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and p44/42MAPK signaling, suppress the expression of structural components and cell adhesion molecules in murine and human keratinocyte cultures.

Omori-Miyake *et al.* (2014) first observed that IL-4 would suppress the expression of Dsg1, Dsc1, keratin 1, and keratin 10 at both mRNA and protein levels in murine and human cultured keratinocytes. The authors confirmed that the regulation by IL-4 was dependent on IL-4 receptor-alpha and STAT6.

Moreover, the suppression of expression of Dsg1, keratin 1, and keratin 10 by IL-4 was prevented by the addition of an MEK inhibitor, suggesting that the suppression was regulated via p44/42MAPK signaling. Addition of the p38MAPK inhibitor did not alter the suppressed expression of Dsg1, keratin 1, or keratin 10 by IL-4, suggesting that p38MAPK is not responsible in this suppression. The suppression of Dsc1 expression by IL-4 was not prevented by the addition of the MEK inhibitor, suggesting the important role of STAT6 signaling in Dsc1 expression.

Similar suppressive effects were also found by the addition of IL-13 in parallel experiments. In contrast, the addition of IL-5 did not cause a suppressive effect. These results suggest that IL-4 and IL-13 have important roles in the pathogenesis of AD.

Omori-Miyake *et al.* (2014) also examined mRNA expression levels of keratins and desmosomal components in IL-4 receptor-alpha chain–deficient keratinocytes. No reduction in mRNA expression for these proteins was observed by the addition of IL-4 and IL-13 in these deficient keratinocytes, confirming that IL-4 and IL-13 exerted their effects via the IL-4 receptor-alpha chain.

Finally, the addition of IL-4 and IL-13 to cultured HaCaT cells led to cell fragmentation through downregulation of expression of Dsg1, Dsc1, keratin 1, and keratin 10. The authors speculated that AD may develop or be exacerbated by the disruption of epidermal stability owing to the suppression of structural components and cell adhesion molecules by Th2 cytokines.

This work is the first comprehensive study of the role of Th2 cytokines on the suppression of structural components and cell adhesion molecules, leading to reduced stability and integrity of keratinocytes. It indicates that in addition to immunological and allergic mechanisms, as well as filaggrin mutation and tight junction–related changes in the skin barrier, the stability and integrity of the epidermis itself, which are regulated by cytokines, may also have important roles in AD.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid for Scientific Research (No. 20390308, 20591331, 21659271, 23591634, 23791298, 23791299, 23791300, 23791301, 24659534, 24591672, 24591640, and 24791185) and Supported Program for the Strategic Research Foundation at Private Universities 2011–2015 from the Ministry of Education, Culture, Sports, Science and Technology, and by 'Research on Measures for Intractable Diseases' Project: matching fund subsidy (H23-028 to K Iwatsuki and H24-038 to TH) from the Ministry of Health, Labour and Welfare.

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Dormant Melanomas or Changing Nevi?

Giuseppe Argenziano[1], Aimilios Lallas[1], Caterina Longo[1], Elvira Moscarella[1], Margherita Raucci[1] and Iris Zalaudek[1,2]

The development of new primary melanomas in patients treated with vemurafenib has been reported recently in a study by Perier-Muzet *et al.* The primary outcome of the study was to describe the dermoscopic changes that prompted excision of those melanomas. However, the crucial point raised by the study is the large number of melanomas that were detected.

Journal of Investigative Dermatology (2014) **134,** 1196–1198; doi:10.1038/jid.2013.512

The study by Perier-Muzet *et al.* (2014) is an important contribution, one that reports the largest series of second primary melanomas in patients treated with vemurafenib for advanced melanomas. The development of multiple

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Clinical Implications

- Changing melanocytic lesions during vemurafenib treatment should be identified, and those that are melanomas diagnosed as soon as possible.
- Digital dermoscopy during follow-up examinations is an efficient method of detecting early melanomas while minimizing unnecessary excisions.

squamous cell carcinomas is a well-known phenomenon occurring in patients treated with vemurafenib, but the appearance of new primary melanomas in those patients has only recently been reported.

