

In This Issue

James T. Elder

The University of Michigan Medical Center and Ann Arbor Veterans Affairs Hospital, Ann Arbor, Michigan

A Novel Role for Neutrophils in Psoriasis?

Keratinocytes are highly sensitive to the proliferative, pro-survival, and pro-migratory effects of multiple epidermal growth factor receptor (EGFR) ligands, including EGF, transforming growth factor- α (TGF- α), heparin-binding EGF-like growth factor (HB-EGF), amphiregulin (AR), and epiregulin. Interestingly, epidermal keratinocytes produce little or no EGF, whereas several of the other growth factors are produced in substantial quantities. All of these ligands are expressed as transmembrane precursors, which must be cleaved in order to interact with EGFR and elicit a proliferative response. In their article on p 338 of this issue, Meyer-Hoffert, Wingertszahn, and Wiedow have demonstrated that human leukocyte elastase (HLE) has the ability to stimulate proliferation-cultured human keratinocytes via cleavage of TGF- α . They demonstrate that the process is independent of protease-activated receptors, but dependent upon the tyrosine kinase activity of the EGFR. They also showed that HLE treatment transiently elevates intracellular calcium levels in an EGFR-dependent fashion; however, proliferation was not directly linked to proliferation in this study.

Although cleavage of HB-EGF and AR by metalloproteinases has previously been implicated in G-protein coupled receptor (GPCR)-mediated activation of epithelial tumor cells, little or no work has been focused on the potential for ErbB ligand cleavage in normal epithelial cells subjected to an inflammatory cell environment. The present findings are of additional interest because elastase is a serine protease, rather than a metalloproteinase, and because they provide a novel mechanism by which the presence of neutrophils in the upper layers of psoriatic epidermis might contribute to epidermal hyperplasia in psoriasis. TGF- α is highly expressed in the suprabasal layers of the epidermis, where neutrophils tend to collect in psoriasis lesions. Thus, however, so far no one has directly demonstrated increased activation of the EGFR in psoriatic lesions, and it is certainly possible that the signaling pathways that regulate keratinocyte proliferation in culture (i.e., EGFR and extracellular regulated kinase (ERK)) are different than those that may predominate *in vivo* (i.e., NF- κ B). Although the apparent lack of apparent EGFR activation in psoriasis is puzzling, recent studies by our laboratory and others have shown that only low levels of keratinocyte EGFR activation are necessary for

full activation of ERK. Moreover, others have demonstrated increased ERK phosphorylation and GTP loading of Ras (a major link between EGFR and ERK activation) in psoriasis lesions. Thus, although important questions remain unanswered, it remains reasonable to suspect that proteolytic release of one or more EGFR ligands may prove to be the "missing link" between immune/inflammatory cell infiltration and epidermal hyperplasia in psoriasis.

Neutrophilic infiltration of the epidermis is a relatively late event in the development of psoriasis lesions, appearing only after the appearance of dermal perivascular lymphocytes and histiocytes, lymphocytic infiltration of the epidermis, and the development of epidermal hyperplasia. Thus, even if EGFR signaling is important for psoriatic epidermal hyperplasia *in vivo*, it is hard to argue that elastase-mediated cleavage of TGF- α would be the initial event leading to epidermal hyperplasia. It could, however, play a very important role in the spreading of psoriatic lesions, as previous studies of patients with actively spreading plaques were found to express greatly elevated levels of HLE. In contrast, HLE levels were in the normal range in guttate psoriasis lesions. This finding does not rule out a role for autocrine ErbB stimulation at earlier stages of lesion development, as AR, HB-EGF, and epiregulin are also overexpressed in psoriasis lesions. Each of these ligands could be cleaved by different mechanisms, with different relationships to the inflammatory infiltrate and/or to keratinocyte signaling. Indeed, transgenic mice expressing AR in keratinocytes have been found to develop inflammatory and hyperplastic skin lesions resembling human psoriasis as well as an inflammatory arthritis.

To date, there are no successful reports of psoriasis treatment by antibodies or receptor tyrosine kinase inhibitors specific for EGFR. Whether this reflects unpublished negative results or simply a paucity of clinical trials is unclear. The finding that only minimal EGFR activation is necessary for full activation of ERK in cultured keratinocytes suggests that therapeutically attainable levels of EGFR blockade may be inadequate to shut down relevant downstream responses. Alternatively, EGFR signaling may prove to be irrelevant to psoriatic epidermal hyperplasia *in vivo*. Only time will tell.

