 Prague Medical Report / Vol. 105 (2004) No. 3, p. 318–324

Microphthalmia Transcription Factor: a Specific Marker for Malignant Melanoma

Vachtenheim J.[1], Borovanský J.[2]
[1]Laboratory of Molecular Biology of the University Hospital Na Bulovce, Charles University in Prague, Czech Republic;
[2]Institute of Biochemistry and Exprimental Oncology of the First Faculty of Medicine, Charles University in Prague, Czech Republic

Received July 22, 2004, Accepted August 31, 2004

Abstract: The transcription factor microphthalmia (MITF) is required for the formation of normal melanocytes during embryonic development and for the expression of pigment cell-specific markers, which are the downstream transcriptional targets of MITF. It also seems to be crucial for the survival of malignant melanocytes. The special interest of this review is the possible utility of MITF as a marker of malignant melanoma. Melanocyte-specific isoform of MITF appears to be a unique molecule in the differential diagnosis of melanocytic tumors.

Key words: MITF – Melanoma – Melanocyte – Microphthalmia – Transcription

This work was supported by a grant from IGA (NR/8026-3), Ministry of Health, Czech Republic.

Mailing address: Jiří Vachtenheim, MD., Laboratory of Molecular Biology, Clinic of Pneumology, Third Faculty of Medicine, Charles University and University Hospital Na Bulovce, Budínova 2, 180 00 Prague 8, Czech Republic, Phone +420 266 082 272, Fax: +420 284 840 840, e-mail: jivach@upn.anet.cz

© Charles University in Prague – The Karolinum Press, Prague 2004

Introduction

Several cell lineages require the expression of the microphthalmia-associated transcription factor (MITF) to develop normally during embryogenesis. These include melanocytes (derived from the neural crest), mast cells, cells of the retina pigment epithelium (RPE), and osteoclasts. MITF has been found to be associated with human Waardenburg syndrome type IIA [1] and many different mutant alleles at the MITF locus have been described in mice with similar phenotypes (defects of pigmentation and hearing, smaller eyes, and osteopetrosis) resembling the human disease [2, 3]. In adult melanocytes, MITF is believed to activate transcription of genes required for melanogenesis, such as tyrosinase, TRP-1 and TRP-2 (tyrosinase-related proteins –1 and –2), [4, 5]. Moreover, MITF activates transcription from promoters of a set of genes which are likewise specific for melanoma cells but are not directly involved in pigment synthesis, like MLANA/MART1/Melan-A, gp100/SILV (also named PMel17), and melastatin/TRPM1 [4, 6, 7, 8]. MITF is a bHLH-LZ (basic-helix-loop-helix-leucine zipper) type transcription factor that binds to E-boxes found in promoters of these pigment cell-specific genes. In almost all studies, only the reporter assays were employed to elucidate how MITF transactivates its target genes. Transcription from endogenous promoters of known MITF targets associated with melanocytic differentiation has only begun to be addressed. Gaggioli et al. [9] recently observed an inhibition of expression of endogenous tyrosinase and TRP-1 genes in melanocytes and mouse B16 melanoma cells after the transfer of a dominant negative MITF mutant lacking the N-terminal transactivation domain. These autors confirmed the involvement of MITF in the activation of these genes in the chromatin context. Thus, apart from the role of MITF in the proper formation of embryonic pigment cells [10], it mediates an essential transcriptional activity in maintaining the differentiation of mature melanocytes, and the function is preserved upon the malignant transformation. As for the survival of developing melanocytes in the mouse embryo, recent data indicate that MITF can play a crucial role in the survival of melanoma cells. MITF has been shown to mediate the signals of the wnt/β-catenin signaling pathway, which is often upregulated in melanomas, to downstream effectors (one of which might be the bcl-2 gene but others are unknown), thus linking aberrant signaling to deregulated proliferation and/or increased survival [11, 12].

Only MITF-M isoform is melanocyte-specific

MITF is expressed as multiple splicing isoforms (termed A, B, C, D, E, H, M, mc) each of which is transcribed from a different, tissue-specific promoter. These isoforms, distinguishable by RT-PCR (reverse transcription-polymerase chain reaction) analysis, differ only in the first, isoform-specific exon, which is linked to common downstream exons [13]. Some of these isoforms are specific for the mast cells (E and mc), or are more ubiquitously expressed (A, C, and H). MITF-M form is expressed only in melanocytes and melanoma cells [13–16]. How the unique

