Emerging Laminin-332–Dependent and –Independent Roles for Integrin α3 in Protumorigenic Signaling

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The epidermal integrin α3β1 promotes skin tumorigenesis in experimental models; yet, the underlying molecular mechanisms remain mostly unclear. In their article, Ramovs et al. (2020a) identify two spatially separated α3β1-dependent signaling branches fostering skin tumor outgrowth. In basal keratinocytes, α3β1/laminin (LN)-332 drives FAK/Src activation, whereas in suprabasal layers, junctional α3β1 and the tetraspanin CD151 mediate signal transducer and protein kinase B (Akt)–dependent survival that is independent of LN-332 binding.

Introduction

The integrin adhesion receptors play pivotal roles in metazoan life, serving to connect cells to their microenvironment. As heterodimeric transmembrane proteins, integrins bind to distinct ligands in the extracellular matrix (ECM), and through complex intracellular molecular interactions, they transduce extrinsic cues to intrinsic biochemical signals (Humphries et al., 2019). Integrins take a center stage in diverse processes including cell attachment and spreading, cell polarization, survival, and motility, and they also underlie tumor formation, progression, and metastasis. Integrin deregulation is observed in a variety of human cancers, and mechanistic studies using loss-of-function and gain-of-function models could reveal key contributions of integrins in different stages of malignant disease (Hamidi and Ivaska, 2018). However, owing to the plethora of integrin heterodimers that are expressed by cells in tumors and their complex environments and considering the emerging number of both matrix-dependent and -independent ligands of integrins, the exact pathomechanisms through which integrins act in cancer remain poorly understood.

Complex roles of integrin α3β1 in skin cancer: Bulge versus nonbulge functions

Using the well-established 7,12-dimethylbenz[a]anthracene (DMBA) - 12-O-tetradecanoylphorbol-13-acetate (TPA) chemical skin carcinogenesis model, the Sonnenberg laboratory previously demonstrated a requirement for the laminin (LN)-binding α3β1 heterodimer for skin tumorigenesis: K14-Cre–mediated epidermal deletion of integrin α3 resulted in strongly reduced tumor growth and higher turnover of differentiated keratinocytes (KCs) in vivo (Sachs et al., 2012). Following their initial hypothesis that α3 loss renders the early egress of long-lived label-retaining cells out of the hair follicle (HF) bulge niche (Sachs et al., 2012), the group recently explored a specific role for integrin α3 in HF bulge stem cells employing K19-Cre–mediated gene deletion. Surprisingly, integrin α3 loss in the keratin (K) 19-derived lineage did not cause significant egress of bulge stem cells under homeostatic conditions. Instead, the authors noted a de novo expression of K15 in nonbulge cells after integrin α3 inactivation (Ramovs et al., 2020b), consistent with their earlier observation of K15-positive cells in α3-deficient HFs and interfollicular epidermis (Sachs et al., 2012). In line with previous reports by others, lineage tracing in the K19-Cre–based model further revealed that the majority of tumor cells were in fact of nonbulge origin (Ramovs et al., 2020b). Therefore, Ramovs, Sonnenberg, and colleagues continued their efforts and assessed integrin α3 functions in the epidermal K14-derived lineage, aiming to understand through which of the many oncogenic pathways this integrin may promote skin tumor initiation and growth.

Integrin α3 is crucial for different protumorigenic signaling pathways in epidermal tumorigenesis

In their Journal of Investigative Dermatology article (Ramovs et al., 2020a), the authors now demonstrate that epidermal integrin α3 promotes the growth and survival of transformed murine skin KCs through the activation of several protumorigenesis pathways including FAK/Src, protein kinase B (Akt), and signal transducer and activator of transcription (STAT)3 during the initiation phase of DMBA-TPA-mediated tumorigenesis in vivo. The authors propose a differential mechanism through which integrin α3 fosters tumor growth in basal versus suprabasal KCs, engaging layer-specific
binding partners. In basal layers, as expected, integrin α3 colocalized with LN-332 and was required for pronounced FAK/Src signaling, whereas in transformed cells after short-term DMBA-TPA treatment, integrin α3 was additionally observed at sites of cell–cell adhesion in suprabasal layers. Notably, suprabasal layers also displayed the activation of STAT3 and Akt, which was interesting because Akt and STAT3 have been linked to the survival of differentiating KCs (Saeki et al., 2012; Segrelles et al., 2007). Using their previously established spheroid model of transformed murine KCs (Sachs et al., 2012) combined with a broad panel of gain-of-function and loss-of-function approaches, the authors went on to show that α3β1-mediated protumorigenic signaling through FAK/Src, STAT3, and Akt was crucial for three-dimensional (3D) spheroid growth in vitro. In this model, α3β1-mediated STAT3 and Akt signaling was codependent, with α3β1-mediated FAK/Src activation potentially acting upstream of STAT3/Akt (Figure 1).

