

The AMPK- OGT axis prevents acquired drug resistance through inhibition of drug induced epigenetic remodelling in melanoma

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Drug resistance is a major hindrance for prolonged survival in melanoma patients. We recently identified chronic stress induced multi-drug tolerant melanoma cells (IDTCs), which were generated by exposing parental cells to sublethal concentrations of dabrafenib and/or trametinib. These cells show distinct epigenetic changes, specifically an increase in H3K4me3 and H3K27me3 marks after 30 to 50 days of continuous drug exposure forming distinct colonies. Activation of the 5' adenosine monophosphate-activated protein kinase (AMPK), as tested by increased phosphorylation of acetyl coA carboxylase a direct downstream target of AMPK, delayed IDTC colony formation. Prolonged AMPK activation by acetylsalicylic acid (Aspirin) prevented the upregulation of H3K4me3 and H3K27me3 in a state of transition from IDTC colonies to permanent drug resistance. Increased AMPK resulted in downregulation of O-GlcNAc transferase (OGT), which was found to be increased in IDTCs, *in vivo* xenografts and RNA-seq matched patient data, concomitantly with a decrease in H3K4me3 and H3K27me3. Silencing of AMPK reversed these changes. TET1 and EZH1, both downstream mediators of OGT function were increased in IDTCs and melanoma patients but transcript levels decreased in permanent resistant cells. *In vivo* OGT knockdown was as effective as AMPK activation with Aspirin which downregulated H3K4me3 and TET1 expression. CHIP-seq analyses showed a differential occupancy of

H3K4me3 in promoters and gene bodies in parental vs IDTCs after 45 days of exposure to Dabrafenib. Distinct spatiotemporal changes in H3K4me3 and H3K27me3 histone marks are features of IDTCs. AMPK activation prevents the occurrence of these histone marks in IDTCs colonies through a signalling network comprising OGT, TET1 and EZH1. Targeting AMPK or elements of the AMPK nexus will prevent the formation of IDTC colonies, increase the efficacy of current therapies and inhibit the emergence of permanent resistance.