From the same group of investigators, Dalle *et al.*, (2011) first described five new early melanomas that developed in four patients undergoing vemurafenib treatment. Subsequently, Zimmer *et al.*, (2012) described 12 new primary melanomas detected in 11 of 19 patients treated with vemurafenib. In the study by Perier-Muzet *et al.* (2014) 14 second primary melanomas were found in 9 of 42 patients treated with vemurafenib over a mean follow-up period of about 7 months.

The intention of the investigators in this study was to describe the dermoscopic changes that prompted excision of the subsequent melanomas. Although more than 1000 lesions (56% of the monitored lesions) changed over time, only 36 were excised. The criteria that prompted excision were not quantitatively different than those occurring in non-excised lesions. However, several qualitative changes were found to be associated more frequently in the excised compared with non-excised lesions, including changes in size, changes in network morphology, and development of new criteria such as pigmented islands, dark areas, and globules.

On the basis of the assumption that no melanomas were left untreated, these dermoscopic characteristics allowed a good performance in terms of ''number needed to excise'' (2.6 nevi excised to find 1 melanoma). This established the efficiency of the method, i.e., digital dermoscopy, which is designed specifically to detect early melanoma while minimizing unnecessary excisions (Salerni *et al.*, 2013).

To our eye, the crucial point raised by this study is, however, related to the very high number of melanomas detected in this and earlier cohorts. The risk for a patient with melanoma to develop a second primary melanoma is about 5% (Moseley *et al.*, 1979), whereas in this study 21% of the patients undergoing vemurafenib treatment developed a second primary lesion. A possible explanation suggested by the authors was the following: ''… these melanomas were biologically present yet dormant, and, moreover, clinically undetectable before the instauration of therapy, and therefore only revealed by the wild type BRAF paradoxical activation by specific V600E BRAF blockers, then picked-up by repeated follow-up''.

In our opinion, an alternative explanation should be considered, namely: at least some of the melanocytic lesions classified as melanoma in patients treated with vemurafenib might be preexisting nevi that mimic melanoma histopathologically due to drug-induced activation. The development of clinical and histopathologic features of melanomas is a well-known phenomenon that may also occur, for example, in nevi after an acute UV irradiation (Tronnier and Wolff, 1995) or in regrowing nevi after incomplete removal (Kornberg and Ackerman, 1975).

There are, indeed, data in the study by Perier-Muzet *et al.* (2014) that point towards this alternative explanation. First, not only the excised melanomas but also a substantial number of nevi (56%) changed in these patients. Also, as pointed out by Haenssle *et al.* (2012), a variety of dermoscopic changes can be observed in melanocytic nevi of patients treated with vemurafenib. On one hand, nevi may involute, especially those originally showing a papillomatous surface, clinically, and a predominant globular pattern, dermoscopically. On the other hand, preexisting nevi may increase in size and become atypical, especially those that are clinically flat and dermoscopically reticular. Finally, new small reticular nevi may develop as well. Notably, papillomatous globular nevi harbor activating BRAF mutations at much higher frequency than flat reticular nevi may develop. Thus, it is reasonable to consider that globular nevi with oncogenic BRAF mutations will involute during treatment with vemurafenib, whereas the new and growing reticular nevi are those carrying wild-type BRAF (Zalaudek *et al.*, 2011). Apart from the latter speculation, it is a matter of fact that vemurafenib treatment induced changes not only in excised lesions that are histopathologically classified as melanoma, but also in a great proportion of melanocytic nevi in these patients.

A second important issue lies in the fact that the 14 melanomas found by Perier-Muzet *et al.* (2014) were all present before treatment was initiated. The authors did not report whether nevus remnants were histopathologically found in those melanomas. Thus, we can presumably exclude the possibility of new melanomas developing within preexisting nevi after treatment initiation. In other words, those melanomas were not induced but just revealed by vemurafenib. If this is true, it means that 21% of all patients with resected primary melanoma do eventually harbor ''dormant'' additional melanomas that will not be discovered unless unmasked by vemurafenib. Although theoretically possible, this scenario seems less plausible than the more simple hypothesis that preexisting nevi may have acquired morphologic features mimicking melanoma due to drug-induced activation.

