Role of MOF in Epigenetic Regulation Via Histone Lactylation of Annulus Fibrosis Cells

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Disclosures: None

INTRODUCTION:

Intervertebral disc degeneration (IDD), a leading cause of lower back pain, is present in more than 90% of spines by age 60. The intervertebral disc is composed of the outer annulus fibrosis (AF) cells and the inner, more hypoxic nucleus pulposus (NP) cells which produce extracellular matrix essential for load bearing of the spine. IDD is characterized by inflammation, extracellular matrix degradation, and decreased disc function. Disc lactic acid, an abundant end-product of anaerobic respiration, is historically thought to be toxic metabolic waste. Recently, lactate has been identified as a novel histone post-translational modification (PTM) on histone lysine residues.[1] Protein PTMs are emerging as possible markers for both diseases such as cancer as well as the process of normal aging, and are impacted by both genetics and the environment. Histone methylation, another form of PTMs, have been shown to change with age and regulate the longevity of several organisms. Our lab has found that there are high levels of histone lactylation in lactate rich regions of the disc, particularly in NP cells, that are not present in vertebral bone or paraspinal muscle. We hypothesize a novel role for lactate in the epigenetic regulation of disc health via histone lactylation. We explored the role of a histone lysine acetyltransferase, MOF, as a cognate histone lysine lactyltransferase (lactate 'writer'). To do this, we first silenced MOF in rat AF cells treated with IL-1β to mimic the inflammatory condition found in IDD. We then quantified the levels of lactylated histones and expression of key matrix homeostasis genes that control IDD.

METHODS:

Primary rat AF cells were isolated from the spines of three 3-month-old male F344 rats as approved by the University of Pittsburgh's IACUC. Cells were cultured in DMEM/F12 media (17.5 mM Glucose, 10% FBS) at 5% O2 (AF). To transfect with MOF siRNA, AF cells were plated in a 24 well plate. 1.5 uL Lipofectamine 3000 and 3 uL siRNA/15 pmol of MOF siRNA was used to transfect cells. Cells were treated with low nutrient media (DMEM, 1mM Glucose, 1% FBS) for 24 hours to mimic physiological conditions. Cells were then treated with 10 mM lactate. AF cells were also treated with -/+ IL-1β (5ng/ml) to model the inflammatory IDD condition for 24 hours. Gene expression of MOF, Col1 (anabolic), MMP13, Cox2 (catabolic), LDHA, MCT1, and MCT4 (lactate metabolism) were measured using RT-PCT. Protein expression of histone lactylation/anti-Pan Kla (PTM-1401) and histone acetylation/anti-Pan Kac (PTM-101) were measured using Western Blot and normalized to anti-histone H3. Significance was determined using a student's t-test (n = 3).

RESULTS SECTION:

MOF was knocked down in both conditions with 50-80% transduction efficiency (Fig 1). Under non-inflammatory conditions, gene expression of MMP13, a major metalloproteinase implicated in IDD; and MCT1, a key lactate importer in disc cells; were both significantly increased following MOF knockdown (Fig 1A). In the inflammatory condition, Col1 expression was significantly increased while expression of the inflammatory marker Cox2 was significantly decreased post MOF knockdown (Fig 1B). Overall gene expression was decreased in inflammatory conditions in rat AF. Under both inflammatory and non-inflammatory conditions, MOF silencing resulted in decreased histone lactylation and acetylation (Fig 2); however, this decrease was not significant.

DISCUSSION:

MOF silencing has differential effects on expression of genes regulating matrix homeostasis in both inflammatory and noninflammatory conditions. In noninflammatory conditions, expression of MMP13, a major matrix catabolism enzyme, was significantly upregulated when MOF was silenced. However, in the inflammatory condition in AF cells, gene expression of anabolism (Col1) was upregulated while catabolism (Cox2) was downregulated when MOF was silenced. These changes may reflect the changes in histone lactylation in AF cells, but further confirmatory experiments are needed.

SIGNIFICANCE/CLINICAL RELEVANCE:

The pathogenesis of IDD and significance of PTMs in regulation of disc health is poorly understood. This study offers a possible mechanism of epigenetic regulation of disc health which can be targeted for clinical therapies.

REFERENCES:

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

Figure 1) Gene expression of matrix homeostasis genes after MOF silencing in Rat AF

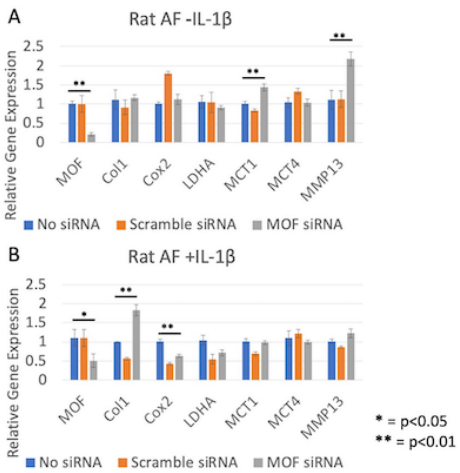

Figure 2) Protein expression of histone lactylation and histone acetylation normalized to H3 in Rat AF (no IL-1β).