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The Impact of MITF on Melanoma Development – News from Bench and Bedside

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Summary

In the current issue, two articles highlight the impact of MITF on melanoma development. In the first, Lister *et al.* (2013) reveal in vivo proof of MITF directly regulating tumor development in *BRAF*^{V600E} melanomas. In the second, Sturm *et al.* (2013) present a clinical trial that emphasizes the importance of the recently discovered E318K MITF germline mutation in patients with multiple primary melanomas.

MITF and its role in *BRAF*^{V600E} melanoma

The master melanocyte transcription factor (MITF) is a member of the microphthalmia-related transcription factor (MiT) family, and it plays key roles in survival, growth and differentiation of melanocytes, retinal pigment epithelium, osteoclasts and other hematopoietic lineages (Haq and Fisher, 2011). Several MiT family members, in addition to MITF, have been associated with cancer—specifically TFE3 (in renal carcinomas and Alveolar Soft Parts Sarcoma) and TFEB (in renal carcinomas). Upstream and downstream alterations of MITF are known to change melanoma phenotype and function. Both, high and low MITF expression levels have been associated with melanoma development.

Recently, the complex interactions among genetic and environmental factors in melanoma have been a focus of intense research. About 50% of melanoma patients exhibit somatic mutations in the *BRAF* gene coding for the B-Raf serine/threonine kinase involved in the Ras/Raf–mitogen-activated protein kinase pathway. So far, over 30 different *BRAF* mutations are known to exist, although *BRAF*^{V600E} is by far the most common mutation. Its discovery was followed by successful application of small molecule inhibitors in clinical trials (Flaherty *et al*., 2012), although drug resistance remains a problem. Since the discovery of MITF's oncogenic amplification in human melanomas (Garraway *et al.*, 2005) and the in vitro studies that corroborated a potential interplay of *BRAF* and MITF (Wellbrock and Marais, 2005), recent studies have assigned *BRAF*^{V600E} a critical role in

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Conflict of interest

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sustaining MITF activity at an intermediate level, enabling tumor growth (Hoek and Goding, 2010). However, in vivo analyses have thus far been lacking.

The published work of Lister *et al.* (2013) begins to close this gap. A temperature-sensitive *mitfa*^{vc7} mutant allele enables MITF activity to be varied within an individual animal by altering water temperature. The *mitfa*^{vc7} allele is a splice site mutation at the intron 6 splice donor site inducing a reduction in melanocyte numbers when zebrafish are reared at less than 26°C, and an almost complete loss of melanocytes at a temperature higher than 28°C. Lister and coworkers crossed this *mitfa*^{vc7} mutant zebrafish with a transgenic *BRAF*^{V600E} fish to generate *BRAF*$^{V600E/V600E}$*mitfa*$^{vc7/vc7}$ (BRAF^{V600E}*mitf*) animals. Comparing *BRAF*^{V600E}*mitf* zebrafish with *BRAF*$^{V600E/V600E}$*p53*$^{M215K/M215K}$ (*BRAF*^{V600E}*p53*) fish, *BRAF*^{V600E}*mitf* mutants showed an expression of the intron 6 mutated *mitfa* transcript and an almost complete loss of the *mitfa* transcript. *BRAF*^{V600E}*mitf* mutants, when exposed to low levels of MITF, developed pigmented lesions; in 18 out of 67 fish they progressed to melanomas, a melanoma incidence similar to that found in *BRAF*^{V600E}*p53* zebrafish (48/177). Not surprisingly, *in vivo* abrogation of MITF revealed an impressive tumor regression in 12 out of 15 fish after 8 weeks. Complete tumor regression occurred in 6 of the 12, as indicated by melanophage infiltration and increased apoptosis in tumor tissues. However, after increasing MITF levels the tumors relapsed at the previous sites. Taken together, these data strongly suggest direct interaction between MITF and growth/survival of BRAF^{V600E} melanomas, as well as a key survival role for MITF within *BRAF*^{V600E}-driven melanomas.