In summary, further research is warranted to clarify to what extent patients treated with vemurafenib are at higher risk for developing new primary melanomas than those who do not undergo this treatment. This is obviously not a trivial issue for dermatologists and dermatopathologists, as changing melanocytic lesions occurring under vemurafenib treatment need to be differentiated and eventual melanomas diagnosed as early as possible. To this end, while these BRAF inhibitors are now being entered into large-scale trials in the adjuvant setting for patients with melanoma, the

study by Perier-Muzet *et al.* highlights the need for careful sequential skin examinations, including dermoscopy and digital monitoring of these patients.

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
This study was supported in part by the Italian Ministry of Health (RF-2010-2316524).

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Indomethacin to the Rescue of TRAIL-Resistant Melanomas

Rajasekharan Somasundaram[1] and Meenhard Herlyn[1]

Patients with melanomas develop resistance to both conventional- and targeted-therapy drugs. Promising clinical responses with immune checkpoint reagents have resulted in renewed interest in the use of biological therapies, although only subsets of individuals are known to respond to these reagents. Tse *et al.* now report on the use of indomethacin, an anti-inflammatory drug, to sensitize therapy-resistant melanoma cells.

Journal of Investigative Dermatology (2014) **134,** 1198–1199; doi:10.1038/jid.2014.1

Melanoma is an aggressive form of skin cancer with few treatment options. Melanomas are comprised of heterogeneous subtypes having distinct molecular signatures (Finn *et al.*, 2012). Due to this complexity, single-agent therapies for melanomas remain largely unsuccessful. This is because most patients with metastatic melanoma quickly develop resistance to both conventional (dacarbazine and temozolomide) and targeted therapies (vemurafenib, dabrafenib (BRAF inhibitors) and trametinib (MEK inhibitor)). Even though targeted-therapy drugs have shown dramatic reductions in tumor burden when compared with conventional chemotherapeutic agents, clinical responses are often relatively short-lived (Flaherty *et al.*, 2013). In contrast to targeted-therapy drugs, clinical responses to immune checkpoint reagents (anti-CTLA4 and anti-PD1) are more durable and long lasting (Pennock *et al.*, 2011; Hamid *et al.*, 2013). However, therapy responses in patients with good clinical outcome depend on the existence of sufficient numbers of pre-sensitized anti-melanoma T cells in the circulation or in tumor infiltrates. Thus, only subsets of patients respond to anti-CTLA4 or anti-PD1 (McDermott and Atkins, 2013). The targeted-therapy drug, vemurafenib, is known paradoxically to activate T cells, increase infiltration of these cells and upregulate melanoma-associated antigens on tumor cells, resulting in improved effectiveness by melanoma-reactive T cells (Cooper *et al.*, 2013). This has led to combination-therapy trials comprising vemurafenib and anti-CTLA4. Early observations suggest that the combination-therapy trials are accompanied by serious side effects of skin and liver toxicities (Ribas *et al.*, 2013). The reasons for these toxicities are poorly understood; however, T cells reacting to normal tissue antigens could be one of the major issues in such trials. Thus, there is a need to find additional reagents that target tumor survival directly. Many tumor types, including melanomas, evade therapies due to a defective ability of cancer cells to undergo apoptosis. Thus, targeting apoptotic pathways may improve drug sensitivities and minimize toxicities that are frequently associated with the other therapies.

Apoptosis is a normal physiological process by which cells undergo programmed cell death in response to intrinsic (mitochondrial-mediated) or extrinsic (tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated) signals (Hersey and Zhang, 2001). Auto-reactive immune cells, virus-infected cells and DNA-defective or dysfunctional cells are eliminated by apoptotic phenomena to maintain tissue integrity. Apoptosis is generally accompanied by three main events: (a) activation of caspase, a primary driver of apoptosis; (b) DNA and protein degradation; and (c) changes in membrane morphology leading to phagocytic elimination by scavenger cells. Activation of caspase is dependent on intrinsic mitochondrial–mediated or classical extrinsic TRAIL– or death receptor (DR)–mediated signaling

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