N-terminus in MITF affects the transactivation by the whole protein is less clear but it appears to be dispensable for the ability of MITF to transactivate the tyrosinase promoter-reporter, and the mast cell-specific N-terminus linked to common exons inhibited the activation of this promoter [16, 17]. The melanocyte-specific MITF promoter is positively regulated by at least four transcription factors: CREB, an effector of the cAMP signalling pathway, Sox 10, a high mobility group type DNA-binding domain transcription factor, LEF-1, a component of the Wnt signalling pathway involving β-catenin, and Pax3, a paired box domain and a homeodomain-containing transcription factor [reviewed in 5]. The sequences and localization of the binding sites in the mouse and human promoter are almost identical suggesting a very similar regulation in both species. The transcription factors bind to their consensus sites that are in a close proximity within the MITF promoter and some of them (e.g. Pax3 and Sox10) may act in a synergistic fashion [reviewed in 5].

Immunohistochemical detection of MITF in malignant melanoma

Immunohistochemical staining of melanoma specimens with the anti-MITF antibody (D5) revealed positivity in virtually all cases [18]. This suggested that MITF could be a promising new marker for primary and metastatic melanomas. The staining was nuclear and skin melanocytes, nevi, and dysplastic nevi were also positive. Clearly, the antibody to MITF was demonstrated to be a more specific tool than the widely used HMB-45 antibody (directed to gp100) and the anti-S100 protein (a calcium-binding protein family member) antibody. A number of subsequent studies evaluated the utility of MITF in the immunohistochemical diagnosis of melanoma. Most of the studies showed that the MITF antibody could be a valuable tool in the diagnosis of melanocytic lesions, being a useful supplement to the detection of gp100, MLANA, or tyrosinase [19–23]. In one report, the expression of MITF correlated with improved survival of melanoma patients [24]. These authors observed that the higher was the number of MITF positive cells in a primary tumor sample, the better was the prognosis.

Melanomas that are more difficult to diagnose histologically, like amelanotic or desmoplastic cell melanomas, were also MITF-positive but the frequency of positive samples was lower [19, 25, 26]. In other studies, however, MITF failed to positively stain a large portion of desmoplastic and spindle-cell melanomas [23, 27–29]. Desmoplastic melanoma is a subtype characterized by a collagenous stroma and spindle-shaped cells which often lack conventional melanoma markers but are S100-positive [30]. It thus appears that this melanoma subtype lacks the expression of MITF, at least in a subset of cases. Previously, we detected three melanoma cell lines which did not express the MITF-M, but expressed low amounts of MITF-A, however it was unknown whether the original specimens were histopathologically diagnosed as desmoplastic [31]. It is further important that MITF has not been found to mislocalize to the cytoplasm in melanomas while

a rare cytoplasmic staining was seen in non-melanoma tumors like breast carcinomas, thus distinguishing a possible nonspecific staining; a false nuclear positivity in non-melanomas was extremely rare [18, 20]. In other reports, the D5 MITF antibody has been found not to be sufficiently specific for melanoma cells and the immunoreactivity was observed in several other cell types (e.g. histiocytes, lymphocytes, osteoclasts, and macrophages) rendering the melanoma diagnosis inconclusive in many cases [28, 32]. As the D5 monoclonal antibody, used in most immunohistochemical studies, is directed to the part of the molecule encoded by exons common to all MITF isoforms [33], the lack of specificity seen in these studies could be due to the staining of ubiquitous isoforms like A and H. In many non-melanocytic human tumor cell lines, MITF-A has been frequently detected [31]. However, an antibody specific to MITF-M is unavailable so far, thus precluding a possible more precise immunohistochemical diagnosis of melanocytic cells.

MITF-M in melanocytic tumors

The presence of a tissue-specific first exon in MITF enables the discrimination of isoforms by using the first exon-specific primers in the RT-PCR analysis.
The MITF-M has been succesfully detected in several reports by this method, confirming its melanocyte origin and absence in other tissues [13, 15–17, 31, 34–36]. The MITF-M isoform has been recently detected also in circulating melanoma cells in blood of melanoma patients, revealing the usefulness of this estimation for the detection of distant metastases, even in cases where tyrosinase is negative in the same sample [36]. The expression of MITF-M has been investigated in clear cell sarcoma (CCS), a malignant tumor sharing many features with melanoma. This unusual tumor (also named melanoma of soft parts) with a characteristic chromosomal translocation creating the chimeric EWS/ATF1 oncogenic protein has a predilection for the deep soft tissues of lower extremities and is associated with melanocytic pigmentation. CCS cells were shown to be MITF immunoreactive [37, 38] and the MITF-M was indeed present in cells of CCS, further confirming the close relationship of these two malignancies [35, 38]. Uveal melanomas, like tumors arising from the skin, were also shown to express the MITF-M isoform [39]. Interestingly, the MITF-M isoform was associated with the specific spindle-cell type morphology when the MITF-M-lacking human melanoma cells were transfected with this isoform and grown in SCID (severe combined immunodeficiency) mice; the growth of these MITF-M-expressing tumors in SCID mice was retarded when compared to controls [34]. Thus, the MITF-M expression may be linked to a specific phenotype in an experimental model. Although exceptionally, some melanomas and melanoma cell lines have the MITF-M repressed [34, 40]. It was shown that the re-expression of MITF-M ectopically in such cell lines could affect the phenotype (see above), but the restoration of the melanocyte-specific differentiation including the induction of silenced markers and MITF transcriptional targets could not be achieved in these

