Transcription 2:1, 19-22; January/February 2011; © 2011 Landes Bioscience

Dual roles of lineage restricted transcription factors

The case of MITF in melanocytes

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Microphthalmia-associated Transcription Factor, MITF, is a master regulator of melanocyte development, differentiation, migration and survival.[1] A broad collection of studies have indicated that MITF directly regulates the transcription of genes involved in pigmentation, which are selective to the melanocyte lineage. In addition, MITF controls expression of genes which are expressed in multiple cell lineages and may also play differential roles in activating vs. maintaining gene expression patterns. In this Point-of-View article, we discuss lineage restricted transcription factor activation of both tissue-specific and ubiquitously expressed genes using melanocytes and MITF as a model system that may eventually provide insights into such processes in multiple cell lineages.

Key words: MITF, melanocyte, epigenetic, gene regulation, lineage specific

Submitted: 08/15/10

Revised: 09/15/10

Accepted: 09/16/10

Previously published online: www.landesbioscience.com/journals/transcription/article/13650

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Cell reprogramming, where the epigenetic signature directing cellular identity can be erased and rewritten, demonstrates the pervasive and far-reaching importance of transcription factors in the development, maintenance and rewiring of cells.[2] In 2006, Takahashi and Yamanaka published their seminal study on cell reprogramming, showing that only four selected transcription factors were required to directly and permanently transform adult mouse fibroblasts into induced pluripotent stem (iPS) cells.[3,4] However, reprogramming can also include the conversion of one somatic cell type to another.

In 1996, it was shown that ectopic expression of a single transcription factor, MITF, may convert fibroblasts into cells with characteristics of pigment-producing melanocytes.[5] Similarly, ectopic expression of *MITF* into Medaka embryonic stem cells drives differentiation into melanocytes.[6] Conversely loss of function mutations in MITF produce depigmentation that is different from albinism,[7] where melanocytes exist but pigment production is defective. Instead, MITF deficiency produces depigmentation due to absence of viable melanocytes.[8-10] Interestingly, besides its role in melanocyte survival/proliferation, it is also clear that MITF transcriptionally regulates expression of numerous pigmentation genes. Thus, MITF appears to be a master regulator of melanocyte development, differentiation, migration, survival and function.

The extent and permanence of genetic, epigenetic and developmental changes emphasize the central role for lineage-specific transcription factors in imprinting a cell's lineage and identity. The study of MITF as a model lineage-restricted transcription factor both illustrates and extends our understanding of epigenetic changes and gene expression during cell development, maintenance and function.

MITF is a basic helix-loop-helix leucine zipper (bHLHzip) protein that recognizes and interacts with canonical E-box promoter/enhancer sequences as an obligate dimer.[11-13] Several related bHLHzip transcription factors are capable of heterodimerizing with MITF and binding to identical DNA sequences. The related factors TFEB, TFE3 and TFEC, together with MITF, are collectively termed the MiT family.[12] Whereas MITF expression appears to be largely restricted to certain specific cell-types, other members of the

Table 1. MITF known target genes in melanocyte lineage

	Gene symbol		Function
(A) lineage specific expressed genes	*TYR/OCA1*[13]	Tyrosinase/oculocutaneous albinism IA	melanin production
	TYRP1[31]	Tyrosinase-related protein 1	melanin production
	DCT/TYRP2[31]	dopachrome tautomerase (dopachrome delta-isomerase, tyrosine-related protein 2)	melanin production
	MLANA/MART1[32]	melan-A	melanosome biogenesis
	SILV/PMEL17/GP100[32]	human homologue of murine *silver*	melanosome biogenesis
	AIM1[33]	absent in melanoma 1	pigmentation pathway
	MLSN1/TRPM1[34]	melastatin/transient receptor potential cation channel, subfamily M, member 1	unknown
(B) ubiquitously expressed genes	*DIA1/DIAPH1*[20]	diaphanous-related formin 1	actin polymerization
	CDK2[17]	cyclin-dependent kinase 2	cell cycle progression
	BCL2[16]	B-cell CLL/lymphoma 2	antiapoptotic factor
	TBX2[15]	T-box transcription factor 2	maintenance of cell identity
	p21/CDKN1A[18]	cyclin-dependent kinase inhibitor 1A	cell cycle progression
	cMET[21]	c-Met receptor tyrosine kinase (hepatocyte growth factor receptor)	proto-oncogene/cell growth, motility and invasion
	p16/INK4a[19]	cyclin-dependent kinase inhibitor 2A	tumor suppressor/cell cycle inhibitor
	DICER[22]	Rnase III endonuclease	microRNA biogenesis

MiT family are thought to be more ubiquitously expressed.

