Role of HDAC Inhibitors in Skeletal Biology

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Abstract

Osteocyte is a cell found in bone tissues, forming extensive networks as it differentiated from osteoblasts, which are involved in bone metabolism by controlling both osteoblast (bone-forming cell) and osteoclast (bone-resorbing cell) functions through osteogenic genes such as SOST, RANK, and DMP1. Osteoclasts secrete sclerostin, a protein that reduces proliferation and differentiation of osteoblasts. Recently, class II HDACs (histone deacetylases) have been reported to control SOST expression and, as such, bone metabolism. Interestingly, an HDAC inhibitor (HDACi), Scriptaid, was reported to induce expression of metabolic genes by disrupting the HDAC co-repressor complex and increasing Metf2 expression in muscles. Exercise induces nuclear export of HDAC 4/5 and activation of Metf2, which can be induced by Scriptaid. Similar mechanisms are also present in osteocytes and maybe in bone. We hypothesized that HDAC inhibitors might increase Metf2 expression and induce SOST expression, which can be detrimental for bone. To investigate the SOST expression, we subjected Ocy454 cells, osteocyte cell line from mice, to an in vivo environment, into differentiation for 10 days. On day 10, cells were treated with TSA and MC1568, both HDAC inhibitors, within a timecourse of 16 hours, 2 hours, 1 hour, and 30 minutes. RNA was isolated and gene expression analyzed by real-time PCR. According to preliminary data, SOST was suppressed by Scriptaid whereas RANKL and GLUT4 (glucose transporter) expressions were increased. This allowed us to conclude that HDAC inhibitors might have anabolic effect on bone but additional studies must be conducted to further analyze this relationship.

Methods

Cell line: Ocy454 cells were routinely maintained in αMEM+10% fetal bovine serum and 1 % antibiotic-antimycotic. Ocy454 cells were plated in 12 well plate at 100k/ml and allowed to reach confluence at the permissive temperature (33C) for 3 days. Subsequently, cells were differentiated at 37C for 7 days. On day 7, the cells were treated with one dose of TSA or MC1568 in a time course of ± 16 hrs, ±2hrs, ±1hr and ±30min.

RNA isolation
Total RNA was isolated using Qiagen RNeasy kit, following manufacturer’s recommendation. RNA was quantified using NanoDrop and 1 μg of RNA was used to generate complementary (c)DNA using Veriti 60 Well Thermal Cycler.

Semi-quantitative RT-PCR
(c)DNA synthesis was performed followed by SYBR Green qPCR (StepOnePlus, Life technology). B-Actin was used for normalization of gene expression. Delta CT was computed within each sample and Delta Delta CT was computed across experimental conditions.

Results

Dose response effects of Scriptaid N=2 samples each treatment group; *P<0.05; **P<0.01 (Scriptaid -h)

Time Course effects of MC1568 N=2 samples each treatment group; DMSO and control are both treated at 16h

Conclusions

Based on the preliminary data, HDAC inhibitors can affect osteocytic gene expression (SOST, RANKL, and GLUT4) transcription rapidly as shown in the time course experiment. Additional experiments are needed in the future to dissect the mechanisms of HDAC inhibitors.

Discussion

Our data indicate that HDAC inhibitors such as TSA and MC1568 functions as a negative regulator of MEF2C-dependent SOST expression in osteocytes. At -1hr, SOST had the maximum suppression against TSA while SOST showed the maximum suppression at -2hr when treated with MC1568. Furthermore, RANKL was upregulated by TSA at -1hr but interestingly was suppressed by MC1568 throughout the time course. GLUT4, the only metabolic gene used as a primer in this experiment, was also upregulated by TSA at -1hr but was suppressed by MC1568 throughout the time course. Though further studies are required to elucidate the relationship of RANKL and GLUT4 with HDAC inhibitors, we report that the suppression of SOST is consistent with the prior information of its effect on the osteoblasts.

References

Acknowledgements

Additional thanks to Yuhei, for providing guidance for basic cell culturing techniques; RNA isolation, cDNA Synthesis and qPCR; Sun, for providing essential details for the experiment and giving me advice for effective experimentation; Tokyo, for supervising my role at the laboratory after given instructions; everyone in the lab, for making my experience enjoyable and informative.