Keeping Order in the Neighborhood: New Roles for TGFβ in Maintaining Epithelial Homeostasis

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TGFβs are thought to have tumor suppressor activity in many organ systems, but receptor inactivation in mouse models has not previously resulted in increased spontaneous tumorigenesis. A study in this issue of *Cancer Cell* shows that mice with a targeted knockout of the type II TGFβ receptor in stratified epithelia specifically develop spontaneous squamous cell carcinomas in the anogenital region, but not in the skin. Loss of TGFβ signaling appears to destabilize the epithelium such that homeostasis fails in the face of persistent proliferative challenge, a normal feature of the anogenital site, and latent invasive and migratory phenotypes are unmasked.

The TGFβs are remarkably pleiotropic molecules, evoking a wide range of biological responses from cells in all major lineages of the body. Within this repertoire of activities, their ability to potently inhibit the proliferation of epithelial cells is particularly striking. Since the TGFβs are widely expressed, and many human cancers show aberrations in TGFβ receptor expression or function, the TGFβ pathway is predicted to play an important tumor suppressor role in many tissues (Pardali and Moustakas, 2007; Bierie and Moses, 2006). Surprisingly however, mice with targeted ablation of TGFβ receptors in several different epithelia have not developed spontaneous tumors in any studies done to date (Lu et al., 2006; Forrester et al., 2005; Munoz et al., 2006; Ijichi et al., 2006), suggesting that much remains to be understood about the functioning of this pathway.

New light has been shed on this question in a study by Elaine Fuchs and coworkers in the current issue of *Cancer Cell* (Guasch et al., 2007). All the known effects of TGFβ funnel through the ligand-binding type II TGFβ receptor (TβRII), which the Fuchs group selectively inactivated in several stratified and glandular epithelia. They found that their conditional knockout (CKO) mice rapidly developed spontaneous squamous cell carcinomas with high penetrance, but interestingly, these tumors arose only in the anogenital region and not at other targeted sites, such as the skin. Their in-depth comparison of the two different sites, integrated with analysis of the wound healing phenotype in the same animals, has yielded new insights into the role of TGFβ in epithelial carcinogenesis.

Early clues came from analysis of the balance between proliferation and apoptosis in asymptomatic CKO epithelia. As expected, deletion of TβRII caused a mild increase in epithelial cell proliferation in both the anogenital epithelia and the skin, confirming that endogenous TGFβ normally provides a tonic growth inhibitory signal at both sites. Despite this increase in proliferation, in young mice normal homeostasis was preserved in all tissues by a compensatory increase in apoptosis. However, in older mice, homeostatic mechanisms broke down specifically in the TβRII null anogenital epithelia, where rapid proliferation persisted unopposed by compensatory apoptosis, and ultimately tumors developed.

So, what is unique about the anogenital region that might provoke this catastrophic failure of homeostasis in the TβRII null epithelium? This region is prone to mechanical trauma in mice, but the authors were able to show that repeated wounding failed to induce carcinomas in the backskin epidermis, suggesting that intermittent trauma alone was not the culprit. Intriguingly, tumors in the anal canal were found to arise primarily at the transitional zone where the stratified squamous epithelium of the anal skin merges with the mucosal epithelium of the large intestine. Classic pathology recognizes that tumors frequently arise in transition zones between different types of epithelia, and it is likely that cells in such zones receive conflicting informational cues from their different neighbors, leading to a chronic state of "identity crisis" that may destabilize normal differentiation and homeostasis programs. Indeed, the Fuchs group found that, even in wild-type mice, the epithelium of the transitional zone in the anus naturally showed many features reminiscent of hyperproliferative epidermis. These included aberrant expression of differentiation markers, enhanced Ras-MAPK signaling, and locally increased inflammation.

