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News and Commentary

A pervasive role for MIG6 in restraining cell proliferation

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The correct execution of morphogenetic patterns and the homeostatic control of tissue integrity require that cell proliferation be tightly balanced against cell differentiation and death. Among cell-intrinsic mechanisms deputed to restraining cell proliferation, negative feedback loops have a prominent role, given their ability to operate on sensing the generation and/or accumulation of intracellular signals.

MIG6/RALT (encoded by the *ERRFI1* gene) is a cytosolic adaptor/scaffold protein (Figure 1a). *ERRFI1* transcription is induced by as diverse stimuli as growth factors, hormones, vitamins, hypoxia, mechanical and metabolic stress,[1] a notion that would predicate MIG6 involvement in a wide range of physiological and physiopathological scenarios. Yet, research on MIG6 has focused primarily on its function in growth control, namely on its capacity of acting as a feedback inhibitor of ErbB and, possibly, MET receptor tyrosine kinases (RTKs),[2,3] with EGFR being the most investigated receptor model. EGFR activation increases *ERRFI1* transcription in a RAS-ERK-dependent fashion, leading to accumulation of the MIG6 protein within 60–90 min.[4] In turn, MIG6 binds to the tyrosine kinase domain of the ligand-occupied EGFRs, causing their catalytic inactivation[5,6] and endocytosis-dependent degradation[7] (Figure 1). This two-tiered mechanism of receptor inhibition is thought to provide for strong and durable attenuation of EGFR mitogenic signaling. Consistently, *Errfi1*$^{-/-}$ mice show a fully penetrant skin phenotype, that is, epidermal hyperplasia and markedly increased susceptibility to skin carcinogenesis, which was formally proved to be caused by excess Egfr signaling.[8] Hence, Mig6 is an essential negative feedback regulator of Egfr in mouse epidermis.

Germline disruption of *Errfi1* in mice also causes variably penetrant phenotypes characterized by hyperplastic lesions, which may progress to frank malignancy, in the endometrium and epithelia lining the gastrointestinal tract, biliary ducts, gallbladder, bronchial airways and mammary gland. In addition, *Errfi1* null mice suffer from an early-onset degenerative joint disease, caused by aberrant proliferation of precursor mesenchymal cells in cartilages.[8–12] An unanswered question is whether these phenotypes are reflective of excess cell proliferation driven by unopposed ErbB or MET signaling. A related issue is whether it is satisfactory to rationalize the biological finalism of MIG6 induction by a plethora of stimuli solely in the context of MIG6-driven RTK inhibition. A few recent publications provide insightful advances concerning the above issues.

Hopkins *et al.*[9] set out to study mammary gland development in *Errfi1* null mice. They observed an altered pattern of morphogenesis characterized by decreased ductal branching and widespread luminal filling of terminal end buds (TEBs). Concentrating on TEBs, they discovered that supernumerary luminal cells accumulated as a consequence of reduced apoptotic cell death. This phenotype was not determined by excess ErbB signaling, but turned out to be caused by defective Abl activity. In detail, Mig6 was found to bind and activate the Abl tyrosine kinase domain. This event, possibly in conjunction with enhanced nuclear localization of Abl, ignited a p73-dependent pro-apoptotic pathway.[9] Of note, Hopkins *et al.*[9] found that Mig6 became competent to activate Abl only in absence of signals generated by active ErbB RTKs via Src family kinases (Figure 1b), which led to the proposal that Mig6 may regulate ErbB or Abl kinase activity in a mutually exclusive fashion. In this model, TEB luminal cells that are pushed away from the basal membrane on epithelial multilayering end up receiving an insufficient ErbB-Src signal, which sets Mig6 free to activate Abl and launch an apoptotic program.