Currently, MITF is known to regulate a number of genes of importance in differentiation and maintenance of the melanocyte lineage (**Table 1**).[1] A tissue restricted transcription factor with the ability to epigenetically transform somatic cells into melanocytes[5] and facilitate functions crucial to lineage development and survival is expected to activate both lineage-specific (A) and ubiquitously expressed genes involved in basic cell maintenance (B).[8-10]

(A) MITF-activated melanocyte specific genes include those controlling differentiation, which, for melanocytes, is largely defined as pigmentation. Such genes include Tyrosinase, *TYRP1*, *TYRP2/DCT*, *MART1/MLANA*, *SILV/PMEL17*, *AIM1* and *TRPM1* (reviewed in ref. 1). This subset of genes, together with its activator MITF, exhibits a lineage specific expression pattern and may be exploited as lineage markers, which aid in the diagnosis of melanocytic neoplasms, especially melanoma.[14]

(B) MITF also appears to regulate the expression, in melanocytes, of genes which are also expressed in many other cell types. Among the known genes in this category are regulators of cell cycle progression, apoptosis, proliferation and survival. In 2000, *TBX2* (T-box transcription factor) became one of the first MITF target genes to be identified that was not involved in pigmentation, but rather in cell survival.[15] Later, additional MITF target genes important for the proliferation and survival of melanocyte were described: the anti apoptotic factor *BCL2*,[16] cyclin-dependent kinase 2 (*CDK2*),[17] the cyclin dependent kinase inhibitors *p21*^{Cip1} (*CDKN1A*)[18] and *p16/INK4A*,[19] diaphanous-related forming 1 (*DIA1*),[20] and the c-*MET* receptor tyrosine kinase.[21] A recent observation by our laboratory revealed that, upon melanocyte differentiation, MITF induces the transcription of DICER, a key RNase III endonuclease crucial to miRNA biogenesis.[22] These studies demonstrated that MITF transcriptionally upregulates *DICER* during cAMP induced melanocyte differentiation, causing DICER-dependent processing of the pre-miRNA-17_92 cluster, thus targeting BIM, which results in enhanced melanocyte survival.[22] Interestingly, MITF-dependent expression of *TBX2* and *DICER* appears to occur after commitment to the melanocyte lineage[15] in the case of *TBX2* or upon cAMP induced differentiation[22] in the case of *DICER*. Although certain MITF target genes are ubiquitously expressed (i.e., not restricted to the melanocyte lineage), their expression in melanocytes is MITF-dependent and this may simultaneously require lineage commitment or differentiation.

When analyzing current information regarding target genes of MITF, several major questions become evident. Firstly, several MITF target genes possess functions that appear contradictory or conflicting. For example, MITF can act as an anti-proliferative transcription factor by modulating p21, but can equally promote cell cycle progression by transcription of the *CDK2* gene. Secondly, its targets are not uniformly responsive to MITF among different tested experimental systems. Thus, the ectopic expression of MITF in fibroblasts drove expression of melanogenic marker genes such as Tyrosinase.[5] In contrast, when Gaggioli, et al. overexpressed MITF in B16 mouse melanoma cells or human melanocytes, Tyrosinase expression remained static. However, transfection with a dominant negative form of MITF inhibits Tyrosinase expression, suggesting that MITF is required but not sufficient for Tyrosinase expression.[23] Interestingly, in reprogramming studies, only a small fraction (2%) of MITF transfected fibroblasts became melanocyte-like cells,[5] suggesting the involvement of additional unknown factors in modulating certain MITF activities. Of course additional technical differences may account for variations in experimental observations, such

as vectors, timing, magnitude of expression, analytical methods, etc.

Taken together, MITF is regulating several apparently distinct biological classes of genes which may offer a unique opportunity to study the mechanisms involved in harnessing an individual transcription factor to control multiple phenotypic outcomes. One interesting case involves the transcription of *CDK2* (cell cycle progression) and *SILVER*/*PMEL17* (differentiation/pigmentation). Although these genes reside in apparently highly distinct cellular pathways, they are thought to be regulated by the identical MITF-targeted promoter/enhancer element because of their nearly overlapping genomic location (head-to-head adjacent configuration). Whereas MITF's control of both genes appears to be melanocyte-specific, the *CDK2* gene is ubiquitously expressed and did not appear to require the MITF-targeted E box element when studied in non-melanocytic cells.[17]

Many mechanisms regulating MITF transcriptional activity remain unknown. It seems plausible that specific checkpoints in melanocyte development and function tune MITF activation of specific genes, but the precise stimuli driving preferential transcription of specific genes have not been mapped. The "dynamic epigenetic" model[20] postulates a threshold of MITF activity in which low levels of MITF activity promote proliferation, whereas higher levels are required for terminal differentiation.[20] However, the factors regulating MITF activity, from extracellular-derived signals to molecular co-factors or chromatin modifications altering promoter accessibility, are mostly unknown.