MITF-negative cells [34, 40]. Therefore, the chromatin-organized target promoters for MITF, which are otherwise MITF-sensitive in differentiated melanoma cells, are refractory to ectopically delivered MITF in MITF-negative melanoma cell lines. These melanomas thus resemble the non-melanocytic tumors rather than the original melanocytes [40].

Conclusion

Given the central role of MITF in the development and differentiation of melanocytes, it is not surprising that the most frequently used markers for melanoma diagnosis are the transcriptional targets of MITF (gp100, MLANA, tyrosinase) or MITF itself. These proteins are also the most specific for melanomas and the melanocyte-specific isoform, MITF-M, is an extremely specific marker for both normal and malignant melanocytes, confirming the melanoma diagnosis and representing an aid in the diagnosis of clear cell sarcoma, some amelanotic melanomas, and rare atypically located non-skin and non-ocular melanomas.

References

1. TASSABEHJI M., NEWTON V. E., READ A. P.: Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene. *Nat. Genet.* 8: p. 251–255, 1994.
2. MOORE K. J.: Insight into the microphthalmia gene. *Trends Genet.* 11: p. 442–448, 1995.
3. STEINGRIMSSON E., MOORE K. J., LAMOREUX M. L., FERRE-D'AMARE A. R., BURLEY S. K., ZIMRING D. C., SKOW L. C., HODGKINSON C. A., ARNHEITER H., COPELAND N. G.: Molecular basis of mouse microphthalmia (mi) mutations helps explain their developmental and phenotypic consequences. *Nat. Genet.* 8: p. 256–263, 1994.
4. BERTOLOTTO C., BUSCA R., ABBE P., BILLE K., ABERDAM E., ORTONNE J. P., BALLOTTI R.: Different cis-acting elements are involved in the regulation of TRP1 and TRP2 promoter activities by cyclic AMP: pivotal role of M boxes (GTCATGTGCT) and of microphthalmia. *Mol. Cell Biol.* 18: p. 694–702, 1998.
5. GODING C. R.: Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage. *Genes Dev.* 14: p. 1712–1728, 2000.
6. BENTLEY N. J., EISEN T., GODING C. R.: Melanocyte-specific expression of the human tyrosinase promoter: activation by the microphthalmia gene product and role of the initiator. *Mol. Cell Biol.* 14: p. 7996–8006, 1994.
7. DU J., MILLER A. J., WIDLUND H. R., HORSTMANN M. A., RAMASWAMY S., FISHER D. E.: MLANA/MART1 and SILV/PMEL17/GP100 are transcriptionally regulated by MITF in melanocytes and melanoma. *Am. J. Pathol.* 163: p. 333–343, 2003.
8. MILLER A. J., DU J., ROWAN S., HERSHEY C. L., WIDLUND H. R., FISHER D. E.: Transcriptional regulation of the melanoma prognostic marker melastatin (TRPM1) by MITF in melanocytes and melanoma. *Cancer Res.* 64: p. 509–516, 2004.
9. GAGGIOLI C., BUSCA R., ABBE P., ORTONNE J. P., BALLOTTI R.: Microphthalmia-associated transcription factor (MITF) is required but is not sufficient to induce the expression of melanogenic genes. *Pigment Cell Res.* 16: p. 374–382, 2003.
10. OPDECAMP K., NAKAYAMA A., NGUYEN M. T., HODGKINSON C. A., PAVAN W. J.,

