Response to Letter to the Editor on “Anti-Psoriatic Drug Monomethylfumurate Increases Nuclear Factor Erythroid 2-Related Factor 2 Levels and Induces Aquaporin-3 mRNA and Protein Expression”

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We thank Lai and colleagues for their interest in our work and appreciate the opportunity to respond to their concerns. We agree with them that the mechanism by which monomethylfumurate acts is as yet unclear, which led us to investigate its effects on nuclear factor erythroid 2-related factor 2 (Nrf2) levels. Indeed, we showed that monomethylfumurate increased Nrf2 protein levels, nuclear translocation, and target gene expression (Helwa et al., 2017); these results are consistent with data reported in other systems (Ananth et al., 2013; Promsote et al., 2014; Ahuja et al., 2016). However, we disagree that oxidative stress simply promotes psoriasis lesions since there seems to be insufficient data to determine whether oxidative stress is an etiologic factor for this disease. Clearly, oxidative stress plays a key role since markers of oxidative stress are elevated in psoriatic patients (Kaur et al., 2013; Peluso et al., 2016; Borska et al., 2017), and reduction of oxidative stress with antioxidant supplementation improves the disease (Kharaeva et al., 2009). Antioxidant molecules can also improve psoriasiform lesions in mouse models of psoriasis (see, e.g., An et al., 2016; Zhang et al., 2016; Chen et al., 2017). While we also agree that T cells are key mediators of psoriasis, there is evidence that other cells may also be involved. Thus, epidermal-specific c-Jun and JunB conditional knockout mice exhibit a psoriasiform skin phenotype that is reduced but not abolished in mice also lacking Rag-2 (Zenz et al., 2005), and therefore T and B cells, suggesting the importance of keratinocytes. Similarly, treatment of mice with clodronate liposomes to deplete

monocytes/macrophages improves psoriasiform lesions in two genetic mouse models of psoriasis (Stratis et al., 2006; Ward et al., 2011), implicating involvement of these immune cells in the disease process as well. Nevertheless, we agree with Lai and colleagues that additional studies with monomethylfumurate should be performed in T cells and other immune system components.

As for the role of Nrf2 in keratinocytes, there is evidence that Nrf2 is involved in keratinocyte proliferation, and Lai and colleagues provide several examples of references reporting such results. However, there are also data in the literature to support a contribution of Nrf2 to keratinocyte differentiation. Thus, Nrf2 levels and activation as well as its targets are increased in a differentiation-dependent manner, and Nrf2 overexpression results in upregulation of the keratinocyte differentiation markers K10 and loricrin (Lee et al., 2014). In addition, the genes encoding LCE1 family members and small proline-rich proteins SPRR2D and SPRR2H are direct targets of Nrf2 (Ishitsuka et al., 2016); these proteins are late keratinocyte differentiation markers that contribute to the cornified envelope and thus the barrier function of the epidermis. Finally, in a transgenic mouse model epidermal-specific expression of a dominant-negative Nrf2 mutant that inhibits the activity of Nrf2 and its family members enhances papilloma formation in a two-stage tumorigenesis model (auf dem Keller et al., 2006), suggesting the ability of this transcription factor family to suppress keratinocyte growth. Thus, together these results are consistent with a differentiative effect of Nrf2 and its targets. As is often the case in biology, the answer as to the exact role of Nrf2 in keratinocytes and skin is likely complex and may be dependent on the levels of Nrf2 relative to other members of the Nrf family and/or additional components of the pathway, the extent of exposure to oxidative stress, and possibly other unknown factors. Alternatively, a fine-tuning of the pathway may be necessary

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This Letter to the Editor is in response to “Anti-psoriatic drug monomethylfumurate increases nuclear factor erythroid 2-related factor 2 levels and induces aquaporin-3 mRNA and protein expression” by Helwa I, Choudhary V, Chen X, Kaddour-Djebbar I, and Bollag WB, found in *J Pharmacol Exp Ther* 2017, **362**:243–253.

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ABBREVIATIONS: AQP3, aquaporin-3; Nrf2, nuclear factor erythroid 2-related factor 2; PLD2, phospholipase D2.

such that both too much and too little Nrf2 may contribute to epidermal pathologies. Clearly, further study is needed.