Could any features of this chronically destabilized state contribute to the spontaneous tumorigenesis at the anogenital site in the CKO mice? Ras pathway activation was an obvious suspect, and the authors showed that, although tumors did not form spontaneously in the TβRII null backskin, TβRII null keratinocytes expressing oncogenic *Ha-Ras* rapidly formed aggressively invasive and metastatic

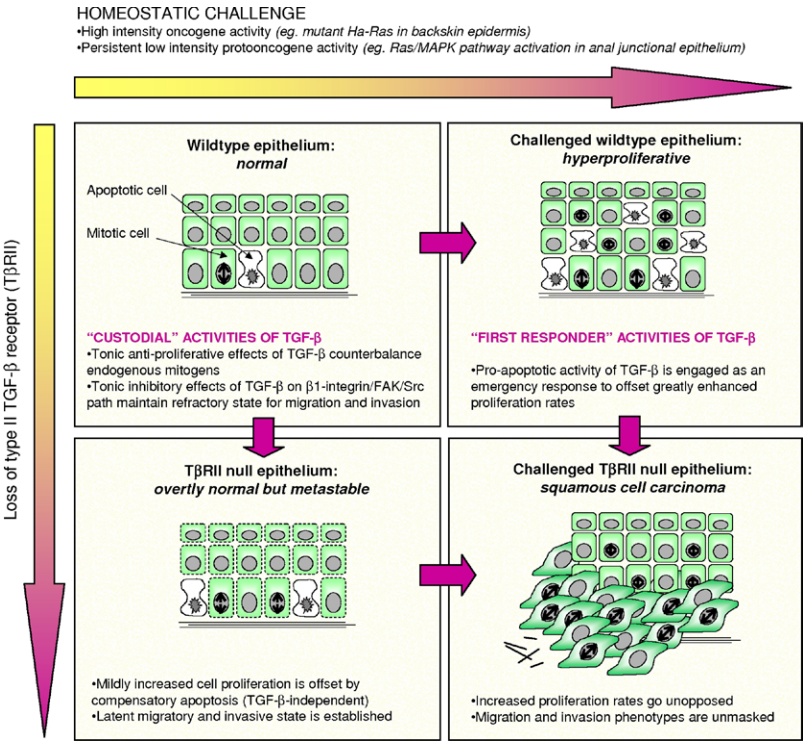

Figure 1. Model for the Effect of TβRII Deletion on Homeostasis in Stratified Epithelia

squamous cell carcinomas. As with the anal tumors, tumor formation in the skin was associated with a dramatic reduction in compensatory apoptosis. Most importantly, Ras activation in keratinocytes was accompanied by an enhanced sensitivity to the proapoptotic effects of TGFβ in vitro, suggesting that TGFβ-induced apoptosis may be critically important in opposing unscheduled increases in cell proliferation.

The data suggest a model in which different activities of TGFβ are called into play under different circumstances (Figure 1). Thus, TGFβ in its role as an antiproliferative factor is engaged in tonic maintenance of cell number in the normal epithelium, and loss of this particular contribution of TGFβ can readily be offset by compensatory apoptotic mechanisms that are TGFβ independent. This role could be viewed as a "custodial" function. In contrast, TGFβ in its role as a proapoptotic factor seems to be a crucial "first responder" in the face of major proliferative challenge. The nature of the challenge could be acute oncogene activation (e.g., mutant *Ha-Ras* in the backskin), or *chronically* elevated signaling through proto-oncogenic pathways (e.g., persistently elevated Ras/MAPK signaling in the junctional zone of the anal canal in older mice). At least in stratified epithelia stressed by hyperactivation of the Ras pathway, this role of TGFβ cannot be compensated for by other pathways, and loss of TGFβ response permits unopposed proliferation.

A surfeit of cells alone does not make a carcinoma, so it was of interest to ask whether loss of TGFβ response destabilizes the tissue in other ways that might also contribute to tumor development. In light of the known parallels between tumors and wounds, the authors analyzed the wound healing phenotype in their mice. Wounds on the skin of the CKO mice healed faster than their wild-type counterparts, reflecting a migratory and invasive advantage in CKO keratinocytes, which depended on activation of the Src/FAK pathway by β1-integrin. Interestingly, the TβRII null anal canal showed evidence of β1-integrin activation and elevated FAK activity even in the asymptomatic state.