ARNHEITER H.: Melanocyte development in vivo and in neural crest cell cultures: crucial dependence on the Mitf basic-helix-loop-helix-zipper transcription factor. *Development* 124: p. 2377–2386, 1997.
11. WIDLUND H. R., HORSTMANN M. A., PRICE E. R., CUI J., LESSNICK S. L., WU M., HE X., FISHER D. E.: Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. *J. Cell Biol.* 158: p. 1079–1087, 2002.
12. WIDLUND H. R., FISHER D. E.: Microphthalamia-associated transcription factor: a critical regulator of pigment cell development and survival. *Oncogene* 22: p. 3035–3041, 2003.
13. SHIBAHARA S., TAKEDA K., YASUMOTO K., UDONO T., WATANABE K., SAITO H., TAKAHASHI K.: Microphthalmia-associated transcription factor (MITF): multiplicity in structure, function, and regulation. *J. Investig. Dermatol. Symp. Proc.* 6: p. 99–104, 2001.
14. AMAE S., FUSE N., YASUMOTO K., SATO S., YAJIMA I., YAMAMOTO H., UDONO T., DURLU Y. K., TAMAI M., TAKAHASHI K., SHIBAHARA S.: Identification of a novel isoform of microphthalmia-associated transcription factor that is enriched in retinal pigment epithelium. *Biochem. Biophys Res. Commun.* 247: p. 710–715, 1998.
15. OBOKI K., MORII E., KATAOKA T. R., JIPPO T., KITAMURA Y.: Isoforms of mi transcription factor preferentially expressed in cultured mast cells of mice. *Biochem. Biophys Res. Commun.* 290: p.1250–1254, 2002.
16. TAKEMOTO C. M., YOON Y. J., FISHER D. E.: The identification and functional characterization of a novel mast cell isoform of the microphthalmia-associated transcription factor. *J. Biol. Chem.* 277: p. 30244–30252, 2002.
17. FUSE N., YASUMOTO K., TAKEDA K., AMAE S., YOSHIZAWA M., UDONO T., TAKAHASHI K., TAMAI M., TOMITA Y., TACHIBANA M., SHIBAHARA S.: Molecular cloning of cDNA encoding a novel microphthalmia-associated transcription factor isoform with a distinct amino-terminus. *J. Biochem. (Tokyo)* 126: p. 1043–1051, 1999.
18. KING R., WEILBAECHER K. N., MCGILL G., COOLEY E., MIHM M., FISHER D. E.: Microphthalmia transcription factor. A sensitive and specific melanocyte marker for Melanoma Diagnosis. *Am. J. Pathol.* 155: p. 731–738, 1999.
19. O'REILLY F. M., BRAT D. J., MCALPINE B. E., GROSSNIKLAUS H. E., FOLPE A. L., ARBISER J. L.: Microphthalmia transcription factor immunohistochemistry: a useful diagnostic marker in the diagnosis and detection of cutaneous melanoma, sentinel lymph node metastases, and extracutaneous melanocytic neoplasms. *J. Am. Acad. Dermatol.* 45: p. 414–419, 2001.
20. DORVAULT C. C., WEILBAECHER K. N., YEE H., FISHER D. E., CHIRIBOGA L. A., XU Y., CHHIENG D. C.: Microphthalmia transcription factor: a sensitive and specific marker for malignant melanoma in cytologic specimens. *Cancer* 93: p. 337–343, 2001.
21. CHANG K. L., FOLPE A. L.: Diagnostic utility of microphthalmia transcription factor in malignant melanoma and other tumors. *Adv. Anat. Pathol.* 8: p. 273–275, 2001.
22. SHEFFIELD M. V., YEE H., DORVAULT C. C., WEILBAECHER K. N., ELTOUM I. A., SIEGAL G. P., FISHER D. E., CHHIENG D. C.: Comparison of five antibodies as markers in the diagnosis of melanoma in cytologic preparations. *Am. J. Clin. Pathol.* 118: p. 930–936, 2002.
23. GRANTER S. R., WEILBAECHER K. N., QUIGLEY C., FISHER D. E.: Role for microphthalmia transcription factor in the diagnosis of metastatic malignant melanoma. *Appl. Immunohistochem. Mol. Morphol.* 10: p. 47–51, 2002.
24. SALTI G. I., MANOUGIAN T., FAROLAN M., SHILKAITIS A., MAJUMDAR D., DAS GUPTA T. K.: Micropthalmia transcription factor: a new prognostic marker in intermediate-thickness cutaneous malignant melanoma. *Cancer Res* 60: p. 5012–5016, 2000.
25. KOCH M. B., SHIH I. M., WEISS S. W., FOLPE A. L:. Microphthalmia transcription factor and

