nickel binding has not been reported. Unfortunately, SC protein extraction must be performed under denaturing conditions, and the presence of resident proteins that need their secondary or tertiary structure for nickel binding could not be evaluated. Moreover, as the cornified envelope resists breakdown into its protein constituents, they are inaccessible for analysis. Finally, the setup did not allow for assessment of nickel binding by free amino acids. In summary, we demonstrate that filaggrin derived from both SC and full epidermis binds nickel. Other epidermal proteins may bind nickel, but filaggrin is a strong, denaturation-resistant chelator, and the relevance of the other proteins, e.g., for the accumulation of nickel in the SC, remains unclear. As filaggrin null mutations are associated with an increased risk of allergic nickel dermatitis in European populations, this study provides a possible link between genetics, protein expression, and function.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Katrine Ross-Hansen[1], Ole Østergaard[2], Julia T. Tanassi[2], Jacob P. Thyssen[1], Jeanne D. Johansen[1], Torkil Menné[1] and Niels H.H. Heegaard[2]

[1]National Allergy Research Centre, Department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Hellerup, Denmark and [2]Department of Clinical Biochemistry, Immunology and Genetics, Statens Serum Institut, Copenhagen, Denmark
E-mail: katrine.ross-hansen@regionh.dk

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Evidence for an Alternatively Spliced MITF Exon 2 Variant

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TO THE EDITOR

Alternative splicing of exon 1 in the microphthalmia-associated transcription factor (*MITF*) gene gives rise to a family of transcription factors that differ only in exon 1 sequence (Steingrimsson *et al.*, 2004). Each MITF isoform is associated with a specific promoter and each unique promoter/isoform combination results in MITF expression in a different cell lineage. In the melanocytic cell lineage, MITF is expressed from the M-promoter, leading to a transcript containing exon 1 M; therefore, this isoform is termed MITF-M. The amplification of *MITF-M* occurs in 10–20% of all melanoma cases, and it has been suggested that MITF-M may be a prognostic marker for poor survival in melanoma (Garraway *et al.*, 2005; Ugurel *et al.*, 2007). Recently, a germline mutation in *MITF* (E318K) has also been shown to contribute to increased susceptibility to melanoma (Bertolotto *et al.*, 2011; Yokoyama *et al.*, 2011). The MITF signaling pathway has further been shown to contribute to melanoma susceptibility. A recent study identified frequent somatic mutations in both MITF and its regulator SOX10, with 14% of primary and 20% of metastatic melanoma containing mutations in these genes (Cronin *et al.*, 2009).

Recently, an isoform of MITF-M that is alternatively spliced at exons 2 and 6 has been reported. This shortened isoform, termed MITF-MDel, was expressed at a detectable level only in melanocytes

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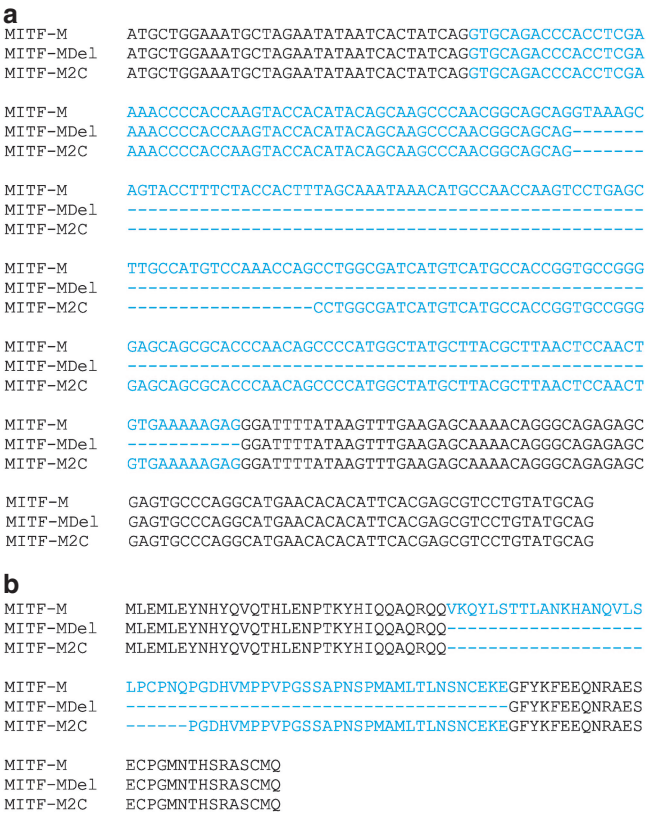

