Title: MITF-Mdel, a Novel Melanocyte/Melanoma-specific Isoform of Microphthalmia-associated Transcription Factor-M, as a Candidate Biomarker for Melanoma

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Dr. Robin Cassady-Cain
In House Editor, BMC Medicine
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Dear Dr. Cassady-Cain:

Thank you for allowing us the opportunity to improve our manuscript. We have made the following revisions as requested:

a) Please add some further context to the background section of your abstract to make it more accessible for a general medical audience.

The following was added to the abstract:

**Background:** Melanoma incidence is on the rise, and advanced melanoma carries an extremely poor prognosis. Treatment options, including chemotherapy and immunotherapy, are limited and offer low response rates and transient efficacy. Thus, identification of new melanocyte/melanoma antigens that serve as potential novel candidate biomarkers in melanoma is an important area for investigation.

b) Please expand your background section of the main text by including an introductory paragraph outlining the link between the gene and melanoma, some brief information about what is known about biomarkers for melanoma, as well as the rationale behind pursuing this molecule as a possible biomarker for melanoma, this is to provide some context for your study for a more general medical audience.

The following was added to the background section:

Several studies provide evidence that MITF serves as an oncogene in human melanoma. MITF amplification was found in 15-20% of metastatic melanoma, and is associated with decreased five-year survival [17]. In addition, transformation of immortalized human melanocytes occurred through the cooporation of MITF and activated BRAF (V600E) [17].

The diagnosis of metastatic melanoma relies most often on S100 and HMB-45 melanoma biomarkers [18]. However, S100 is highly sensitive but not very specific as it also stains other nonmelanoma cancers. In contrast, HMB-45 is highly specific for melanoma but not very sensitive since it may miss a significant of melanoma cases. Therefore, the combination of both S100 and HMB-45 is often used to improve their
diagnostic utility [18]. MITF has been shown to be superior to the S100 and HMB-45 combination in both sensitivity and specificity in the diagnosis of melanoma [18].

One of the most difficult decisions in the treatment of melanoma is whether a particular patient needs adjuvant therapy. Current approaches to adjuvant therapy in melanoma, including the use of high dose interferon-α, are associated with significant toxicity but with modest benefits. Therefore, it is important to have ways to identify which patients are at higher risk of relapse and therefore may benefit from adjuvant therapy. One of the strategies that have been widely studied is the detection of circulating tumor cells, using a variety of molecular biomarkers. The most commonly used markers are the melanoma/melanocyte tissue-differentiation antigens, including tyrosinase and MART-1. However, the lower than expected frequency of detection of circulating tumor cells using these assays may limit their clinical utility [19]. Unlike other biomarkers in melanoma, MITF is expressed at various levels in almost all melanoma specimens [18, 20]. This is most likely due to its essential function in the survival of the melanocyte lineage [18, 20]. In addition, MITF detection after treatment was a significant independent prognostic factor for relapse-free and overall survival [21]. Therefore, MITF or MITF isoforms have the potential of being an important biomarker for melanoma.

c) Nucleic acid sequences, protein sequences, and atomic coordinates should be deposited in an appropriate database in time for the accession number to be included in the published article, please deposit your sequence data in the appropriate database (DNA Data Bank of Japan (DDBJ), European Molecular Biology Laboratory (EMBL/EBI) Nucleotide Sequence Database, or GenBank (National Center for Biotechnology Information)) and include the accession number in the manuscript.

The following was added to the results section (page 9):

MITF-Mdel sequence was deposited in Genbank (Accession number GU355676).

We hope that our paper is now suitable for publication in your journal at this time.

Sincerely,

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