Invasive skin carcinoma—Ras and α6β4 integrin lead the way

Although the genesis of invasive squamous cell carcinoma (SCC) from stratified epithelia of the skin is considered to be a complex, multistage process, recent work on human epidermis reveals that sustained Ras signaling coupled with suppression of Ras-induced growth arrest is sufficient to drive the entire process and that the α6β4 integrin and its laminin 5 ligand are essential components of this process.

Understanding the mechanisms that contribute to the genesis of invasive carcinoma from healthy epithelia is a problem of significance and considerable challenge. In the epidermis, the most common site of human cancer, this process involves disruption of the tightly regulated cycle of epidermal growth and differentiation, resulting in hyperproliferation and subsequent invasion into dermis. Enter the Ras GTPase, one of the most widely studied and important molecules in cancer, which has the potential to affect epithelial function and carcinogenesis in numerous and profound ways (Shields et al., 2000). Although the involvement of Ras in human SCC seems likely, given data from mouse models of SCC (Yuspa, 1998) and the finding that activating Ras mutations occur in some spontaneous SCCs (Pierceall et al., 1991), the fact that expression of active Ras induces growth arrest in human epidermal cells complicates the nature of its involvement (Dajee et al., 2003). In principle, the existence of a secondary mechanism that prevents Ras-induced growth arrest would liberate the tumorigenic functions of Ras. Recent studies from Khavari’s group (Dajee et al., 2003; Lazarov et al., 2002) validate this idea and provide insights into the mechanisms that underlie the formation of invasive SCC in humans.

Using a model system that involves the retroviral expression of specific genes in primary human keratinocytes and the subsequent use of these cells for grafting to regenerate human skin on immune-deficient mice, Khavari’s group discovered that expression of oncogenic Ras (Ras Gly12Val mutant) alone induces growth arrest and graft failure because it suppresses expression of CDK4, a cyclin-dependent kinase that inactivates Rb. However, coexpression of oncogenic Ras with molecules that impede Ras-induced growth arrest results in epidermal carcinoma resembling SCC. Invasive epidermal carcinoma resulted from the coexpression of oncogenic Ras with either CDK4 (Lazarov et al., 2002) or IκBα (Dajee et al., 2003), a molecule that complexes with NF-κB and prevents it from entering the nucleus and mediating transcription. Interestingly, CDK4 expression appears to be central to the mechanism of tumorigenesis in both studies, because IκBα induces CDK4 expression, providing a mechanism by which NF-κB inhibition prevents Ras-mediated growth arrest (Figure 1).

The results obtained with IκBα are of particular interest to many in the NF-κB field, because it had been known that NF-κB inhibits epidermal proliferation and that inhibition of NF-κB predisposes murine skin to tumorigenesis (Dajee et al., 2003), functions that contrast with the protumorigenic function of NF-κB seen in other tumors (Karin et al., 2002). In fact, expression of IκBα alone in the graft model induced a mild hyperplasia substantiating the finding that NF-κB inhibits epidermal proliferation. One conclusion that emerges from their work is that cooperativity between Ras signaling and NF-κB function drives the formation of SCCs: IκBα expression enables epidermal cells to circumvent Ras G1 restraints, and Ras prevents the susceptibility to apoptosis caused by inhibition of NF-κB function. An unfortunate consequence of these findings is that NF-κB inhibitors, which are being developed for cancer therapy, may actually promote the formation of epidermal carcinomas. A striking finding in the Khavari studies is that the epidermal carcinomas are highly invasive. The mechanisms involved in the formation of carcinomas and in the progression to invasive carcinoma are rather distinct, implying that the cooperative effects of Ras signaling and either CDK4 or IκBα expression transcend cell cycle regulation and alter other aspects of epithelial function. This implication is validated by their finding that a marked decrease in E-cadherin expression was seen in both the Ras/CDK4 and Ras/IκBα tumors. Loss of E-cadherin is known to facilitate an invasive phenotype by promoting an epithelial-mesenchymal transition, a process that can be driven by Ras (Oft et al., 2002). Other changes observed in these tumors include increased expression of matrix metalloproteases and other factors that promote invasiveness.
VEGF, changes that are also associated with invasive carcinoma. Although these recent studies have focused on cell cycle regulation, a challenge ahead is to establish mechanistic links between Ras signaling, IkBα, or CDK4 expression, and specific functions associated with invasion. A key issue in this direction is whether IkBα, for example, contributes to invasive functions directly. Convincing data provided by Dajee et al. (2003) indicate that the effects of IkBα expression are limited to NF-κB inhibition. Unless NF-κB mediates the transcription of genes that are “antiinvasive,” a likely assumption is that the invasive phenotype observed in the oncogenic Ras/IκBα tumors is the result of sustained Ras signaling. One potentially fruitful line of research would be to investigate endogenous TGF-β signaling in the oncogenic Ras/IκBα tumors, given that elevation of Ras and Smad2 levels is sufficient to generate invasive SCC in mice (Oft et al., 2002).

Another important finding reported by Dajee et al. (2003) is the necessity of the α6β4 integrin for epidermal carcinoma formation. This integrin is essential for the integrity of the epidermis because it engages laminin 5 in the basement membrane that separates the epidermis from the dermis, and it provides a link to the intermediate filament cytoskeleton in epidermal cells in rigid structures termed hemidesmosomes (Mercurio and Rabinovitz, 2001). It has been implicated also in epidermal proliferation (Mainiero et al., 1997). In addition, many studies have established a role for α6β4 in more dynamic functions associated with invasive carcinoma, including migration and survival (Mercurio and Rabinovitz, 2001; Shaw et al., 1997). Findings that are consistent with the fact that expression of α6β4 increases markedly in SCC but not in basal cell carcinomas (Rossen et al., 1994), which also originate from epidermal cells but are relatively nonaggressive. Given these prior studies, their observation that antibodies specific for either α6β4 or laminin 5 prevent the genesis of invasive SCC is reassuring. In a more definitive experiment, Dajee et al. (2003) observed that keratinocytes deficient in the expression of either the β4 subunit or laminin 5 (isolated from patients with blistersing skin diseases) were unable to form tumors upon expression of oncogenic Ras/IκBα, but expression of the respective genes restored their ability to form invasive tumors. These latter data provide the most definitive evidence to date for the involvement of α6β4 in the genesis of invasive carcinoma. It will be informative to discern how the multiple functions of α6β4 (e.g., proliferation, migration, survival) contribute to the formation of invasive epidermal carcinomas.

Despite the findings that activating Ras mutations occur in some spontaneous SCCs and that IkBα expression is increased in some SCCs compared to normal epidermis, Dajee et al. (2003) indicate correctly that these specific alterations may not account for the formation of most SCCs. Rather, they posit that “these proteins may engage programs of carcinogenesis that are accessible to several oncogenic factors.” One could postulate that alterations in the expression or activation of Ras effectors contribute to SCC. In this direction, for example, it is worth noting that a high frequency of activating mutations in B-Raf, a Ras effector, occurs in premalignant and malignant melanomas (Pollock et al., 2003). Nonetheless, the recent findings of the Khavari group substantiate the central role of the Ras pathway in the genesis of invasive epidermal carcinoma, and they reveal the necessity of additional mechanisms such as NF-kB inhibition that release Ras-mediated growth arrest. They also present compelling evidence for the involvement of the α6β4 integrin and one of its laminin ligands in epidermal carcinogenesis. Future studies that link components of the Ras pathway to specific aspects of epidermal transformation and invasion and that define the interactions between Ras and α6β4 more rigorously should be rewarding.

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