ORIGINAL ARTICLE

High *MITF* Expression Is Associated with Super-Enhancers and Suppressed by CDK7 Inhibition in Melanoma

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Cutaneous melanoma is an aggressive tumor that accounts for most skin cancer deaths. Among the physiological barriers against therapeutic success is a strong survival program driven by genes such as *MITF* that specify melanocyte identity, a phenomenon known in melanoma biology as lineage dependency. *MITF* overexpression is occasionally explained by gene amplification, but here we show that super-enhancers are also important determinants of *MITF* overexpression in some melanoma cell lines and tumors. Although compounds that directly inhibit *MITF* are unavailable, a covalent CDK7 inhibitor, THZ1, has recently been shown to potently suppress the growth of various cancers through the depletion of master transcription-regulating oncogenes and the disruption of their attendant super-enhancers. We also show that melanoma cells are highly sensitive to CDK7 inhibition both in vitro and in vivo and that THZ1 can dismantle the super-enhancer apparatus at *MITF* and *SOX10* in some cell lines, thereby extinguishing their intracellular levels. Our results show a dimension to *MITF* regulation in melanoma cells and point to CDK7 inhibition as a potential strategy to deprive oncogenic transcription and suppress tumor growth in melanoma.

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INTRODUCTION

Melanoma is an extremely aggressive form of skin cancer that originates from melanocytes, which are neural crest-derived pigment cells that migrate widely during embryogenesis to take up residence in a variety of anatomical compartments. Central to the biology of melanocytes and melanoma cells is *MITF,* a master transcription factor-encoding gene, which is essential for maintaining melanoblast proliferation, driving the differentiation of the melanocyte lineage in early development, and regulating the transcriptional program necessary for melanin synthesis (Opdecamp et al., 1997; Wellbrock and Arozarena, 2015; Yasumoto et al., 1994). The importance of *MITF* as a critical cell identity gene in melanocytes is preserved in melanomas, where it is often overexpressed and functions as a oncogenic transcription factor important for maintaining tumor survival, enhancing proliferation, and promoting differentiation (Cirenajwis et al., 2015; Harbst et al., 2012; Hsiao and Fisher, 2014). However, the underlying mechanism by which *MITF* levels are sustained is not fully understood, because only 10−20% of melanoma tumor specimens exhibit amplification of the MITF locus (Wellbrock and Arozarena, 2015).

Recently identified regulatory domains termed super-enhancers (SEs) provide insight into possible epigenetic mechanisms affecting MITF expression plasticity in melanoma. SEs are clusters of enhancers bound by an extreme density of transcription factors and cofactors, including CDK7, and tend to be associated with genes that control and define cell identity. SEs are also acquired by tumor cells at key oncogenes, are capable of energizing gene expression, and, importantly, are exquisitely sensitive to transcriptional disruption (Hnisz et al., 2013; Loven et al., 2013; Whyte et al., 2013). This last characteristic of SEs makes them an ideal proxy target for their attendant genes. Transcription factors that seem to offer therapeutic

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Abbreviations: ChIP-seq, chromatin immunoprecipitation sequencing; CTD, carboxy-terminal domain; GI_{50}, concentration at which half maximal inhibition of cell proliferation is achieved; GSEA, gene set enrichment analysis; MITF-hi, high MITF expression level; MITF-lo, low MITF expression level; Pol II, polymerase II; SE, super-enhancer; Ser, serine; siRNA, small interfering RNA

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opportunities, such as MITF, have historically been difficult to target with small molecule inhibitors, but an alternative approach is to selectively down-regulate the expression of these proto-oncogenic transcription factors by targeting enzymatic cofactors central to transcriptional regulation.

Recently, various groups have demonstrated the ability to preferentially affect expression of key tumor identity and oncogenic transcription factors using a first-in-class covalent CDK7 inhibitor, THZ1 (Chipumuro et al., 2014; Christensen et al., 2014; Kwiatkowski et al., 2014; Wang et al., 2015). Unique among the CDK family, CDK7 serves as a critical regulator of the cell cycle and gene transcription (Fisher, 2012; Schachter and Fisher, 2013). In the nucleus, CDK7 forms the kinase core of the RNA polymerase II (Pol II) general transcription factor IIH and phosphorylates the Pol II carboxy-terminal domain (CTD) at serine (Ser)5/Ser7, thereby promoting transcriptional initiation (Akhtar et al., 2009; Fisher, 2005; Glover-Cutter et al., 2009). CDK7 may also indirectly promote elongation via phosphorylation of CDK9, a subunit of P-TEFb that regulates transcriptional elongation by phosphorylating Pol II CTD at Ser2 (Larochelle et al., 2012).

In this study, we investigate the role SEs play in driving *MITF* expression in melanomas that lack high-level amplification of the *MITF* locus. Furthermore, we show the therapeutic potential of abrogating up-regulated *MITF* transcription in MITF-dependent melanoma cells by disrupting the SE complexes via covalent targeting of CDK7, an important component of the transcriptional apparatus.

