pain, NRSF induced downregulation of MOR is involved in the reduction of morphine analgesia. Epigenetically, up-regulation of MOR could substantially improve the effect of system delivery of morphine. The results indicate that NRSF plays an important role in the modulation of MOR transcription and may represent a novel analgesic target for bone cancer pain. What should be noted is that the expression of opioid receptor is not only regulated by transcription but are also controlled by extensive post-transcriptional processing. Further studies are needed at both the preclinical and clinical levels to develop pharmacological therapy and to effectively block/relieve bone cancer pain with the goal of increasing the functional status and quality of life of humans with bone cancer pain.

http://dx.doi.org/10.1016/j.jot.2016.06.089

367 miR-138-5p TARGETS MACF1 TO INHIBIT BONE FORMATION

Airing Qian*, Zhihao Chen*, Fan Zhao*, Chao Liang*, Lifang Hu*, Chong Yin*, Peng Shang*, Ge Zhang*
*Key Laboratory for Space Biosciences & Biotechnology, Institute of Special Environmental Biophysics, School of Life Sciences, Northwestern Polytechnical University, Xi’an 710072, China
**Institute for Advancing Translational Medicine in Bone and Joint Diseases, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Introduction: MicroRNAs (miRNAs) play important roles in the regulation of target gene expression to coordinate a broad spectrum of biological processes. There is increasing evidence that multiple miRNAs serve as important regulators of osteoblast differentiation and bone formation. Significantly, recent studies have discovered that miR-138-5p is upregulated in osteoporosis model. The RPM machine as an unloading model was used to culture cells. And the expression of miR-138-5p altered with bedridden time and was negatively correlated with the expression of the bone formation marker genes ALP and the bone forming cells in hind limb unloading (HLU) and 20-month aging mice. In RPM condition, the expression of miR-138-5p was negatively correlated with the bone formation marker gene ALP and ALP positive cells in hind limb unloading mice.

Subjects and Methods: Bone specimens from 70 osteoporotic individuals with bedridden states were collected. Twenty-one-month mice and HLU mice were used as the osteoporosis model. The RPM machine as an unloading model was used to culture cells. The expression of miR-138-5p in the bone formation in HLU mice and 20-month aging mice were used as the osteoporosis model. The RPM machine as an unloading model was used to culture cells. The expression of miR-138-5p in regulating the bone formation in HLU mice and 20-month aging mice were used as the osteoporosis model. The RPM machine as an unloading model was used to culture cells.

Results: We assessed the expression of miRNAs involved in bone formation in bone specimens from 70 osteoporotic individuals with bedridden states in clinical settings. The expression of miR-138-5p altered with bedridden time and was negatively correlated with the expression of the bone formation marker genes ALP in bedridden women and men. Moreover, consistent results were found in bone tissue and ALP positive cells in hind limb unloading (HLU) and 20-month aging mice. In RPM condition, the expression of high miR-138-5p expression increased gradually and ALP activity decreased in osteoblasts after RPM treatment for 12, 24, 48 hours. Target prediction analysis tools and luciferase activity were used to confirm microtubule actin crosslinking factor 1 (MACF1) as a direct target of miR-138-5p, and miR-138-5p inhibited MACF1 expression and osteoblast differentiation in vitro. We treated mouse preosteoblast MC3T3-E1 cells with antagonim-138-5p and cultured cells under RPM condition. The results indicated that miR-138-5p functions as a mechanical unloading sensitive miRNA and plays a negative role in RPM-induced osteoblast differentiation reduction. Predominantly, we found an inhibitory role of miR-138-5p in regulating the bone formation in HLU mice and in vivo pre-treatment with antagonim-138-5p partly recovered the bone loss caused by hind limb unloading.

Discussion and Conclusion: Taken together, these results suggest that in vivo inhibition of miR-138-5p by anti-miR-138-5p could represent a potential therapeutic strategy for ameliorating bone loss.

Funding/support: This work was supported by the National Natural Science Foundation of China (31400725, 31570940).