Figure 1. Microphthalmia-associated transcription factor (MITF)-M isoform alignments. (**a**) Alignment of exons 1–3 of *MITF-M* (NM_000248.3) with *MITF-Mdel* (GU355676.1) and *MITF-M2C*. Alternative exons are indicated in blue and deleted bases with a dash (–). (**b**) MITF-M2C protein prediction aligned with MITF-M (NM_000239.1) and MITF-Mdel (ABD90411). Alignment corresponds to exons 1–3, and alternative exons are indicated in blue and deleted amino acids with a dash.

and melanoma cells and was predicted to be a prognostic biomarker for melanoma (Wang *et al.*, 2010). The functional significance of MITF-MDel in either melanocyte or melanoma development is still unknown and debated.

Exon 2 of MITF-M contains a ''cryptic splice donor site'', which divides the exon into 2 A (60 bp) and 2B (168 bp). MITF-MDel was found to be lacking exon 2B (Hallsson *et al.*, 2000; Wang *et al.*, 2010). Mice carrying a homozygous germline deletion of exon 2B ($MITF^{mi\text{-}bws}$) have a mild pigmentation phenotype, whereas others have shown that exon 2B is dispensable for melanocyte development (Hallsson *et al.*, 2000; Bismuth *et al.*, 2005; Bauer *et al.*, 2009). Phosphorylation of a conserved serine residue (S73) contained within exon 2B has been proposed to both enhance the transcriptional activity and decrease the stability of the protein (Wu *et al.*, 2000). It is therefore possible that the absence of S73 could contribute to the pigmentation defects observed in $MITF^{mi\text{-}bws}$ mice and to any functional consequences of MITF-MDel expression.

While cloning *MITF-M* from cultured human melanocytes, we found a third shortened transcript that we sequenced and aligned against both *MITF-M* and *MITF-MDel* (Figure 1a). This revealed a 75 bp in-frame deletion in exon 2 commencing at the cryptic splice donor site at the start of exon 2B; here, we call this alternatively spliced isoform *MITF-M2C*. Alignment of the predicted protein sequence for MITF-M2C with MITF-M and MITF-MDel showed a deletion of 25 amino acids with those outside of the deletion remaining unaffected (Figure 1b). No previously identified functional protein components are contained within the deletion, and it is therefore possible that this transcript does not encode a protein with unique function. Importantly, the key residue for phosphorylation of S73 remains encoded in the *MITF-M2C* transcript. We cannot, however, rule out any change in the secondary structure and, therefore, potential function in the MITF-M2C protein.

To determine whether *MITF-M2C* was widely expressed, we performed quantitative real-time reverse-transcriptase–PCR (qRT–PCR) to measure each of the exon 2 variants across a panel of cell lines and patient tumor specimens. The PCR experiment was designed with one common forward primer and three separate reverse primers, which differentiated between the transcripts on the basis of the sequence across the exon 2 splice junction (Supplementary information online). Analysis of 19 human cell lines, including 18 metastatic melanoma, one primary melanoma (MM200) and normal human melanocytes, revealed a low-level expression of *MITF-M2C* across all cell lines tested (Figure 2a). Metastatic melanoma samples representing brain, skin (regional lymph node), and kidney metastases derived from 6 patients similarly showed a low-level expression of *MITF-M2C* in all specimens (Figure 2b). Sequencing of the *MITF-M2C* PCR product confirmed specific amplification and shows the *MITF-M2C* splice junction (Figure 2c). Western blot analysis provides some evidence that the protein may be expressed in both melanocytes and melanoma cell lines (Supplementary Figure S1 online). We were able to detect minor bands of a smaller apparent molecular weight than MITF-M. As the antibody used to detect MITF was raised against the N-terminal region, which is common to all three isoforms, we are unable to definitively identify and distinguish MITF-M2C and MITF-MDel. However, the relative abundance of the detected proteins was consistent with levels detected by qRT-PCR, supporting our tentative identification of these isoforms.

