Perturbation of Histone Lactylation on Gene Expression in Rat Nucleus Pulposus Cells

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Disclosures: None

INTRODUCTION:

Intervertebral disc degeneration (IDD) is present in nearly all spines by age 60 and is associated with pain, disability, and up to $100 billion annually in healthcare costs in the US. Cells of the hypoxic nucleus pulposus (NP) produce an abundance amount of lactate as well as extracellular matrix aggrecan that is essential for the load bearing function of the spine. IDD is characterized by decreased NP aggrecan due to disrupted matrix homeostatic balance. The molecular mechanisms that cause this disruption in matrix homeostasis are still unknown. Found to play a role in progression of many age-related diseases, epigenetic modifications include histone post-translation modifications (PTMs) and DNA methylation. Recently, histone lactylation via histone lysine residues has been identified as a novel histone PTM.[1] Our lab has identified high levels of histone lactylation in lactate-rich regions of the disc, particularly NP cells, not present in vertebral bone or paraspinal muscle. However, the histone lysine delactylases and lactyltransferases (lactate 'erasers' and 'writers', respectively) are not yet identified in disc cells. In this study, we tested one potential cognate histone lysine delactylase inhibitor (SD939/Pracinostat) that inhibits all the HDAC isoforms, and one key cognate histone lysine lactyltransferase (MOF). We treated rat NP cells with SD939 (blocks delactylation on histones) and MOF siRNA (blocks lactylation on histones) to study the effects on expression of genes controlling extracellular matrix homeostasis and lactate metabolism.

METHODS:

Primary rat NP cells were isolated from the spines of 3-month-old male F344 rats as approved by the University of Pittsburgh's IACUC. Cells were cultured in DMEM/F12 media with 10% FBS at 2% O2. For eraser inhibition in which histone lactylation is expected to increase, 10x of the IC50 of SB939 (15000 nM) were used to treat rat NP cells for 24 hours. For writer inhibition to decrease histone lactylation, 3 uL siRNA/15 pmol of MOF siRNA and 1.5 uL lipofectamine was used to transfect NP cells in a 24-well plate. Cells were treated with low nutrient media (DMEM, 1mM Glucose, 1% FBS) for 24 hours to mimic physiological conditions. NP cells were then treated with 10 mM lactate to mimic physiological condition. Gene expression of ACAN, MMP13, Cox2 (inflammatory marker), LDHA (enzyme converting pyruvate to lactate enzyme), MCT1 (lactate importer), MCT4 (lactate exporter), and MOF were measured using RT-PCR. Significance was determined using a student's t-test (n = 9 for SB939, n = 4-10 for MOF siRNA).

RESULTS SECTION:

NP cells treated with MOF silencing RNA and SB939 for 24 hours exhibited no cytotoxicity effects. With both histone eraser and histone writer inhibition, ACAN expression was significantly decreased while MMP13 and Cox2 expression was significantly increased. LDHA, MCT1, and MCT4 gene expression all increased with SB939 treatment (histone eraser inhibition) but decreased with MOF siRNA treatment (histone writer inhibition)

DISCUSSION:

Perturbation of histone lactylation, either by its suppression or enhancement, leads to a decrease in the anabolic gene ACAN and an increase in the key catabolic MMP13 gene, indicating that epigenetic control via lactylated histones is vital for maintaining the balance of matrix homeostasis . Opposing effects of MOF siRNA and SB939 on LDHA, MCT1, MCT4 also suggest that histone lactylation controls expression of genes involved in NP lactate metabolism.

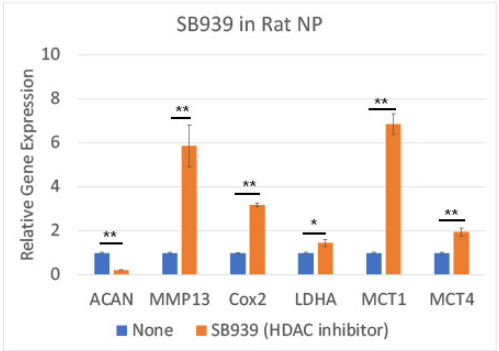
SIGNIFICANCE/CLINICAL RELEVANCE:

This study revealed the role of histone lactylation in epigenetic control of matrix homeostasis and lactate metabolism NP cell, providing a new epigenetic mechanism of regulation of NP cell function which can be therapeutically targeted to prevent disease progression in IDD.

REFERENCES:

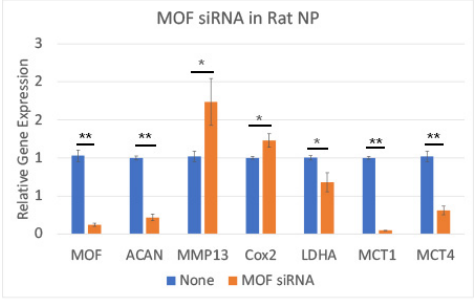
1. Zhang, D. *et al.* Metabolic regulation of gene expression by histone lactylation. *Nature* **574,** 575–580 (2019).

Figure 1) Effect of SB939 on Gene Expression in Rat NP



* = p<0.05, ** =p<0.01

Figure 2) Effect of MOF siRNA on Gene Expression in Rat NP



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