Recent epigenetic studies may shed light on these questions. In high resolution chromatin analyses from our lab, Ozsolak et al. combined nucleosome positioning data with chromatin immunoprecipitation-chip profiles and revealed that MITF predominantly binds nucleosome-free regions, supporting the model that nucleosomes limit sequence accessibility[24] (or the transcription factor may confer nucleosome phasing by its DNA binding). These studies suggest the involvement of nucleosome positioning in MITF interactions with chromatin, although numerous details remain to be determined, including the uniformity (or variation) of these patterns among distinct subsets of MITF target genes.

Chromatin modification is likely key to the regulation of MITF transcriptional activity. MITF can likely relax chromatin by recruiting the histone acetyl transferase CBP/p300 to the Tyrosinase promoter.[25,26] It is currently unclear whether endogenous target genes other than Tyrosinase are subject to this transcriptional mechanism. It was also described that MITF recruits the SWI/SNF complex to the promoters of differentiation-related targets Tyrosinase and *TRP1*, but not to cell maintenance genes *TBX2* and *BCL2*.[27,28] This mechanism is suggested to drive selective expression of MITF target genes. SWI/SNF complexes are ATP-dependent chromatin-remodeling enzymes that alter the position of nucleosomes along the chromosome and, as a consequence, affect promoter accessibility to regulatory factors.[29,30] In their work, Keenen et al. indicate that epigenetic modulation contributes to direct expression of distinct gene classes. MITF may therefore both control and be controlled by its access to nucleosome-free stretches of target sequence. Clearly, additional studies are required to fully explain how chromatin structure affects MITF access and interactions with its targets through different developmental stages.

The map of genes expressed at a given time is crucial for maintaining normal cell function. Studies of MITF have yielded new molecular intricacies, regulatory paradoxes and a multitude of unanswered questions, illuminating the likely complexity of regulatory layers required to induce expression of the right target at the right time in the life of a cell. As greater insights are gleaned into the mechanistic underpinnings of epigenetic regulation, cellular reprogramming and lineage determination, MITF may emerge as an attractive model transcription factor and simultaneously provide valuable information of relevance to human diseases, including pathologic pigmentation patterns and melanocytic neoplasms.

References

1. Levy C, Khaled M, Fisher DE. MITF: master regulator of melanocyte development and melanoma oncogene. Trends Mol Med 2006; 12:406-14.
2. Hanna J, Carey BW, Jaenisch R. Reprogramming of somatic cell identity. Cold Spring Harb Symp Quant Biol 2008; 73:147-55.
3. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126:663-76.
4. Meissner A, Wernig M, Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. Nat Biotechnol 2007; 25:1177-81.
5. Tachibana M, Takeda K, Nobukuni Y, Urabe K, Long JE, Meyers KA, et al. Ectopic expression of MITF, a gene for Waardenburg syndrome type 2, converts fibroblasts to cells with melanocyte characteristics. Nat Genet 1996; 14:50-4.
6. Bejar J, Hong Y, Schartl M. Mitf expression is sufficient to direct differentiation of medaka blastula derived stem cells to melanocytes. Development (Cambridge) 2003; 130:6545-53.
7. Newton JM, Cohen-Barak O, Hagiwara N, Gardner JM, Davisson MT, King RA, et al. Mutations in the human orthologue of the mouse underwhite gene (uw) underlie a new form of oculocutaneous albinism, OCA4. Am J Hum Genet 2001; 69:981-8.
8. Hodgkinson CA, Moore KJ, Nakayama A, Steingrimsson E, Copeland NG, Jenkins NA, et al. Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell 1993; 74:395-404.
9. Steingrimsson E, Arnheiter H, Hallsson JH, Lamoreux ML, Copeland NG, Jenkins NA. Interallelic complementation at the mouse Mitf locus. Genetics 2003; 163:267-76.
10. Widlund HR, Fisher DE. Microphthalamia-associated transcription factor: a critical regulator of pigment cell development and survival. Oncogene 2003; 22:3035-41.
11. Bentley NJ, Eisen T, Goding CR. Melanocyte-specific expression of the human tyrosinase promoter: activation by the microphthalmia gene product and role of the initiator. Mol Cell Biol 1994; 14:7996-8006.
12. Hemesath TJ, Steingrimsson E, McGill G, Hansen MJ, Vaught J, Hodgkinson CA, et al. Microphthalmia, a critical factor in melanocyte development, defines a discrete transcription factor family. Genes Dev 1994; 8:2770-80.
13. Yasumoto K, Yokoyama K, Shibata K, Tomita Y, Shibahara S. Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene. Mol Cell Biol 1994; 14:8058-70.
14. King R, Weilbaecher KN, McGill G, Cooley E, Mihm M, Fisher DE. Microphthalmia transcription factor. A sensitive and specific melanocyte marker for MelanomaDiagnosis. Am J Pathol 1999; 155:731-8.
15. Carreira S, Liu B, Goding CR. The gene encoding the T-box factor Tbx2 is a target for the microphthalmia-associated transcription factor in melanocytes. J Biol Chem 2000; 275:21920-7.
16. McGill GG, Horstmann M, Widlund HR, Du J, Motyckova G, Nishimura EK, et al. Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. Cell 2002; 109:707-18.
17. Du J, Widlund HR, Horstmann MA, Ramaswamy S, Ross K, Huber WE, et al. Critical role of CDK2 for melanoma growth linked to its melanocyte-specific transcriptional regulation by MITF. Cancer Cell 2004; 6:565-76.

