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Identification of *BRAF* mutations in eruptive melanocytic nevi: new insights into melanomagenesis?

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Abstract

Eruptive melanocytic nevi (EMN) is an unusual phenomenon characterized by the abrupt, simultaneous appearance of hundreds of melanocytic nevi on previously uninvolved sun-exposed skin. The mechanisms underlying this phenomenon are not well understood, but have been associated with both systemic immunosuppression and bullous dermatoses. The paper under evaluation brings new insight into the molecular events underlying EMN development in a patient receiving 6-mercaptopurine immunosuppressive therapy for ulcerative colitis. Sequencing of DNA from 20 eruptive nevi revealed the presence of *BRAF* V600E mutations in 85% of the lesions tested. The role of mutated *BRAF* in the initiation and progression of melanoma in conjunction with the strong correlation between nevus number and melanoma risk suggests the need for photoprotection in patients receiving thiopurine therapy. The study under evaluation further points to the possible interaction between environmental mutagens and UV radiation in the acquisition of *BRAF* mutations that may in turn increase the risk of melanoma development.

Keywords

BRAF; immunosuppression; MAPK; melanoma; nevi; thiopurines; UV

Summary of methods & results

The purpose of the study was to investigate the molecular mechanisms underlying the development of eruptive melanocytic nevi (EMN) in a white male patient receiving 2 years of 6-mercaptopurine (6-MP) therapy for ulcerative colitis [1]. Over the course of the 6-MP treatment, the patient experienced an explosive growth of >200 melanocytic lesions on intermittently sun-exposed body sites. The nevi first presented as a series of red macules, which later became raised and pigmented [1]. The appearance of new nevi abated upon discontinuation of therapy. In total, 20 of these lesions were excised over a 5-year period, and formalin-fixed, paraffin-embedded tissue was selectively harvested by laser-capture microdissection. Samples were analyzed by allele-specific PCR, with follow-up verification by direct DNA sequencing. The T1799A single-nucleotide substitution (the mutated

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nucleotide target of *BRAF* V600E) was identified in 85% of the lesions evaluated. A number of the melanocytic lesions evaluated (20%) showed 'mild atypia', and one EMN was noted to be Spitz-like [1]. Most of the EMN were found to occur on intermittently sun-exposed sites, particularly the patient's upper arms, chest, back, abdomen and flank. It was therefore surmised that the development of EMN in this 6-MP-treated patient was linked to the acquisition of *BRAF* V600E mutations and that this resulted from the synergistic interaction between UV light exposure and 6-MP.

Expert commentary

Melanoma is the most devastating form of skin cancer and a leading cause of cancer death, particularly in young adults. As even relatively small melanomas can readily metastasize, early disease detection is critical. The causes of melanoma are complex and are linked to both solar UV radiation exposure as well as genetic factors [2]. Epidemiological studies have shown that individuals with fair skin, an inability to tan, blue eyes and a tendency to freckle, are more susceptible to melanoma. A history of intermittent, severe sunburn, particularly at an early age, also increases the risk of melanoma. Another predictive factor for melanoma development is the presence of large numbers of nevi [3]. Common acquired nevi are melanocytic proliferations typically acquired during the first three decades of life on areas of sun-exposed skin [4]. Although most nevi are benign, they can, in rare cases, develop into melanoma. There is a good correlation between nevus number and melanoma development, with individuals harboring >120 nevi having a 20-fold increased risk of melanoma [4,5]. Eruptive melanocytic nevi (EMN) is a rare condition characterized by the rapid appearance of hundreds of nevi, often in a grouped distribution. The condition was first described in 1868, and has been linked to bullous dermatoses and systemic immunosuppression, such as that seen following organ transplantation [6,7]. The scarcity of EMN and the limited number of cases reported in the literature has made it difficult to assess whether EMN constitutes an increased risk for melanoma [7].

Sekulic and colleagues make an addition to our knowledge about EMN by demonstrating the presence of activating *BRAF* V600E mutations in the majority of lesions arising on an EMN patient receiving thiopurine therapy [1]. The role of mutated *BRAF* in the initiation and progression of melanoma is now well established, with >50% of all cutaneous melanomas known to harbor *BRAF* V600E mutations [8,9]. Mutated *BRAF* drives melanoma progression through activation of the Ras/Raf/MEK/ERK MAPK pathway that contributes to the oncogenic phenotype of melanoma through increased cell proliferation, invasion and survival [9,10]. Interestingly, the acquisition of mutant *BRAF* seems to be an early event in melanoma development, with >80% of benign nevi also found to harbor the *BRAF* V600E mutation [11]. This apparent paradox, in which most melanocytic nevi harbor *BRAF* V600E mutations but do not progress to melanoma, was resolved by the discovery in 2006 that most nevi display the hallmarks of senescence and stain highly for the tumor suppressor p16 as well as the senescence marker β -galactosidase [12,13]. Overexpression of *BRAF* V600E in primary human melanocytes leads to an initial burst of cell proliferation followed by growth arrest and the adoption of a senescence-like phenotype [12]. Spitz nevi (benign nevi clinically and histologically resembling melanoma and thus often misdiagnosed) also exhibit high MAPK activity, but low rates of proliferation – again linked to high p16 expression [14]. Taken together, it seems likely that, although mutant *BRAF* is acquired at the precancerous stage, this usually leads to the activation of senescence pathways that prevent any further progression to malignancy [15]. The senescence status and level of growth arrest observed in the nevi of the EMN patient described by Sekulic *et al.* was not reported, but may have given some insight into the potential for future melanoma development.