The data suggest that another very important role of endogenous TGFβ in normal epithelia may be to limit the migratory and invasive potential of these cells. Through tonic suppression of β1 integrin activation, wild-type cells may be rendered relatively "deaf" to promigratory signals emanating from the stroma and elsewhere. In contrast, TβRII null cells are poised to leave the neighborhood, and their latent migratory phenotype can readily be unmasked by oncogene activation and/or tissue disruption. Thus, not only is an intact TGFβ pathway critical for the emergency response to undesirable increases in cell proliferation, but also the data suggest an important new tumor suppressor role for the pathway in maintaining "locostasis"—ensuring that cells stay in their proper location. Superficially, this result seems at variance with an extensive literature on TGFβ as a promigration and proinvasion factor in tumorigenesis (Pardali and Moustakas, 2007). However, other biological responses to TGFβ can switch radically as molecular context changes (Sanchez-Capelo, 2005), and the migratory response may simply be an additional example of this general phenomenon.

A number of interesting questions are raised by this work. How applicable are these findings to other epithelia, and to other oncogenic insults? Are there other chronically destabilized epithelia that might be prone to spontaneous tumorigenesis on loss of TGFβ response? What is the relative importance of the proapoptotic and antimigration/invasion effects of TGFβ compared with other potential tumor suppressor activities, such as induction of replicative senescence and maintenance of genomic stability? Why does loss of TβRII not affect proliferation or apoptosis of the stem cells in the hair follicle bulge region, despite evidence for TGFβ pathway activity in this compartment? Much remains to be learned, but the Fuchs article clearly demonstrates that loss of TGFβ response can destabilize tissue homeostasis at multiple levels, leading to a tumor-prone state.

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Bmi1 and Cell of Origin Determinants of Brain Tumor Phenotype

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Glioblastomas frequently express oncogenic EGFR and loss of the *Ink4a/Arf* locus. *Bmi1*, a positive regulator of stem cell self renewal, may be critical to drive brain tumor growth. In this issue of *Cancer Cell*, Bruggeman and colleagues suggest that brain tumors with these molecular alterations can be initiated in both neural precursor and differentiated cell compartments in the absence of *Bmi1*; however, tumorigenicity is reduced, and tumors contain fewer precursor cells. Surprisingly, tumors appear less malignant when initiated in precursor cells. *Bmi1*-deficient tumors also had fewer neuronal lineage cells, suggesting a role for *Bmi1* in determination of cell lineage and tumor phenotype.

Glioblastomas remain among the most aggressive human cancers. The application of the conceptual and methodological framework of neural stem cell biology to brain cancer (Bachoo et al., 2002; Holland et al., 1998) and the identification of human brain tumor initiating cells (Singh et al., 2004) has opened up fresh approaches to interrogate the cell of origin for brain tumors. Particularly in mouse model systems, investigators can address the effect of expression of oncogenes or loss of tumor suppressors in normal precursor or differentiated cell compartments.

Current understanding of the mechanisms of tumor progression and initiation remain limited, particularly the cell context of recognized molecular signaling pathways implicated in the disease, such as aberrant EGFR signaling. Does oncogene expression drive a stem cell or a progenitor compartment in the tumor? Determinants of tumor phenotype and relationship to prognosis are also poorly understood. How do distinct molecular alterations specify the ultimate histopathologic tumor picture? How does the expression of neural precursor or differentiated lineages in the tumor correlate with tumor behavior? Are tumors that express more markers of differentiation less aggressive? As well, the relationship of tumor behavior to the putative cell of origin is not understood. Do tumors arise in a stem cell or a more differentiated cell compartment, and does the behavior and phenotype of the tumor depend on the cell compartment of origin? Are tumors that arise in stem cell compartments more malignant than those arising in progenitors, or vice versa? These questions come in to focus in the study by Bruggeman and colleagues (Bruggeman et al., 2007).

Bmi1 has been implicated in control of stem cells in multiple tissues, particularly as a positive regulator of self renewal, and *Bmi1*-deficient mice have deficiencies in their stem cell compartments, including the brain (Molofsky et al., 2003; Park et al., 2003). *Bmi1* promotion of proliferation and self renewal is thought to relate to suppression of the *Ink4a/Arf* locus (Bruggeman et al., 2005), although other loci have recently been shown to be targeted as well (Fasano et al., 2007). *Ink4a/Arf* loss itself, consistent with its tumor suppressor role, causes increased neural stem cell activity in vivo (Molofsky et al., 2006). Although *Ink4a/Arf* is lost genetically in a large fraction of human glioblastoma samples, mice deficient for *Ink4a/Arf* rarely develop spontaneous brain tumors.

The current study by Bruggeman et al. (2007) attempts to further probe the functional role of *Bmi1* together