Integrin α3 mediates LN-332–independent survival of differentiating KCs

An important finding of this study resulted from the expression of an α3 mutant that is unable to bind to LN-332. Remarkably, this mutant was still able to restore STAT3/Akt activation and 3D growth of Itga3-knockout (KO) spheroids, almost comparable to that of its wild-type (WT) counterpart. This unconventional, ligation-independent feature of integrin α3 was confirmed by the use of a function-blocking integrin α3 antibody, which did not compromise STAT3/Akt activation and 3D growth of WT spheroids. These data at least in part implicated LN-independent roles of integrin α3 in tumor promotion (Figure 1).

Partners at junctions: Integrin α3 and tetraspanin CD151 mediate cell–cell contact integrity and suprabasal cell survival

The observation of an integrin α3 pool at cell–cell contacts in transformed skin falls into an exciting and yet largely unresolved territory. On the basis of the LN-332–independent role of integrin α3 in STAT3/Akt activation, the authors wondered whether junction-localized integrin α3 acts in concert with its previously identified binding partner tetraspanin CD151. In previous work and resembling integrin α3 loss (Sachs et al., 2012), a protumorigenic role of CD151 in DMBA-TPA-driven skin cancer (Li et al., 2013; Sachs et al., 2014) as well as a genetic interaction between integrin α3 and CD151 (Sachs et al., 2014) have been demonstrated. Integrin α3 can also bind to E-cadherin, and this has been linked to the stability of cell–cell adhesions in a manner dependent on CD151. Interestingly, Itga3-KO epidermis exhibited reduced CD151 levels, and CD151 deletion resulted in reduced suprabasal STAT3 and Akt activity, suggesting a molecular link between junctional α3/CD151 and survival signaling in differentiated KCs. Notably, CD151 loss was also associated with altered E-cadherin distribution and antibody-mediated blockade of E-cadherin phenocopied CD151 deletion with respect to impaired survival signaling, supporting a role of cell–cell contact integrity in α3/CD151-dependent survival signaling. The authors further observed aberrant LN-332 expression in inner, suprabasal layers of CD151-KO spheroids, which may explain the remaining dependency on FAK/Src signaling for robust 3D growth and balanced differentiation in the absence of CD151 in spheroids.

Final remarks

Many previous studies have contributed to a better understanding of the role of integrin adhesion receptors in tumorigenesis, accumulating a vast body of evidence regarding functions of individual integrin subunits and some of their interaction partners in specific oncogenic signaling pathways (Hamidi and Ivaska, 2018). In this study by Ramovs et al. (2020a), the authors compared α3-dependent tumor initiation events in vivo after DMBA-TPA treatment with data from the elegant spheroid model that allowed comprehensive manipulation and restoration of key protumorigenic pathways (Src, FAK, Akt, STAT3, TGF/Smad2) in vitro. Their work provided mechanistic insights into how integrin α3 regulates KC turnover during tumor initiation, with surprisingly distinct roles of integrin α3 in basal and suprabasal layers (Figure 1).

