See related article on pg 2148

COMMENTARY

HB-EGF, the Growth Factor that Accelerates Keratinocyte Migration, But Slows Proliferation

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Among epidermal growth factors, heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) is expressed unlike others, and produces unusual effects on keratinocytes. A new report illustrates the development of a motile phenotype characterized by signs of epithelial–mesenchymal transition, reduced proliferation, and altered expression of epidermal markers. We comment on differences between endogenous HB-EGF and recombinant factor, about opportune and inopportune situations of HB-EGF overexpression by epidermal keratinocytes, as well as about the consequences on epidermal tissues.

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Among members of the epidermal growth factor (EGF) family of growth factors produced in the epidermis, the heparin-binding EGF-like growth factor (HB-EGF) is one of the ligands for the EGF receptor (EGFR) in keratinocytes (Piepkorn *et al.*, 1998). In this cell type, HB-EGF may function differently from other members of its family, as it induces unique consequences. In a new study based on lentiviral infection of immortalized hTERT keratinocytes that conditionally induces the expression of HB-EGF, Stoll *et al.* (2012) report some specific behaviors: enhanced expression of HB-EGF produces a motile phenotype in keratinocytes, with poor cell–cell adhesion and elongated morphology instead of their usual typical epithelial cobblestone pattern. The report also illustrates that this phenotype has similarities with the epithelial–mesenchymal transition that is observed during carcinogenesis in transformed epithelial cells. Surprisingly, exposure of keratinocytes to exogenous recombinant HB-EGF, even at elevated concentrations, is unable to fully induce the same phenotype, whereas exposure to culture medium conditioned by HB-EGF-overexpressing keratinocytes leads to a motile phenotype, thereby demonstrating the involvement of an additional released (soluble) growth factor. Neutralization of HB-EGF in the conditioned medium using a specific antibody, or inhibition by GM6001 of the activity of proteases required for the release of soluble HB-EGF from its membrane precursor, causes disappearance of the motile phenotype. Thus, these surprising observations reveal on one hand that a recombinant growth factor able to bind EGFRs with high affinity is poorly active in modifying keratinocyte phenotype into a motile one, whereas on the other hand, low concentrations of the endogenous growth factor that is released into the extracellular medium is sufficient to trigger the motile phenotype.

Evidence for differences between recombinant exogenous HB-EGF and endogenous HB-EGF

Ligand-dependent activation of the EGFR is likely required absolutely to induce the motile phenotype, because blockade of shedding, neutralization of HB-EGF, or inhibition of the receptor's tyrosine kinase activity impedes development of the motile phenotype. Thus, what differences between recombinant HB-EGF and endogenous factor explains the data? Stoll *et al.* (2012) propose that posttranslational modifications or consequences of the shedding of HB-EGF precursor would make the difference. They also argue against a role for the transmembrane precursor of HB-EGF. Indeed, HB-EGF membrane and cytoplasmic moieties are left in the producing cell after shedding of the extracellular soluble growth factor. These moieties could have interfered with cell signaling after autocrine activation of the EGFR at the surface of keratinocytes. Instead, collaboration with a cofactor, also released by keratinocytes overexpressing HB-EGF, and which could drive HB-EGF to signal differently, cannot be excluded. Published data suggest that hyaluronic acid for instance may be a candidate (Barnes *et al.*, 2010).

HB-EGF slows keratinocyte proliferation and favors a motile phenotype

In other respects, Stoll *et al.* (2012) further illustrate reduced cell growth in keratinocytes induced to overexpress HB-EGF. This is a rather surprising observation with respect to the potential autocrine role of HB-EGF on keratinocytes (Piepkorn *et al.*, 1998), which is mainly thought to stimulate cell proliferation and tissue hyperplasia, after retinoid treatment for instance (Rittié *et al.*, 2006).

Thus, unusual consequences of HB-EGF production and release can be expected in epidermal tissues. Exposure of keratinocytes to HB-EGF increases their expression of HB-EGF. With respect to wound healing, acceleration of keratinocyte migration and quicker epidermal repair were observed upon incubation with HB-EGF (Shirakata *et al.*, 2005). Accordingly, we reported elevated HB-EGF expression at the margins of healing epidermal wounds (Mathay *et al.*, 2008), and similar elevations in HB-EGF expression are now reported to occur in the regenerating epidermis after laser capture microdissection (Stoll *et al.*, 2012). Such a beneficial effect of HB-EGF during tissue repair appears to be in perfect concordance with its induced expression *in vivo* or *in vitro* (Stoll *et al.*, 2012). It now appears that, unlike other growth factors involved in tissue repair, such as platelet-derived growth

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Clinical Implications

- When overexpressed by keratinocytes, HB-EGF deregulates the cell phenotype.
- HB-EGF promotes epidermal wound healing while retarding keratinocyte proliferation.

factor, the main effect of HB-EGF lies in the induction of a motile phenotype, uncoupled from cell proliferation, which leads keratinocytes to re-epithelialize skin wounds before they become proliferative.