The results presented here show that this previously unreported splice variant of *MITF-M* is expressed at a lower level in a variety of metastatic melanoma cell lines and tumors. These results also indicate that MITF-M2C may be a common feature of cells and tissues that express *MITF-M*. On the basis of the

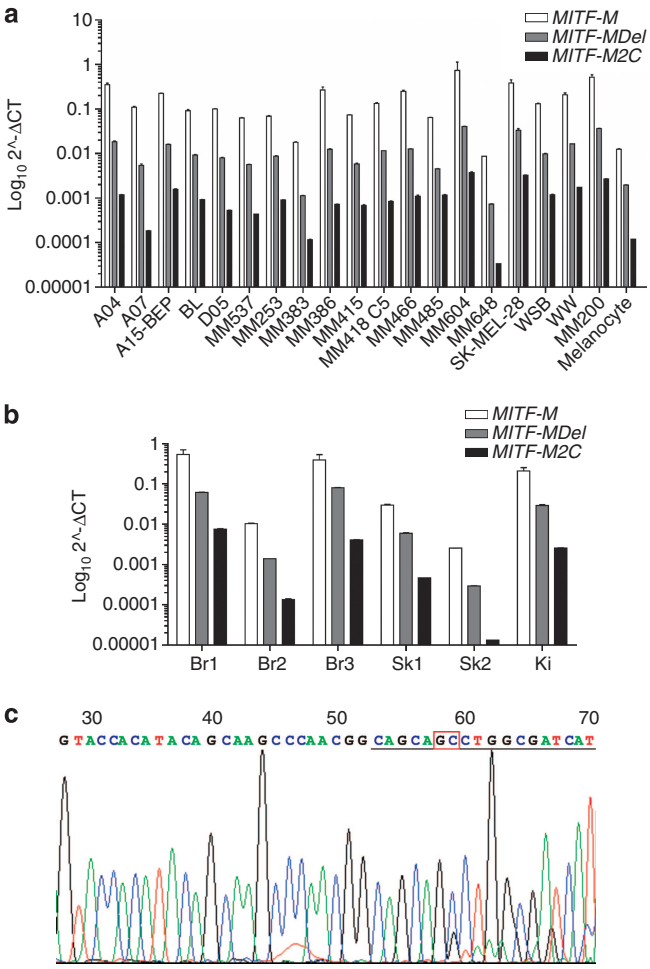

Figure 2. Detection of microphthalmia-associated transcription factor (*MITF*)-*M* isoforms in human melanocytes and melanoma cell lines. Quantitative reverse transcription PCR for *MITF-M* (white), *MITF-Mdel* (gray), and *MITF-M2C* (black), normalized to glyceraldehyde-3-phosphate dehydrogenase. (**a**) Human melanoma and melanocyte cell lines. MM200 was derived from a primary tumor. (**b**) Human metastatic melanoma tumors isolated from the brain (Br), skin/lymph node (Sk), and kidney (Ki). Error bars represent standard deviation, $n = \geqslant 2$. (**c**) An *MITF-M2C* reverse transcription PCR product was sequenced to confirm specific amplification of this isoform. The chromatogram shows the splice boundary, the location of the reverse PCR primer is underlined in black, and the bases at the splice junction are boxed in red.

current understanding, we feel that there may be little physiological significance of MITF-M2C expression in either melanogenesis or melanoma development. However, given the low expression level of this transcript, it will be interesting to see whether MITF-M2C is detected in high-throughput sequencing studies in melanoma tumor samples.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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***Jacinta L. Simmons*[1], *Carly J. Pierce*[1] and *Glen M. Boyle*[1]**

[1]*Cancer Drug Mechanisms Group, Department of Cell and Molecular Biology, QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia*
E-mail: Glen.Boyle@qimrberghofer.edu.au

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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