18. Carreira S, Goodall J, Aksan I, La Rocca SA, Galibert MD, Denat L, et al. Mitf cooperates with Rb1 and activates p21^{Cip1} expression to regulate cell cycle progression. Nature 2005; 433:764-9.
19. Loercher AE, Tank EM, Delston RB, Harbour JW. MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. J Cell Biol 2005; 168:35-40.
20. Carreira S, Goodall J, Denat L, Rodriguez M, Nuciforo P, Hoek KS, et al. Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. Genes Dev 2006; 20:3426-39.
21. McGill GG, Haq R, Nishimura EK, Fisher DE. c-Met expression is regulated by Mitf in the melanocyte lineage. J Biol Chem 2006; 281:10365-73.
22. Levy C, Khaled M, Robinson KC, Veguilla RA, Chen PH, Yokoyama S, et al. Lineage-specific transcriptional regulation of DICER by MITF in melanocytes. Cell 2010; 141:994-1005.
23. Gaggioli C, Busca R, Abbe P, Ortonne JP, Ballotti R. Microphthalmia-associated transcription factor (MITF) is required but is not sufficient to induce the expression of melanogenic genes. Pigm Cell Res 2003; 16:374-82.
24. Ozsolak F, Song JS, Liu XS, Fisher DE. High-throughput mapping of the chromatin structure of human promoters. Nature Biotechnol 2007; 25:244-8.
25. Sato S, Roberts K, Gambino G, Cook A, Kouzarides T, Goding CR. CBP/p300 as a co-factor for the Microphthalmia transcription factor. Oncogene 1997; 14:3083-92.
26. Price ER, Ding HF, Badalian T, Bhattacharya S, Takemoto C, Yao TP, et al. Lineage-specific signaling in melanocytes. C-kit stimulation recruits p300/CBP to microphthalmia. J Biol Chem 1998; 273:17983-6.
27. de la Serna IL, Ohkawa Y, Higashi C, Dutta C, Osias J, Kommajosyula N, et al. The microphthalmia-associated transcription factor requires SWI/SNF enzymes to activate melanocyte-specific genes. J Biol Chem 2006; 281:20233-41.
28. Keenen B, Qi H, Saladi SV, Yeung M, de la Serna IL. Heterogeneous SWI/SNF chromatin remodeling complexes promote expression of microphthalmia-associated transcription factor target genes in melanoma. Oncogene 29:81-92.
29. Dechassa ML, Sabri A, Pondugula S, Kassabov SR, Chatterjee N, Kladde MP, et al. SWI/SNF has intrinsic nucleosome disassembly activity that is dependent on adjacent nucleosomes. Mol Cell 38:590-602.
30. Whitehouse I, Flaus A, Cairns BR, White MF, Workman JL, Owen-Hughes T. Nucleosome mobilization catalysed by the yeast SWI/SNF complex. Nature 1999; 400:784-7.
31. Bertolotto C, Busca R, Abbe P, Bille K, Aberdam E, Ortonne JP, et al. Different cis-acting elements are involved in the regulation of TRP1 and TRP2 promoter activities by cyclic AMP: pivotal role of M boxes (GTC ATG TGC T) and of microphthalmia. Mol Cell Biol 1998; 18:694-702.
32. Du J, Miller AJ, Widlund HR, Horstmann MA, Ramaswamy S, Fisher DE. MLANA/MART1 and SILV/PMEL17/GP100 are transcriptionally regulated by MITF in melanocytes and melanoma. Am J Pathol 2003; 163:333-43.
33. Du J, Fisher DE. Identification of Aim-1 as the underwhite mouse mutant and its transcriptional regulation by MITF. J Biol Chem 2002; 277:402-6.
34. Miller AJ, Du J, Rowan S, Hershey CL, Widlund HR, Fisher DE. Transcriptional regulation of the melanoma prognostic marker melastatin (TRPM1) by MITF in melanocytes and melanoma. Cancer Res 2004; 64:509-16.