Stoll *et al.* (2012) further report that the induced HB-EGF expression in keratinocytes is concomitant with the inhibition of the expression of keratins, especially the suprabasal epidermal keratins 1 and 10. Such a downregulation of keratin 10 expression by HB-EGF in cultured keratinocytes, as well as the lack of expression of keratin 10 by keratinocytes producing HB-EGF in the migrating epidermal margins, has been reported to occur during healing (Mathay *et al.*, 2008). In addition, other reports confirm reduced expression of suprabasal keratins when HB-EGF expression is upregulated. For instance, Stoll and Elder (1998) have reported stimulated HB-EGF expression after exposure of epidermal keratinocytes to retinoic acid. Concomitant inhibition of keratin 10 expression by retinoic acid in autocrine cultures of keratinocytes has been demonstrated (Giltaire *et al.*, 2009). Furthermore, cholesterol depletion in keratinocyte plasma membranes also results in overexpression of HB-EGF, associated with the inhibition of keratin 10 expression (Mathay *et al.*, 2008, 2011).

HB-EGF expression in challenged keratinocytes

Thus, it appears that epidermal keratinocytes challenged by various alterations in their environment behave in a similar manner and exhibit stimulation of their endogenous expression of HB-EGF. This is concomitant with stimulation of the expression of other gene products, such as interleukin-8 after cholesterol depletion (Mathay *et al.*, 2011) or exposure to sensitizing chemicals (Frankart *et al.*, 2012), matrix metalloproteases 1 and 10 (Mathay *et al.*, 2011; Stoll *et al.*, 2012), and even more surprisingly, involucrin (Mathay *et al.*, 2008; Giltaire *et al.*, 2009; Mathay *et al.*, 2011). Now, Stoll *et al.* (2012) reveal that keratinocytes overexpressing HB-EGF are very likely triggered toward a motile, abnormally differentiated, but also poorly proliferative, phenotype. These results seem fully consistent with observations made during cutaneous healing at wound margins, where HB-EGF-producing keratinocytes are rather motile, exhibit altered differentiation, and do not proliferate (while in this location). Nevertheless, it is interesting to note that treatment conditions, such as exposure to retinoic acid, or disease conditions, such as atopic dermatitis and psoriasis, associated with an elevated expression of HB-EGF by keratinocytes, also exhibit altered differentiation, but are characterized *in vivo* by epidermal hyperplasia (Rittié *et al.*, 2006; Mathay *et al.*, 2011). This suggests that some of the observations made for keratinocytes cultured in low-calcium medium may not correspond to the behavior of cohesive keratinocytes *in vivo*. In fact, except for the wound healing, overexpression of HB-EGF could probably reflect an inopportune epidermal response.

The role of keratinocytes in overexpression of HB-EGF is complex. Although Stoll *et al.* (2012) contribute by uncovering new features linked to this epidermal factor, some partners and involved strategies remain hidden, complicating our current understanding of growth factor biology.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Barnes L, Tran C, Sorg O *et al.* (2010) Synergistic effect of hyaluronate fragments in retinaldehyde-induced skin hyperplasia which is a Cd44-dependent phenomenon. *PLoS ONE* 5:e14372

Frankart A, Coquette A, Schroeder KR *et al.* (2012) Studies of cell signaling in a reconstructed human epidermis exposed to sensitizers: IL-8 synthesis and release depend on EGFR activation. *Arch Dermatol Res* 304:289–303

Giltaire S, Herphelin F, Frankart F *et al.* (2009) The CYP26 inhibitor R115866 potentiates the effects of all-trans retinoic acid on cultured human epidermal keratinocytes. *Br J Dermatol* 160:505–13

Mathay C, Giltaire S, Minner F *et al.* (2008) Heparin-binding EGF-like growth factor is induced by disruption of lipid rafts and oxidative stress in keratinocytes and participates in the epidermal response to cutaneous wounds. *J Invest Dermatol* 128:717–27

Mathay C, Pierre M, Pittelkow MR *et al.* (2011) Transcriptional profiling after lipid raft disruption in keratinocytes identifies critical mediators of atopic dermatitis pathways. *J Invest Dermatol* 131:46–58

Piepkorn M, Pittelkow MR, Cook PW (1998) Autocrine regulation of keratinocytes: the emerging role of heparin-binding, epidermal growth factor-related growth factors. *J Invest Dermatol* 111:715–21

Rittié L, Varani J, Kang S *et al.* (2006) Retinoid-induced epidermal hyperplasia is mediated by epidermal growth factor receptor activation via specific induction of its ligands heparin-binding EGF and amphiregulin in human skin in vivo. *J Invest Dermatol* 126:732–9

Shirakata Y, Kimura R, Nanba D *et al.* (2005) Heparin-binding EGF-like growth factor accelerates keratinocyte migration and skin wound healing. *J Cell Sci* 118:2363–70

Stoll SW, Elder JT (1998) Retinoid regulation of heparin-binding EGF-like growth factor gene expression in human keratinocytes and skin. *Exp Dermatol* 7:391–7

Stoll SW, Rittié L, Johnson JL *et al.* (2012) Heparin-binding EGF-like growth factor promotes epithelial-mesenchymal transition in human keratinocytes. *J Invest Dermatol* 132:2148–57