melanoma cell adhesion molecule expression distinguish desmoplastic/spindle cell melanoma from morphologic mimics. *Am. J. Surg. Pathol*. 25: p. 58–64, 2001.
26. KING R., GOOGE P. B., WEILBAECHER K. N., MIHM M. C., JR., FISHER D. E.: Microphthalmia transcription factor expression in cutaneous benign, malignant melanocytic, and nonmelanocytic tumors. *Am. J. Surg. Pathol.* 25: p. 51–57, 2001.
27. GRANTER S. R., WEILBAECHER K. N., QUIGLEY C., FLETCHER C. D., FISHER D. E.: Microphthalmia transcription factor: not a sensitive or specific marker for the diagnosis of desmoplastic melanoma and spindle cell (non-desmoplastic) melanoma. *Am. J. Dermatopathol.* 23: p. 185–189, 2001.
28. MIETTINEN M., FERNANDEZ M., FRANSSILA K., GATALICA Z., LASOTA J., SARLOMO-RIKALA M.: Microphthalmia transcription factor in the immunohistochemical diagnosis of metastatic melanoma: comparison with four other melanoma markers. *Am. J. Surg. Pathol.* 25: p. 205–211, 2001.
29. XU X., CHU A.Y., PASHA T. L., ELDER D. E., ZHANG P. J.: Immunoprofile of MITF, tyrosinase, melan-A, and MAGE–1 in HMB45-negative melanomas. *Am. J. Surg. Pathol.* 26: p. 82–87, 2002.
30. LONGACRE T. A., EGBERT B. M., ROUSE R. V.: Desmoplastic and spindle-cell malignant melanoma. An immunohistochemical study. *Am. J. Surg. Pathol.* 20: p. 1489–1500, 1996.
31. VACHTENHEIM J., NOVOTNA H.: Expression of genes for microphthalmia isoforms, Pax3 and MSG1, in human melanomas. *Cell Mol. Biol. (Noisy -le-grand)* 45: p. 1075–1082, 1999.
32. BUSAM K. J., IVERSEN K., COPLAN K. C., JUNGBLUTH A. A.: Analysis of microphthalmia transcription factor expression in normal tissues and tumors, and comparison of its expression with S–100 protein, gp100, and tyrosinase in desmoplastic malignant melanoma. *Am. J. Surg. Pathol.* 25: p. 197–204, 2001.
33. HEMESATH T. J., PRICE E. R., TAKEMOTO C., BADALIAN T., FISHER D.E.: MAP kinase links the transcription factor Microphthalmia to c-Kit signalling in melanocytes. *Nature* 391: p. 298–301, 1998.
34. SELZER E., WACHECK V., LUCAS T., HEERE-RESS E., WU M., WEILBAECHER K. N., SCHLEGEL W., VALENT P., WRBA F., PEHAMBERGER H., FISHER D., JANSEN B.: The melanocyte-specific isoform of the microphthalmia transcription factor affects the phenotype of human melanoma. *Cancer Res.* 62: p. 2098–2103, 2002.
35. LI K. K., GOODALL J., GODING C. R. LIAO S. K., WANG C. H., LIN Y. C., HIRAGA H., NOJIMA T., NAGASHIMA K., SCHAEFER K. L., LEE K. A.: The melanocyte inducing factor MITF is stably expressed in cell lines from human clear cell sarcoma. *Br. J. Cancer.* 89: p. 1072–1078, 2003.
36. SAMIJA I., LUKAC J., MARIC-BROZIC J., KUSIC Z.: Microphthalmia-associated transcription factor and tyrosinase as markers of melanoma cells in blood of patients with melanoma. *Croat. Med. J.* 45: p. 142–148, 2004.
37. GRANTER S. R., WEILBAECHER K. N., QUIGLEY C., FLETCHER C. D., FISHER D. E.: Clear cell sarcoma shows immunoreactivity for microphthalmia transcription factor: further evidence for melanocytic differentiation. *Mod. Pathol.* 14: p. 6–9, 2001.
38. ANTONESCU C. R., TSCHERNYAVSKY S. J., WOODRUFF J. M., JUNGBLUTH A. A., BRENNAN M. F., LADANYI M.: Molecular diagnosis of clear cell sarcoma: detection of EWS-ATF1 and MITF-M transcripts and histopathological and ultrastructural analysis of 12 cases. *J. Mol. Diagn.* 4: p. 44–52, 2002.
39. MOURIAUX F., VINCENT S., KHERROUCHE Z. ET AL.: Microphthalmia transcription factor analysis in posterior uveal melanomas. *Exp. Eye Res.* 76: p. 653–661, 2003.
40. VACHTENHEIM J., NOVOTNA H., GHANEM G.: Transcriptional repression of the microphthalmia gene in melanoma cells correlates with the unresponsiveness of target genes to ectopic microphthalmia-associated transcription factor. *J. Invest. Dermatol.* 117: p. 1505–1511, 2001.