The E318K Mutation in Melanoma

So far, relatively few mutations have been liked to familial melanoma. In addition to CDKN2A and CDK4, a human germline mutation of MITF has now been identified. Performing whole-exome sequencing of patients from several melanoma families, Yokoyama *et al.* (2011) identified an individual carrying a germline variant (coding DNA sequence c.G1075A; protein sequence p.E318K; rs149617956) in MITF. Linkage analysis of 31 families subsequently identified to carry the variant, generated a log of odds score of 2.7 under a dominant model, indicating E318K as a possible intermediate risk variant. Large-scale screening of an Australian cohort confirmed the impact of the E318K variant. Whereas the MITF E318K variant was found in 14 out of 1,953 controls, it was present in 34 of the 2,059 patients. The MITF E318K variant was particularly augmented in cases displaying multiple primary melanomas and in those with a family history of melanoma. Consistent with a gain-of-function state for MITF, the variant was also reported to be associated with non-blue eye color. The gain-of-function was shown to correspond to loss of a previously described SUMOylation site at Codon 318 on MITF (Miller *et al*., 2005), which is otherwise antagonistic to MITF's transcriptional function. Subsequent replication of these findings in two independent population-based case-control samples from the U.K. confirmed that the MITF E318K variant occurred much more commonly in cases than controls. Bertoletto *et al.* (2011) sequenced the MITF gene in 62 patients with both melanoma and renal cell cancer and discovered the same heterozygous germline mutation resulting in substitution of glutamic acid 318 with lysine (E318K) as well as similar alterations in MITF SUMOylation and transcriptional activity. Later, an Italian study examined 667 melanoma

patients, revealing a 3-fold higher risk for developing melanoma, and a 6.4-fold higher risk for developing multiple primary melanomas for MITF E318K carriers compared to control populations. Furthermore, this mutation has been associated with an increased nevus number and non-blue eye color. Additionally, carriers with a personal and/or family history of pancreatic cancer and kidney patients seemed to suffer from a significantly higher risk of developing melanoma (Ghiorzo *et al*., 2012). However, a clear phenotypic characterization of nevi and tumor patterns in MITF E318K melanoma patients has been lacking so far.

In the current issue Sturm *et al.* (2013) screened 288 volunteer patients for the MITF E318K mutation and identified six carriers. Whereas all of these patients were fair skinned and had suffered from multiple primary melanomas, none showed a mutation in the CDK2A locus and two out of the six patients were found to be red haired MC1R R/R homozygotes. Eye imaging revealed an almost equal number of blue and non-blue eye color carriers. Furthermore, patients carrying the MITF E318K mutation showed a significantly higher number ($p=0.008$) of nevi larger than 5mm. Out of the total of 13 melanomas excised from the E318K carriers, histopathology identified 4 amelanotic melanomas. The corresponding incidence of 30% was clearly higher than the generally reported frequency of 2 to 8%, suggesting a possible impact of the MITF E318K mutation on the development of amelanotic tumors. As 3 4 melanomas were obtained from a MITF E318K carrier patient showing a MC1R homozygous R/R genotype, suggesting that interplay of MITF E318K mutants and MC1R variants might contribute to the formation of melanomas, especially amelanotic melanomas. However, further large-scale studies are needed.

Taken together, these translational studies highlight the crucial impact of MITF on melanoma risk and development. In 2010, a phase one study evaluating drug tolerance of an histone deacetylase inhibitor inhibitor, shown to suppress MITF expression (Yokoyama *et al*., 2008) was initiated (http://www.clinicaltrials.gov, Identifier: NCT01065467, accessed 10 August 2013). Further identification of MITF modulating small molecules and a better understanding of the cellular and molecular relationship between MC1R variants, E318K and BRAF/NRAS mutations will be necessary to fully understand MITF's role(s) in melanoma pathogenesis and to optimize strategies from bench to bedside.

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Clinical Implications

- Screening for CDKN2A, CDK4, and the recently discovered E318K mutation should be performed in all patients presenting with a history of familial melanomas or a history of multiple primary melanomas, due to the potential ability to identify family members with elevated risk.
- Regarding melanoma development, and especially in amelanotic melanomas, special attention should be paid to MC1R-mutant red haired patients bearing the E318K mutation.
- In a zebrafish model, abrogation of MITF activity in *BRAF*^{V600E} melanomas revealed complete melanoma regression, highlighting possible therapeutic opportunities for humans. Further investigation of small molecule modulators of MITF may lead to useful methods of screening and treatment.