

# THE ROLE OF HEME OXYGENASE IN METASTATIC MELANOMA TUMORIGENICITY

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## ABSTRACT

Reactive oxygen species (ROS) are highly reactive, tumorigenic molecules. In response to ROS accumulation, or oxidative stress, the transcription factor Nrf2 promotes expression by binding antioxidant response elements (AREs) found in the promoter of target genes. Traditionally, Nrf2 has been considered inhibitory of cancer by promoting the expression of phase II detoxifying enzymes, drug transporters, anti-apoptotic proteins, and proteasomes, which facilitate the removal of ROS and promote cell survival. Recently, however, overexpression of Nrf2-target genes has been implicated in promoting several cancer hallmarks and facilitating cancer development.

Significant focus has been given to the role of Keap1/Nrf2 as a sensor for oxidative stress. Much less attention has been paid to the role of Bach1, a transcriptional repressor that competes with Nrf2 for ARE binding. The best-characterized Bach1 target is Heme Oxygenase-1 (HMOX1). While heme oxygenase inhibits cancer by preventing ROS-induced damage, mounting evidence suggests that *HMOX1* overexpression at later stages in cancer development may promote cancer progression. Heme oxygenase catalyzes the degradation of heme and has two isoforms. HMOX1 is inducible by heme and oxidative stress while HMOX2 is constitutively expressed.

Stage IV metastatic melanoma has a median survival of only 6 to 10 months. Unfortunately, current therapeutic approaches provide limited benefit in overall survival, highlighting the need for the identification of novel therapeutic targets. Activating mutations in B-Raf are found in approximately 70% of malignant melanomas. Using an anchorage-independent melanosphere assay, which is indicative of the tumorigenicity of melanoma cells, we found that activation of B-Raf, but not N-Ras, is a driver of melanosphere formation. We provide evidence that derepression of Bach1 by treatment with cobalt protoporphyrin IX (CoPP) is sufficient for melanosphere formation, and that melanosphere formation induced by either CoPP treatment or B-Raf activation is dependent on heme oxygenase activity. Global transcriptome analysis revealed enrichment for genes involved in focal adhesion and extracellular matrix (ECM)-receptor interactions following either B-Raf activation or treatment with CoPP. We propose a mechanism by which heme oxygenase promotes melanosphere formation, and by extension, enhanced tumorigenicity, by modulating expression of genes involved in focal adhesion and ECM-receptor interactions. Heme oxygenase activity may provide a novel therapeutic target for the treatment of metastatic melanoma.