Vectoring in on Melanoma

Melanoma continues to taunt us with its spontaneous regressions, and yet we are not yet able to harness the power of the immune system to cause this joyous event at will.

Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HPV, human papillomavirus

Evidence from animal models and human clinical trials strongly suggests that these remissions are due to immunologic attack against the tumor. The ability to optimize these immunological responses is therefore a key to improved melanoma survival. Increasingly, we appreciate that adaptive immune responses depend upon the quality of the antecedent innate immune response. It is quite remarkable that CpG oligonucleotides, which are much more common in pathogen genomes than in the human genome, serve to enhance the innate immune response by interacting with the toll-like-receptor 9 on antigen-presenting cells, thereby serving to boost the acquired immune response. The use of such oligonucleotides is not new; however, previously, they have usually been injected separately from the expression vector encoding the antigen. On p 371 of this issue, Schneeberger, Wagner, and colleagues demonstrate that by incorporating CpG oligonucleotides into the plasmid vector used to express melanoma antigens, they can boost an antigen-specific, cell-mediated response against melanoma cells in a mouse model of cancer vaccination. This maneuver should ensure an optimal adjuvant effect of CpG DNA, because these sequences are forced to be present in the same cell that expresses the melanoma antigen.

It is important to recognize that this model has been chosen precisely because this combination of melanoma cells and host strain are known to be immunogenic. Unfortunately, many human melanomas can outwit the immune response by downregulating the HLA antigens required to present antigen, and probably by other mechanisms as well. Nevertheless, the results presented here are quite robust and encouraging that similar strategies will be of benefit in human melanoma, especially when combined with the ability to inject DNA capable of producing an optimal cytokine brew for encouraging expression of MHC antigens and otherwise encouraging the anti-tumor response.

Warts and Skin Cancer: Only Skin Deep?

Certain subtypes of human papillomavirus (HPV) (notably HPV-16) have clearly been shown to have carcinogenic properties in cervical neoplasia, and HPV sequences have been reported in a high percentage of premalignant and malignant skin tumors. The association is particularly strong in renal transplant patients, suggesting that the impaired immune system of the transplant patient might be unable to prevent HPV infection, allowing the virus to gain a foothold in the skin. But the HPV types reported in these cancers have

been of many different types, and high levels of viral DNA positivity have been reported in normal appearing skin of the same patients. On p 388 of this issue, Forslund, Dillner, and colleagues utilize PCR and DNA sequencing to compare the prevalence, amount, and type of HPV DNA sequences found on the exterior surface of various benign and malignant skin tumors (seborrheic keratosis, actinic keratosis, squamous cell carcinoma, and basal cell carcinoma) to those found in the underlying tumor. To accomplish this, skin lesions were tape-stripped prior to biopsy, and the viral DNA amplified from the tape-strippings and punch biopsy of the lesion after tape-stripping were compared with those obtained from swabs taken from normal-appearing perilesional skin, forehead skin, and buttocks skin. Interestingly, they found that the surface of lesional skin was far more likely to harbor HPV DNA sequences than the underlying lesion (69% vs 12%, $p < 0.001$), with similar findings for all lesion types examined. Moreover, the prevalence and approximate amount of HPV DNA that could be amplified from the tape-strippings was very similar to that found in swabs from normal-appearing skin, independent of lesion type or body site. Also, HPV DNA sequences were much more abundant in tape-strippings and skin swabs than in the underlying tumors.

These findings might be explained by viral DNA amplification in the upper epidermal layers in the context of virus production (indeed, intact virions were identified in a swab of normal skin). But in approximately half of the cases in which comparison was possible, the HPV types identified in the biopsy of the underlying lesion after tape-stripping were different than those identified in the overlying tape-strippings. This finding is not consistent with viral replication, and suggests that the surface of our skin may be a reservoir for HPV virions. These may have replicated elsewhere on our own skin or hair, or may be drawn from the skin of others. This very high prevalence of virus carriage without apparent infection is a testament to the power of the stratum corneum (along with other innate and acquired immune mechanisms) to ward off HPV infection and to the power of PCR.

These results raise an important note of caution in attempting to forge a causal link between HPV and skin cancer. They, however, also provide a way to focus future efforts to establish HPV causality in skin cancer on the relatively small number of tumors that continue to carry HPV after surface-stripping.