c-MAF-driven metabolic reprogramming mediates H3K27 hyperacetylation to regulate super enhancer-associated genes

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Introduction

Multiple Myeloma (MM) is a neoplasm of plasma cells characterized by the uncontrolled proliferation of abnormal plasma cells in the bone marrow that is incapable of producing functional antibodies. Current treatment regime involves single or combination of novel drug classes such as proteasome inhibitors (bortezomib), immunomodulatory drugs (lenalidomide) and monoclonal antibodies (daratumumab), which have significantly improved survival outcomes in patients. However, this disease still represents an important clinical challenge as it mainly affects the elderly population and frequent development of drug resistance subsequent to initial treatment response. Overexpression of transcription factor c-MAF is found in about 50% of multiple myeloma cases, and associated with the prognostically unfavorable t(14;16) translocation subtype. Genetically, c-MAF-driven metabolic reprogramming with metabolite availability that act as substrates in histone modifications, but how this translates into specificities in gene regulation is unclear. Here, we report a novel involvement of c-MAF in metabolically-driven histone acetylation, including the superenhancer mark H3K27ac, through altering acetyl-CoA metabolism.

Knockdown of c-MAF silencing affects oxidative phosphorylation through decreased TCA cycle flux and mitochondrial dysfunction

Figure 1. (A) Mass-spectrometry based metabolomics of RPMI8226 cells after stable expression of shRNA targeting c-MAF. (B) Epigenetic-associated metabolites and metabolites of the TCA cycle were significantly reduced after cMAF knockdown. (C) Seahorse assay to measure oxygen consumption rate (OCR) of scrambled vs MAF knockdown cells. (D) mRNA expression of TCA cycle enzymes revealed downregulation upon cMAF knockdown. (E) Protein expression of TCA cycle enzymes revealed downregulation upon cMAF knockdown. (F) Examination of the electron transport chain machinery by western blot of the complex I-V members saw a decrease in complexes I and II with loss of cMAF. Experiments were performed with three biological repeats and a representative experiment was shown. *P < 0.01, **P < 0.01, Student’s t-test.

Alteration of c-MAF expression results in altered metabolism with mitochondrial dysfunction

Figure 2. (A) We assessed a panel of histone H3 acetyl marks in two stable c-MAF knockdown clones using ChIP-Seq. Knockdown of c-MAF broadly suppresses most acetylation marks and this was reproducible in both cell lines. (B) Overexpression of c-MAF in non-(14;16) myeloma cells could increase a subset of these acetyl histone marks. (C) In test whether c-MAF promotes epigenetic modifications in a metabolism-dependent manner, we treated RPMI8226 and OCI-MY5 with Metformin, which targets the electron transport chain to disrupt mitochondrial oxidative phosphorylation. Myeloma cells were susceptible to inhibition by Metformin with a corresponding reduction in acetyl-CoA.

Conclusion

To sustain high levels of acetylation, c-MAF acquired the metabolic flexibility to utilize glutamine in addition to glucose, feeding into the tricarboxylic acid (TCA) cycle as acetyl-CoA sources. Loss-of-function studies indicated that c-MAF is important for citrate-derived acetyl-CoA and H3K27ac levels through metabolic enzymes: citrate synthase (CS) and ATP-citrate lyase (ACL). Furthermore, blocking citrate export from mitochondria via CRISPR/Cas9 targeting of SLC25A1 reproducibly reduced the expression in H3K27ac. Silencing of c-MAF also displayed defective mitochondrial oxidative phosphorylation attributed to reduced metabolic flux through TCA cycle and downregulation of mitochondrial complex enzyme activity, expression, and activity. These data were obtained using knockout or CRISPR/Cas9 KD of citrate synthase and reveal a subset of 4 genes (ZC3H3, MEIS2, CRIP1, and STX16A2) for further validation.

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Figure 3. (A) The nutrient sources that contribute to the acetyl-CoA pool are primarily glucose, with the supplementation of glutamine to provide the necessary molecules to produce acetyl-CoA. (B) Inhibition of histone deacetylases (iHDACs) using Panobinostat (LBH589) rescues cMAF-mediated H3K27ac and viability. Knockdown of SLC25A1 reduces H3K27ac, confirming the involvement of citrate as a requirement for histone acetylation. (C) Citrate can be exported from mitochondria via the mitochondrial tricarboxylate transporter (SLC25A1). Knockdown of SLC25A1 reduces H3K27ac, confirming the involvement of citrate as a requirement for histone acetylation. (D) Inhibition of hexokinase dimerization (IID32) using Panobinostat (LBH589) rescues cMAF-mediated H3K27ac and viability.

Figure 4. (A) Heat map showing c-MAF-specific superenhancer genes that were identified in our previous study by RNA-seq and overlapping with cMAF RNA-seq data generated in the RPMI8226 cMAF knockdown cells. (B) This SE gene list is very robust as we were able to validate all of them with THZ1 or JQ1 treatment. These genes were then subjected to metabolic disruptions using metformin or CRISPR KD of CS, the enzyme that produces citrate and ACLY, the enzyme that converts glucose-derived citrate into acetyl-CoA. (C) Citrate can be exported from mitochondria via the mitochondrial tricarboxylate transporter (SLC25A1). Knockdown of SLC25A1 reduces H3K27ac, confirming the involvement of citrate as a requirement for histone acetylation. (D) Inhibition of hexokinase dimerization (IID32) using Panobinostat (LBH589) rescues cMAF-mediated H3K27ac and viability.

Figure 5. (A) Gene track of ZC3H3 for H3K27ac signal and CTCF-seq demonstrated an enrichment of H3K27ac in B cells but not in the controls such as normal plasma cell or B cells. Red underline indicate superenhancer regions. (B) Dependency experiments using two independent ZC3H3 shRNAs indicated that loss of ZC3H3 had a significant effect on cell viability and colony formation in t(14;16) cells RPMI8226 and OCI-MY5.

Figure 6. (A) Seahorse assay to measure oxygen consumption rate (OCR) of scrambled vs MAF knockdown cells. (B) Examination of the electron transport chain machinery by western blot of the complex I-V members saw a decrease in complexes I and II with loss of cMAF. Experiments were performed with three biological repeats and a representative experiment was shown. *P < 0.01, **P < 0.01, Student’s t-test.

Figure 7. (A) Knockdown of c-MAF broadly suppresses most acetylation marks and this was reproducible in both cell lines. (B) Overexpression of c-MAF in non-(14;16) myeloma cells could increase a subset of these acetyl histone marks. (C) In test whether c-MAF promotes epigenetic modifications in a metabolism-dependent manner, we treated RPMI8226 and OCI-MY5 with Metformin, which targets the electron transport chain to disrupt mitochondrial oxidative phosphorylation. Myeloma cells were susceptible to inhibition by Metformin with a corresponding reduction in acetyl-CoA marks. (D) Myeloma cells were susceptible to inhibition by Metformin with a subset of these acetyl histone marks. (E) Knockdown of c-MAF broadly suppresses most acetylation marks and this was reproducible in both cell lines. (F) Overexpression of c-MAF in non-(14;16) myeloma cells could increase a subset of these acetyl histone marks. (G) In test whether c-MAF promotes epigenetic modifications in a metabolism-dependent manner, we treated RPMI8226 and OCI-MY5 with Metformin, which targets the electron transport chain to disrupt mitochondrial oxidative phosphorylation. Myeloma cells were susceptible to inhibition by Metformin with a corresponding reduction in acetyl-CoA marks.