Finally, contrary to the statement by Lai and colleagues in their *Letter to the Editor*, K10 is not considered to be a “hyperproliferation-associated keratin” but rather a keratin linked to normal differentiation (Moll et al., 2008). [Indeed, note that the articles cited by Lai and colleagues include these phrases: “normal keratins K14 and K10” and “differentiation marker K10” from the abstract of Elango et al. (2015) and “Keratin 6 (K6) and keratin 10 (K10) are markers for epidermal hyperproliferation and differentiation, respectively” from the abstract of Mommers et al. (2000), whereas K10 is not mentioned in the article by Ramot et al. (2013) since only keratin 6 and 16 are discussed]. Therefore, we argue that the ability of Nrf2 to induce aquaporin-3 (AQP3), which increases K10 promoter activity (Bollag et al., 2007), as well as mRNA and protein levels when AQP3 is re-expressed in AQP3 knockout keratinocytes (Choudhary et al., 2015), also argues for an effect of Nrf2 in differentiation. Nevertheless, we acknowledge that a controversy exists as to the exact role of AQP3 in the epidermis, since Verkman and colleagues have suggested a pro-proliferative role for AQP3 in keratinocytes and skin cancer (Hara-Chikuma and Verkman, 2008a,b; Verkman, 2008). In contrast, we and other investigators have reported data supporting a prodifferentiative role of AQP3 in keratinocytes. Thus, for instance, we observed an increase in the promoter activities of two differentiation markers (K10 and involucrin) upon cotransfection of keratinocytes with AQP3 but not empty vector (Bollag et al., 2007). In addition, siRNA-mediated knockdown experiments in human keratinocytes demonstrate that cells with decreased AQP3 levels exhibit a reduction in their K10 upregulation in response to an inducer of differentiation (Kim and Lee, 2010). Further support for involvement of AQP3 in differentiation is provided by the facts that differentiating-stimulating PPAR γ agonists upregulate AQP3 expression and levels (Jiang et al., 2011) and that AQP3 is induced at early time points of the differentiation mediated by high cell density in human keratinocytes, with a similar time course to that observed for the increase in keratin 1 expression (Guo et al., 2013).

We believe that the resolution to this controversy concerning the function of AQP3 in keratinocytes may be related to whether this glycerol channel is associated with phospholipase D2 (PLD2) to produce phosphatidylglycerol (or is not associated with PLD2 to instead increase glycerol levels for energy production) (Hara-Chikuma and Verkman, 2008b,c). Thus, we previously demonstrated that PLD2 can use the glycerol transported by AQP3 in a transphosphatidyl reaction to form phosphatidylglycerol (Zheng et al., 2003). PLD2 colocalizes with AQP3 in caveolin-rich membrane microdomains in keratinocytes and these two proteins can be coimmunoprecipitated from lysates of these cells (Zheng and Bollinger Bollag, 2003); therefore, we proposed that AQP3 interacts with PLD2 to funnel glycerol to PLD2 for the production of phosphatidylglycerol (Qin et al., 2011). Subsequent studies in which we manipulated this signaling module indicate that this unit can inhibit proliferation and promote early differentiation of epidermal keratinocytes (Bollag et al., 2007). Indeed, direct addition of phosphatidylglycerol, but not a related phospholipid, can inhibit proliferation and/or trigger differentiation of keratinocytes (Bollag et al., 2007; Xie et al., 2014). This idea is also consistent with

our findings that AQP3 re-expression in AQP3 knockout keratinocytes increases the protein and mRNA expression of several keratinocyte differentiation markers in a PLD2 activity-dependent fashion (Choudhary et al., 2015). Nevertheless, we continue to examine the role of AQP3 in keratinocytes, the skin, and skin diseases because of the ongoing debate.

In summary, then, it appears that much of the data concerning the involvement of Nrf2 in keratinocyte proliferation and differentiation is conflicting, with results supporting its participation in both processes. In other words, the role of Nrf2 in keratinocytes and the epidermis is complicated, requiring further investigation by interested dermatologic researchers dedicated to deciphering this important signaling system in the skin.

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