Commentary

A transactivation switchboard in wound healing

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In receptor-coupled signaling, the phenomenon called transactivation develops when one or more receptors activate yet another. This process has been demonstrated most exhaustively in relation to G-protein coupled receptors (GPRCs) (1) when, upon appropriate stimuli, engagement of given GPRCs may cause the transactivation of other receptor signaling systems. Importantly, certain cytokines, chemokines and agonists of receptor tyrosine kinases (RTKs) may also exert similar effects (2). Among these, the epidermal growth factor receptor (EGFR) family, a key signal transduction system in skin biology, constitutes one of the most well-documented targets of the process.

EGFR signaling is indispensable for controlling survival, migration, and proliferation of epidermal keratinocytes (3, 4). Activation of EGFR induces pro-survival programs and protects from apoptosis which processes are equally important in wound healing and hyperproliferative skin diseases (5, s1, s2). *In vitro* cell culture experiments have proven that this pathway is responsible for the autonomous replicative capacity of keratinocytes, as EGFR inhibitors block their proliferation (s3). Another key process of wound healing is the migration of keratinocytes to the wound site, which is greatly increased by EGFR ligands (s4). Wounding typically causes the release of membrane bound EGFR ligands and, furthermore, the sustained production of matrix metalloproteinase MMP-1 which is required for migration in the absence of the basal membrane (s5). Interestingly all of these effects may be induced not only by ligands of EGFR, but also by activators of other GPCRs and RTKs that subsequently activate EGFR via ligand-dependent or -independent mechanisms - collectively referred to as transactivation.

EGFR transactivation takes place when GPCR or RTK ligands either cause the release of enzymes that cleave the mature forms of EGFR ligands from the cell membrane or initiate kinase cascades that result in the ligand-independent activation of EGFR. Ligand-dependent transactivation of EGFR has been proven to be instrumental in both normal cell functions [e.g. migration of smooth muscle cells (s6)] and disease states [breast cancer (s7) prostate cancer: (s8), reviewed in (s9) and (6)]. Ligand-independent activation, while also reported, is less well defined and investigated, with much more significant gaps in our knowledge in this regard (7).

A paper by Matus et al. in a recent volume of *Experimental Dermatology* (8) assessed the role of EGFR transactivation subsequent to the engagement bradykinin receptor B1 (BRDKRB1), a GPCR that is expressed in keratinocytes during wound healing. In this work, the authors present compelling evidence that, in both *in vivo* or *in vitro* models, although the proliferation of keratinocytes was unaffected, wound healing was nonetheless improved upon BRDKB1 activation. The mechanism behind the enhanced wound closure is most likely the increased production and secretion of MMP-2 and 9 from keratinocytes

as well as a direct effect on keratinocyte migration. These results coincide with previous reports on MMP function, where these proteins were shown to inhibit proliferation and promote differentiation and survival (s10, s11). As presented in the paper, secretion of MMPs is initiated both by ligand dependent and independent transactivation of EGFR signaling. Namely, BRDKRB1 stimulation induced the production of ADAM17, which leads to the shedding of membrane-bound ligands. In parallel to the ligand-dependent transactivation of the system, the engagement of intracellular Src kinases directly phosphorylates intracellular sites on EGFR which leads to ligand-independent activation of the receptor.

Based on these results, the bradykinin system now joins the family of GPCRs that converge on EGFR via ligand-dependent and/or independent transactivation. Other notable mediators that result in similar transactivation include, among others, acetylcholine, catecholamines, angiotensin II, endothelin-1, lysophosphatidic acid and thrombin (7). Although these mediators have been described to be present in acute wounds and evidence points to their capability of transactivating EGFR, their actual role on wound healing is surprisingly diverse. For example, acetylcholine – which influences adhesion, migration, proliferation and differentiation of keratinocytes via initiating muscarinic M1 and M3 receptor coupled MAP kinase activation through EGF transactivation – is generally considered to facilitate wound closure, while adrenergic signaling, which activates EGFR through both ligand dependent and independent pathways, is in general detrimental to wound healing (s12, s13). Other mediators released from wound sites that are capable of transactivating EGFR include angiotensin II, which causes induction of cell migration via autocrine transforming growth factor β signaling. Further, thrombin influences multiple cellular processes in the skin by activating proteinase activated receptors; however, overall it was shown to inhibit wound healing *in vivo* (s14). Whether this effect is dependent on EGFR transactivation in skin is uncovered to date, but since MMP expression and EGFR transactivation has been demonstrated on multiple cell types [including but not limited to osteoblasts, cardiomyocytes, vascular smooth muscle cells and lung adenocarcinoma cells; (9)] it is quite probable that it is present here as well.

Based on the wide variety of activators and pathways that are capable of ultimately also acting through the EGFR pathway and, furthermore, the diverse responses initiated by these activators, EGFR can be regarded as a cellular integrator switchboard of cutaneous metabotropic receptor coupled signaling. Although these results collectively expand the family of EGFR ligands, the role of transactivation in a clinical setting is still not determined and invites further research into this exciting field.

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Conflict of interests
The authors have declared no conflicting interest.

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