repair mechanisms, reduces inflammation, and promotes cell survival (McElroy *et al.*, 2012). The reduction in EGFR signaling observed by Campbell *et al.* (2014) is therefore likely to be primarily responsible for the lifelong diarrhea affecting the child, which is analogous to the inflammatory bowel disease seen in the ADAM17-null individuals described previously (Blaydon *et al.*, 2011).

In addition to direct stimulation by its seven ligands, EGFR signaling can also be activated indirectly by the binding of unrelated ligands to their receptors on EGFR-expressing cells, such as by vascular endothelial growth factor-A and keratinocyte growth factor (FGF7). In these processes, binding of the ligands to their receptors triggers the shedding of an EGFR ligand (typically HB-EGF or TGFα) by an ADAM protease in an Src- or ERK-dependent manner; this ligand can then act as an autocrine growth factor to activate the EGFR. As such, signaling through the EGFR underlies the activity of numerous growth factors that are not direct EGFR ligands. For example, migration of epithelial and endothelial cells in response to FGF7 or vascular endothelial growth factor-A, which bind to specific, non-EGFR-related receptor tyrosine kinases, requires Src-dependent activation of ADAM17 and shedding of HB-EGF (Maretzky *et al.*, 2011). Therefore, signaling through the EGFR is important in cell migration and proliferation in a manner that can be independent of its ligands. EGFR transactivation also underlies the gastro-protective effects of prostaglandins, which are lipid mediators known to have key roles in gastrointestinal defense and regeneration (Pai *et al.*, 2002). Prostaglandin inhibition by either nonsteroidal inflammatory drugs or genetic disease results in severe intestinal disease.

This study provides an interesting contrast to other, previously reported, inherited epidermal diseases that also affect EGFR signaling. In this study, the disease-associated EGFR mutation increases the susceptibility of the EGFR to endocytosis, thereby resulting in reduced downstream EGFR signaling. By contrast, autosomal dominant punctate palmoplantar keratoderma has been associated with mutations in the clathrin-coated vesicle-associated protein AAGAB, leading to reduced EGFR endocytosis and increased EGFR signaling (Pohler *et al.*, 2012). Similarly, the inherited cutaneous disease tylosis with esophageal cancer is characterized by upregulated ADAM17 activity and EGFR ligand shedding, also resulting in palmoplantar keratoderma (Brooke *et al.*, 2014).

Finally, the described mutation in this case is located in the extracellular domain III of the EGFR. Therapeutic mAbs targeting the EGFR used for metastatic cancer, such as cetuximab, bind to this domain. The papulopustular eruptions described with mAbs are generally more severe than those caused by EGFR tyrosine kinase domain inhibitors, illustrating the importance of the extracellular domain III in skin homeostasis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Blaydon DC, Biancheri P, Di WL *et al.* (2011) Inflammatory skin and bowel disease linked to ADAM17 deletion. *N Engl J Med* 365:1502–8

Brooke MA, Etheridge SL, Kaplan N *et al.* (2014) iRHOM2-dependent regulation of ADAM17 in cutaneous disease and epidermal barrier function. *Hum Mol Genet*; e-pub ahead of print 18 March 2014, doi:10.1093/hmg/ddu120

Campbell P, Morton P, Takeichi T *et al.* (2014) Epithelial inflammation resulting from an inherited loss-of-function mutation in EGFR. *J Invest Dermatol* 134:2570–8

Chalaris A, Adam N, Sina C *et al.* (2010) Critical role of the disintegrin metalloprotease ADAM17 for intestinal inflammation and regeneration in mice. *J Exp Med* 207:1617–24

Franzke CW, Cobzaru C, Triantafyllopoulou A *et al.* (2012) Epidermal ADAM17 maintains the skin barrier by regulating EGFR ligand-dependent terminal keratinocyte differentiation. *J Exp Med* 209:1105–19

Iwamoto R, Mekada E (2006) ErbB and HB-EGF signaling in heart development and function. *Cell Struct Funct* 31:1–14

Maretzky T, Evers A, Zhou W *et al.* (2011) Migration of growth factor-stimulated epithelial and endothelial cells depends on EGFR transactivation by ADAM17. *Nat Commun* 2:229

McElroy SJ, Hobbs S, Kallen M *et al.* (2012) Transactivation of EGFR by LPS induces COX-2 expression in enterocytes. *PLoS One* 7:e38373

Pai R, Soreghan B, Szabo IL *et al.* (2002) Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med* 8:289–93

Pohler E, Mamai O, Hirst J *et al.* (2012) Haploinsufficiency for AAGAB causes clinically heterogeneous forms of punctate palmoplantar keratoderma. *Nat Genet* 44:1272–6

See related article on pg 2589

Metformin: A Potential Drug to Treat Hyperpigmentation Disorders

Elisabeth S. Belisle[1] and Hee-Young Park[1]

Hyperpigmentation disorders are generally difficult to treat because of the limited availability of effective therapeutics with minimal side effects. In this issue, Lehraiki *et al.* report that metformin, an antidiabetic drug, inhibited melanogenesis, *in vitro* and *in vivo*, and they suggest that metformin may be used to treat hyperpigmentation disorders. This commentary reviews the molecular mechanisms through which metformin inhibits melanogenesis and examines metformin as a potential drug to treat hyperpigmentation.