Whereas their findings shed light into integrin α3–dependent signaling in the early stages of the disease, it should be noted that integrins often have opposing roles in different stages and/or types of cancers (Hamidi and Ivaska, 2018). Therefore, more work is required to dissect the relevance of α3β1/CD151 and α3β1/LN-332 complexes in later stages of tumorigenesis. Interestingly, despite the reduced tumor load in epidermal α3 integrin-deficient mice, those tumors that formed showed increased progression (Sachs et al., 2012). In contrast, CD151 deletion only affected tumor incidence and growth but did not promote progression (Li et al., 2013; Sachs et al., 2014), which at least suggests an uncoupling of α3β1 and CD151 in progressing disease. On the basis of the findings by Ramovs et al. (2020a), new questions arise. For example, how are α3β1/CD151 at intercellular adhesions molecularly linked to the activation of STAT3 and phosphoinositide 3-kinase/Akt signaling? Beyond the comprehensive knowledge of matrix-based integrin-binding partners, it will therefore be important to gain better insight into junctional α3β1-interacting molecules—in addition to cadiherin-catenin and tetraspanins—to dissect the upstream and downstream regulators of ligation-independent integrin pools at intercellular adhesions. Apart from these emerging intrinsic roles of integrin α3 in protumorigenic signaling, further clarification regarding the extent to which α3 loss shapes the tumor microenvironment through nonautonomous mechanisms is also required. In the K19-Cre–based model (Ramovs et al., 2020b), the Sonnenberg laboratory observed an α3-dependent secretion of CNN2 (connective tissue GF) by bulge cells, promoting colony formation and 3D growth and suggesting that CNN2 contributes to a tumor-permissive environment. In a recent independent study Longmate et al., (2020) acutely deleted integrin α3 in established tumors, which led to the regression of tumors, thus emphasizing the role of integrin α3 in tumor maintenance. These authors identified paracrine effects on stromal cells and reported an α3β1-dependent KC
secretome comprising factors known to modulate inflammatory cells, for example, tumor-associated macrophages. Together, these recent studies demonstrate additional extrinsic functions of integrin α3 in tumorigenesis, and it will be interesting to learn how much tumor-cell intrinsic and secreted factors contribute to α3-dependent tumor growth. Finally, whereas the rapid tumor regression upon acute integrin α3 deletion is clearly of interest for future therapeutic interventions, further work needs to decipher how α3β1 eventually switches from protumorogenic to anti-invasive functions in the course of tumor progression. Although currently unknown, it is tempting to speculate that integrin-dependent secretion signatures may contribute to the multiclonoality seen in skin tumors.

Reeves et al. (2018) recently showed that Ras mutant clones recruit neighboring nonmutant cells into growing benign tumors and that clonal diversity switches in the course of tumor progression. Importantly, different integrins have also been linked to acquired anticancer drug resistance, and previous strategies targeting integrin receptor functions have not been highly successful (reviewed in Hamidi and Ivaska [2018]). The recent insights into protumorogenic signaling networks downstream of α3β1 may therefore benefit the design of future targeting approaches at the level of integrin effectors rather than ECM-based integrin-mediated adhesion.

CONFLICT OF INTEREST
The authors state no conflicts of interest.

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REFERENCES
MMP-9 Mediates Cross-Talk between Neutrophils and Endothelial Cells in Psoriasis

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In their report, Chen et al. provide new insights into psoriasis pathogenesis, showing that neutrophil infiltration of skin lesions increases vascular endothelial cell (VEC) activation, leading to cutaneous vasodilation and enhanced vascular permeability. In patients with psoriasis, neutrophil-derived matrix metalloproteinase 9 (MMP-9) plays a pivotal role in VEC barrier dysfunction, via extracellular signal–regulated kinase-1/2 and p38 pathways. Pharmacologic inhibition of MMP-9 in two different models confers reduced cutaneous vasodilation, vascular permeability, and inflammation, suggesting MMP-9 as a target in psoriasis pathogenesis.


Psoriasis is a chronic inflammatory skin disease characterized by hyperproliferation and abnormal differentiation of keratinocytes, infiltration of inflammatory cells, and neoangiogenesis (Boehncke and Schön, 2015). Although the precise etiology of the disease remains unclear, a combination of genetic and environmental factors is known to trigger abnormal immune-mediated responses involving the IL-23/IL-17 pathway. IL-17A primarily acts on keratinocytes, leading to the production of cytokines and chemokines, including TNF-α and CXCL-1/IL-8, that initiate neutrophil infiltration of skin (Martin et al., 2013). Recent studies have shown that infiltrated neutrophils might contribute to the pathogenesis of psoriasis by generating ROS and releasing neutrophil extracellular traps (NETs) (von Stebut et al., 2020). Notably, the therapeutic depletion of neutrophils significantly relieves the symptoms of patients with pustular psoriasis (Ikeda et al., 2013).

This study is of interest because it improves the mechanistic understanding of the role of neutrophils in the immunopathogenesis of psoriasis (Figure 1b) and may highlight a new therapeutic strategy for psoriasis treatment.

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