Investigating the role of Mdm2 and EZH2 as epigenetic writers in muscle phenotype and differentiation

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Introduction: Skeletal muscle can have an oxidative, glycolytic or mixed metabolic phenotypes. Different muscles can express these phenotypes by expressing different genes. Epigenetic marks on the DNA regulate how genes are expressed. While activating epigenetic marks on genes appear to support a glycolytic phenotype, the role of silencing epigenetic marks remains largely unknown. Therefore, this study investigates the role of silencing marks. A recent report indicates that the E3 Ubiquitin Ligase Mdm2 and the histone methyltransferase EZH2 interact to place silencing marks on genes. We have previously observed that Mdm2 is highly expressed in oxidative muscles. We hypothesize that the interaction between Mdm2 and EZH2 could determine muscle phenotype by regulating silencing marks.

Methods: To investigate this hypothesis, we collected the soleus (oxidative), plantaris (glycolytic), and gastrocnemius (mixed) muscles from 14-month-old C47B6 female mice. In vitro, we differentiated C2C12 myoblasts into myotubes over 5 days. Mdm2, EZH2 and the silencing mark H3K27me3 protein levels were measured by western blotting. Interestingly, we detected H3K27me3 and EZH2 proteins at the expected molecular weights, as well as an additional band at a higher molecular weight, suggestive of ubiquitination.

Results: The oxidative soleus muscle had higher levels of Mdm2, but lower levels of EZH2 compared to the plantaris and gastrocnemius muscles. The soleus had the highest level of the silencing mark H3K27me3, at both the higher and lower bands. During C2C12 differentiation, Mdm2 and H3K27me3 protein levels increased. Meanwhile, protein levels of the lower EZH2 band decreased, while the higher band became more prevalent during differentiation.

Discussion: The global increase in H3K27me3 observed in the soleus and during differentiation supports the idea that silencing marks might support the oxidative phenotype. Our current data brings additional questions regarding the role of ubiquitination of H3 and EZH2 in the muscle.