RESULTS

MITF expression in tumors and melanoma cell lines

To explore the relationship between *MITF* copy number and RNA expression, we examined 287 melanoma tumor specimens from The Cancer Genome Atlas and found that only 3 of the 15 tumors exhibiting high *MITF* expression harbored *MITF* amplification (i.e., >4 copies) (Figure 1a). Cases of elevated *MITF* in the absence of amplification imply alternative mechanisms of *MITF* up-regulation. Recently, heightened transcription of key oncogenes in tumor cells has been linked to the presence of SEs, which have been shown to both influence cell identity and promote the expression of master oncogenes and networks of oncogenic transcription factors (Mansour et al., 2014). We thus set out to determine if SEs could be operative in some *MITF*-overexpressing lines.

To identify a set of *MITF* high- and low-expressing cells, we performed quantitative real-time reverse transcriptase–PCR analysis on 18 melanoma lines and found 8 lines with high *MITF* (MITF-hi) (>2-fold mean normalized *MITF* levels) and 5 lines with low *MITF* (MITF-lo) (<$^1/_4$-fold mean normalized *MITF* levels) expression levels (Figure 1b). Analysis of *MITF* copy number in these lines showed only two lines with evidence of *MITF* gene amplification (Figure 1b, red circles). H3K27ac chromatin immunoprecipitation sequencing (ChIP-seq) profiling, which marks active enhancers, indicated the presence of SEs at the *MITF* gene locus in MITF-hi cells and substantially less enhancer signal at the same location in an MITF-lo cell line (LOXIMVI) and a normal human dermal fibroblast line (Figure 1c) (Kaufman et al., 2016). Western blot analysis also showed higher protein levels of MITF in SK-MEL-5, UACC257, and SK-MEL-30 compared with the MITF-lo LOXIMVI and BJ fibroblast lines (see Supplementary Figure S1 online). Among all genes in the MITF-hi lines, relative ranking of enhancer size (Figure 1d) indicated that the *MITF* locus harbored some of the largest SEs in these cells (rank #2 in UACC257, #4 in SK-MEL-5, and #11 in SK-MEL-30) (see Supplementary Tables S1–S8 online). Using a public dataset, SEs were found to exist proximal to *MITF* in 4 of 10 short-passage cultures derived from melanoma specimens, providing a clinical correlate to our cell line data (Figure 1e, and see Supplementary Tables S5–S8 online) (Verfaillie et al., 2015).

We next examined whether SEs existed near *SOX10*, which is a known transcriptional regulator of *MITF* that has also been implicated as an important driver of melanoma proliferation and survival (Bondurand et al., 2000; Cronin et al., 2013; Graf et al., 2014; Harris et al., 2010; Kubic et al., 2008; Shakhova et al., 2012; Wellbrock and Arozarena, 2015). In all three MITF-hi cell lines, but not the MITF-lo LOXIMVI line, *SOX10* appeared to be associated with exceptionally large enhancers (see Supplementary Figure S2 online) (Kaufman et al., 2016), although only in UACC257 and SK-MEL-30 met the criteria for SE designation. Protein levels of SOX10 were also elevated in these lines compared with the LOXIMVI and BJ lines (see Supplementary Figure S1). These results suggest that *SOX10* and *MITF* could fuel a transcriptional dependency in the MITF-hi lines. To test this possibility, we individually suppressed these two genes in SK-MEL-5, SK-MEL-30, and UACC257 (Figure 2a, and see Supplementary Figure S3 online) and investigated cellular responses. Depletion of *SOX10* led to a notable loss of MITF in SK-MEL-5 and SK-MEL-30, and suppression of *MITF* resulted in a partial depletion of SOX10 (Figure 2a, and see Supplementary Figure S3). Consistent with the loss of these key transcription factors, cell growth (Figure 2b) and colony formation (Figure 2c) of MITF-hi cell lines were significantly impaired when either *MITF* or *SOX10* was depleted; this was not observed in the MITF-lo LOXIMVI line (see Supplementary Figure S4 online). These results indicate that *MITF* and *SOX10* are dependency genes associated with SEs in MITF-hi melanomas.

THZ1 suppresses melanoma growth in vitro and in vivo

In other malignancies, the presence of SE-associated dependency genes has provided a therapeutic opportunity to target master oncogenes through CDK7 inhibition with the first-in-class CDK7 inhibitor THZ1 (Chipumuro et al., 2014; Christensen et al., 2014; Kwiatkowski et al., 2014; Wang et al., 2015). Because MITF-hi cells appear to rely on SE-mediated *MITF* transcription for survival, we examined the effect of THZ1 in the MITF-hi lines more closely. Given THZ1's known specificity for CDK7 and its known effects on transcription, we first sought evidence of intracellular Pol II targeting by THZ1. The compound diminished the phosphorylation of initiation-associated Ser5/7 and elongation-associated Ser2 phospho-residues on Pol II CTD by 6 hours (see Supplementary Figure S5 online). CDK7-mediated phosphorylation of CDK9's activation loop (Thr186) was also inhibited by 24 hours. For both Pol II and CDK9 targets, there were concomitant decreases in protein levels by

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