BRAF V600E mutant melanomas tend to occur on sun-exposed skin [16,17], and are well correlated with skin phenotypes that have poor UV protection and an increased likelihood of solar-induced DNA damage (pale skin, poor tanning response, red hair coloration, freckling). Under normal physiological conditions, exposure of the skin to UV radiation activates *p53* in skin keratinocytes, leading to the increased transcription of α -melanocyte-stimulating hormone [18]. This in turn activates melanocortin receptor 1 signaling in melanocytes and initiates a cascade of biochemical events that ultimately leads to increased melanin synthesis [18,19]. Melanin plays a key role in protecting skin cells against UV-induced DNA damage, and there is evidence that a poor tanning response (e.g. in individuals with certain melanocortin receptor 1 polymorphisms) is associated with an increased likelihood of *BRAF* mutant melanoma development [2]. In a similar vein, the study of Sekulic *et al.* proposes that enhanced levels of DNA damage following thioguanine treatment and UV exposure may contribute to the acquisition of *BRAF* mutations in melanocytes [1]. A mechanism is suggested whereby thiopurine treatment leads to the accumulation of 6-thioguanine (6-TG) nucleotides in the DNA of treated patients, which then acts as a photosensitizer, causing the generation of reactive oxygen species that damage the surrounding DNA [1]. In support of this, the authors note the presence of two potential 6-TG binding sites that flank the commonly mutated T1799 residue of *BRAF*. Although suggested by the authors, it was not clear from the data presented whether 6-MP therapy did in fact increase levels of DNA damage in the melanocytes of the patient with EMN [1]. It is also unclear whether 6-MP therapy enhanced the *BRAF* mutation rate seen in the EMN patient under evaluation, particularly as most common nevi would also be expected to harbor the *BRAF* V600E mutation. This issue will only be resolved by measuring and comparing the *BRAF* mutation frequency rates in EMN patients who received and did not receive 6-MP treatment.

Although not discussed in great depth by Sekulic and colleagues, it is also possible that the immunosuppressive effects of 6-MP may contribute to the EMN observed. Impairment of immune function associated with HIV infection, immunosuppressive drugs following organ transplantation and chemotherapy are all known risk factors for both EMN and melanoma development [6,7]. Conversely, reactivation of immune function using anti-CTLA4 antibodies and IL-2 is known to be of therapeutic benefit for some melanoma patients [20,21]. Recent preclinical studies on mouse melanoma have also pointed to a previously unreported role for UV exposure in mediating immunosuppression that leads to melanoma initiation, through the release of IFN- γ [22].

Five-year view

Whereas the presence of a single nevus confers an unknown but presumably minimal risk, large numbers of nevi, particularly those showing atypia, are predictive for increased risk of melanoma development [3]. The finding that immunosuppressive therapies may contribute to the acquisition of *BRAF* mutations through a synergistic interaction with UV radiation is of potential clinical importance. It further demonstrates that patients receiving thiopurine therapy should be protected from UV exposure. It is worth noting that *BRAF* V600E mutations are also found in and contribute to the progression of thyroid, ovarian and colorectal carcinomas [23-25]. Whether or not thiopurine therapy would enhance the *BRAF* mutation rate in these UV-protected tissues remains to be determined. Another important implication of the study by Sekulic *et al.* is the possibility that other environmental mutagens may cooperate with UV exposure to drive *BRAF* mutation acquisition and melanoma development in susceptible individuals. Although UV exposure is known to be a major risk factor for melanoma development, this by no means accounts for all melanoma risk and there is speculation that other, as yet undiscovered, environmental factors may also contribute. Clearly this is an area deserving of further research.

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Key issues

- Eruptive melanocytic nevi (EMN) is a rare condition characterized by the sudden appearance of hundreds of benign melanocytic lesions.
- The number of nevi (particularly >120 nevi) correlate with increased melanoma risk.
- The molecular events underlying EMN are currently unknown.
- The study under evaluation reports on a patient with EMN following treatment with the immunosuppressive agent 6-mercaptopurine.
- Genomic DNA was extracted from 20 EMN over a period of 5 years. *BRAF* mutational testing of melanocytes harvested from tissue sections by laser capture microscopy revealed the presence of *BRAF* V600E mutations in 85% of samples.
- Thiopurines are known to act as photosensitizers through the generation of reactive oxygen species (ROS). It is suggested that 6-mercaptopurine and UV radiation can synergize to increase the *BRAF* mutational rate.
- It is recommended that patients receiving thiopurine therapy be protected from UV exposure.