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Metformin suppresses the levels of key melanogenic proteins

Metformin is an antidiabetic drug that is known to exert biological effects by inhibiting the energy-sensitive AMP-activated protein kinase (AMPK)–mammalian target of rapamycin (Viollet *et al.*, 2012). Recently, metformin was shown to decrease intracellular levels of cyclic adenosine monophosphate

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Clinical Implications

- Metformin downregulates the expression of microphthalmia-associated transcription factor.
- Metformin has effects on key melanogenic proteins and signaling pathways.
- Metformin is a potential therapeutic agent to treat hyperpigmentation disorders.

(cAMP) (Miller *et al.*, 2013). Because cAMP is a well-known modulator of melanin synthesis (melanogenesis), Lehraiki *et al.* (2014) investigated whether metformin can modulate melanogenesis.

When melanocytes were treated with metformin, basal levels of total melanin were reduced significantly. In addition, metformin blocked foreskolin and α-melanocyte-stimulating hormone (α-MSH)-induced increases in the levels of melanin (Lehraiki *et al.*, 2014). In order to elucidate the mechanisms by which metformin inhibits melanogenesis, the effects of metformin on the levels of three key melanogenic proteins—tyrosinase, tyrosinase-related protein-1 (TRP-1), and tyrosinase-related protein-2 (TRP-2)—were examined using immunoblot analyses. There are two types of melanin: dark, brown-black eumelanin and light, red-yellow sulfur-containing pheomelanin. Synthesis of both types of melanin involves a rate-limiting catalytic step in which the amino acid tyrosine is oxidized by tyrosinase to L-DOPA. This first step is thought to be the critical rate-limiting step in melanogenesis, as inhibition of this reaction blocks melanin synthesis. L-DOPA is then oxidized into DOPA-quinone and converted to 5,6-dihydroxyindole or 5,6-dihydroxyindole-2-carboxylic acid by TRP-2. The role of TRP-1 in human melanogenesis is not well characterized, but genetic mutations in TRP-1 in humans result in hypopigmentation, suggesting that TRP-1 has a key role in melanin synthesis (reviewed in Park and Yaar, 2012). Consistent with the effects of metformin on total levels of melanin, metformin decreased the basal levels of tyrosinase, TRP-1, and TRP-2, and it blocked foreskolin and α-MSH-induced increases in these three key melanogenic proteins (Lehraiki *et al.*, 2014).

Metformin downregulates the expression of microphthalmia-associated transcription factor (MITF) through a cAMP-dependent pathway

MITF, a basic–helix–loop–helix and leucine zipper transcription factor, has been termed the ''master gene'' for melanocyte survival, and it is a key factor regulating transcription of the major melanogenic proteins such as tyrosinase, TRP-1, TRP-2, protein kinase C-β (PKC-β), and MART-1 (reviewed in Park and Yaar, 2012). Moreover, it is well documented that the expression of MITF is upregulated by a cAMP-dependent pathway. When α-MSH binds to its melanocortin 1 receptor, intracellular levels of cAMP are elevated through the activation of the membrane-associated adenylate cyclase enzyme (see Figure 1). The cAMP-dependent protein kinase A enzyme is then activated, and it translocates to the nucleus and phosphorylates the cAMP-responsive element-binding protein (CREB). CREB then binds its DNA consensus sequence CRE in the promoter region of the MITF gene, thereby inducing MITF transcription (see Figure 1). Taking advantage of this knowledge, Lehraiki *et al.* (2014) treated melanocytes with metformin and examined its effects on the intracellular levels of cAMP, activation of protein kinase A, and phosphorylation of CREB, as well as on the levels of MITF. Their results showed that metformin reduced basal levels and inhibited foreskolin- and α-MSH-induced increases in the activities of protein kinase A and CREB phosphorylation, as well as increased cAMP accumulation and the levels of MITF. Thus, the authors concluded that metformin inhibits melanogenesis by downregulating the expression of MITF through a cAMP-dependent pathway.

Although the AMPK pathway has not been implicated in regulating melanogenesis, it has been well documented that AMPK is one of the major mediators of metformin's biological effects. Lehraiki and colleagues thus examined a possible role for AMPK in inhibiting melanogenes by metformin. Melanocytes were transfected with control or dominant-negative AMPK, as well as control siRNA or siRNA against AMPK. Interestingly, neither dominant-negative AMPK nor siRNA against AMPK blocked the inhibitory effects of metformin on melanogenesis. These results demonstrated that AMPK is not involved in mediating the inhibitory effects of metformin on melanogenesis.

Studies have shown that Wnt/β-catenin also has an important role in the expression of MITF (Bellei *et al.*, 2010). Treatment of melanocytes with metformin resulted in downregulation of phosphorylated β-catenin in both basal and forskolin-stimulated conditions, but the transcriptional activity of β-catenin induced by foreskolin was not affected by metformin, suggesting that the Wnt/β-catanin pathway is not involved in mediating the inhibitory effects of metformin on melanogenesis.

Both PKC and cAMP-dependent pathways were implicated in the crosstalk that regulates melanogenesis (Ao *et al.*, 1998). Endothelin-1 and histamine were also shown to utilize both PKC and cAMP-dependent pathways to exert their regulatory effects on melanocyte function (reviewed in Park and Yaar, 2012). Therefore, it is possible that metformin utilizes both PKC and cAMP-dependent pathways to exert its biological actions. Indeed, it was shown that metformin could inhibit the activation of PKC-β (Batchuluun *et al.*, 2014), and PKC-β has been well documented to regulate melanogenesis (reviewed in Park and Yaar, 2012). PKC is a serine/threonine kinase C that is activated by diacylglycerol, a component cleaved from the plasma membrane when cell surface receptors interact with their ligands. Diacylglycerol induces PKC translocation to membranes, where the latter is activated to