Hypoxia-induced transcriptional repression of the melanoma-associated oncogene MITF

Erez Feige,1 Satoru Yokoyama,2,3 Carmit Levy,3,4 Mehdi Khaled,2,4 Vivien Igras,5,6 Richard J. Lin,4 Stephen Lee,5 Hans R. Widlund,3,5 Scott R. Grantier,2, Andrew L. Kung,5 and David E. Fisher2,6

*Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA 02115; ‡Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital, Boston, MA 02114; †Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, ON, Canada K1H BMS; and ‡Department of Pathology, Brigham and Women’s Hospital, Boston, MA 02115

AUTHOR SUMMARY

The regulation of gene expression by sequence-specific DNA-binding proteins or transcription factors, which control the formation of a cRNA copy of a DNA sequence, is one of the most fundamental mechanisms involved in the determination of cellular behavior. Transcription factors are sensitive to external and intracellular cues and turn specific genes "on" or "off," thereby controlling critical cellular processes such as proliferation, differentiation, and survival. In multicellular organisms, different cell types often exhibit dependency on lineage-specific transcription factors. MITF, a transcription factor associated with microphthalmia (a disorder of the eye), is one such protein, and it plays a key role in cells of several lineages. Different gene-regulatory regions (i.e., promoters) control the expression of MITF isoforms that differ in their N-termini (one of the ends of the protein chain), in a tissue-specific manner. Some variants of MITF are important for the development and function of bone-eroding cells (osteoclasts), a few types of cells involved in immune responses, and the retinal pigment epithelium (1). On the contrary, the M-MITF isoform is specifically expressed in cells that synthesize the pigment melanin (melanocytes) and is implicated in the survival, proliferation, and differentiation of these cells (2). Importantly, the effects of MITF are not merely limited to normal developmental and physiological conditions. The MITF gene is a lineage-specific oncogene; that is, it is capable of promoting cancer in cases of melanoma and clear-cell sarcoma (i.e., melanoma of soft parts) (3). The mechanisms of MITF regulation are therefore of great physiological and clinical importance. We characterized a detailed endogenous mechanism of MITF regulation, which suggests that MITF gene amplification in metastatic melanoma provides these cells with a survival advantage. Our findings indicate that a major hypoxia response pathway inhibits MITF expression, thereby limiting cell proliferation and survival under low oxygen stress. In addition, our results suggest that a small-molecule strategy for suppression of the MITF oncogene in vivo will be clinically useful for treating various pathologies.

We examined MITF expression in metastatic melanoma bi-specimens and observed MITF protein expression restricted to a rim of cells surrounding blood vessels. This observation prompted us to hypothesize that MITF expression is regulated by oxygen supply and is perhaps decreased under conditions of low oxygen levels (i.e., hypoxic conditions). This hypothesis was supported by reciprocal staining patterns of MITF and hypoxia-inducible factor 1α (HIF1α) in melanoma specimens. We compared the expression levels of MITF and some of its target genes to those of hypoxia-induced genes and found that MITF levels were inversely correlated with the expression of hypoxia markers. To further explore whether MITF expression might be affected by a hypoxic environment, we subjected cultured melanocytes, melanoma cell lines, and other MITF-expressing cells to low oxygen conditions or to treatment with small molecules (e.g., inhibitors of the prolyl-hydroxylase enzymes) that mimic cellular hypoxic responses by stabilizing HIF1α. These treatments significantly reduced M-MITF expression at both the RNA and protein levels. The same findings were obtained when HIF1α was ectopically introduced into cells by adenoviral infection. On the contrary, “switching off” of HIF1α rescued MITF from hypoxia-induced down-regulation, indicating that MITF suppression is mediated by HIF1α.

In a series of experiments, we demonstrated that the decrease in MITF levels is a consequence of reduced transcription rate...
caused by repression of the MITF promoter. Based on the rate of the decrease in MITF levels and the literature on HIF1, we speculated that HIF1 does not act directly on the MITF promoter, but acts indirectly by inducing a repressor of transcription. Thus, we tested whether transcriptional repressors that are known HIF1α target genes can reproduce the effect of HIF1 on MITF expression. Using various techniques, we identified a transcriptional repressor (DEC1) that is recruited to the MITF promoter under hypoxic conditions, and determined that it is both necessary and sufficient to suppress MITF (Fig. P1). To test the effects of MITF down-regulation on melanoma cells, we generated a stable melanoma cell line in which MITF expression is regulated by a constitutive promoter that is not sensitive to hypoxia. We then compared the growth of this line to a control under hypoxic and normoxic (i.e., normal oxygen level) conditions, or in the presence of a prolyl-hydroxylase inhibitor (DMOG). Whereas the control line exhibited growth arrest and decreased cell number, constitutive MITF expression rescued the cells and enabled growth and survival. In vivo, DMOG treatment reduced melanoma tumor burden in nude mice injected with control cells, but not in mice injected with cells constitutively expressing MITF, emphasizing the dependency of these tumors on MITF expression. These results suggest the potential use of the hypoxia-activated HIF1–DEC1 regulatory axis for targeting MITF in vivo.

What is the physiological relevance of these findings? Hypoxic niches are a common characteristic of most solid tumors. In melanoma, these may lead to local down-regulation of MITF and limit cell proliferation and survival. MITF amplification, which is found in 15% to 20% of metastatic melanomas and is correlated with decreased patient survival, may thus be a protective mechanism of this lineage against hypoxia-induced stress. Our findings strengthen the hypothesis that MITF is a critical factor in malignancy and suggest that MITF suppression may be clinically advantageous. As a therapeutic approach for cancer, the use of prolyl-hydroxylase inhibitors may initially seem counterintuitive because of the role of HIF1 in the formation of new blood vessels. However, our data imply that, in melanoma, or at least in a subset of these cancers, dependency on MITF might be sufficiently strong to render MITF suppression as a clinically useful strategy. In summary, our data demonstrate that the MITF promoter is repressed by the HIF1–DEC1 axis, and that this regulatory pathway could serve as a potential therapeutic target for suppressing MITF expression in various cancers, such as melanoma and clear-cell sarcoma, and in osteoporosis (by targeting osteoclast MITF) and allergies (by targeting MITF in an inflammatory cell).