The Hopkins *et al.*[9] paper provided the first mechanistically defined evidence that MIG6 may exert a pro-apoptotic function. Excitingly enough, a coeval study by Chen *et al.*[13] reported that in *Errfi1*$^{-/+}$ mice the rate of apoptosis of pancreatic β-cells experiencing endoplasmic reticulum (ER) stress (that is, under experimental conditions that mimic type 2 diabetes) is lower than in control littermates. Complementary experiments in cultured cells showed that MIG6 overexpression increased the rate of apoptotic cell death, whereas reducing MIG6 protein abundance by RNAi had the opposite effect. Importantly, induction of ER stress in cultured cells caused accumulation of *ERRFI1* mRNA.[13] Although Chen *et al.*[13] did not provide molecular details on how MIG6 favors apoptosis in ER-stressed β-cells, it is tantalizing that previous work had proposed a role for ABL in the induction of apoptosis in cells experiencing ER stress.[14] Hence, it will be important to test whether MIG6 may instigate apoptosis in all stressing conditions characterized by increased MIG6

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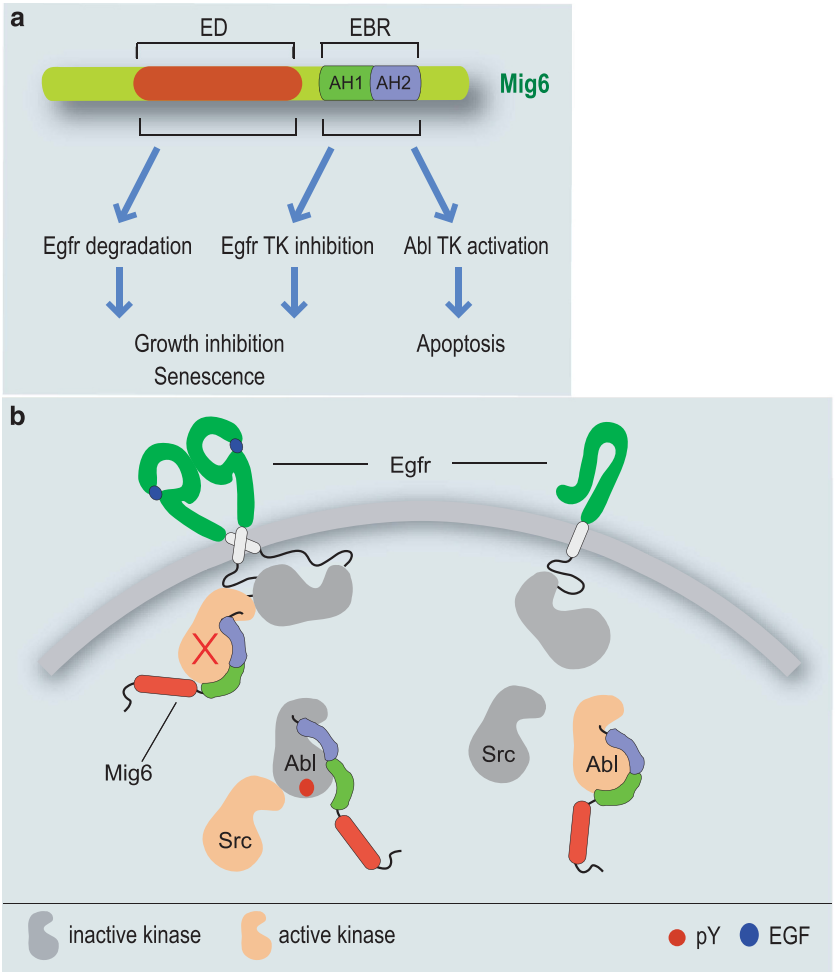

Figure 1 (**a**) Schematic outline of MIG6 structural features. The endocytic domain (ED) couples MIG6 to endocytic proteins, thus instructing the endocytosis-dependent degradation of MIG6-bound EGFR; the ErbB-binding region (EBR) consists of the AH1 (green) and AH2 (violet) subdomains and directs MIG6 binding to the EGFR (catalytic inhibition) and ABL (catalytic activation) tyrosine kinase (TK) domains. (**b**) Regulation of EGFR and ABL kinase activity by MIG6. Top: ligand-induced dimerization of EGFR leads to the formation of asymmetric kinase dimers in which the C-lobe of a donor kinase (gray) contacts the N-lobe of an acceptor kinase, causing allosteric activation of the latter (yellow). MIG6 binds to the C-lobe (via AH1) and active site (via AH2) of the activated kinase domain. This results in immediate catalytic blockade of EGFR. Bottom: the MIG6 EBR may also bind to ABL, with the AH1 and AH2 subdomains involved in contact with the C-lobe and active site of the ABL kinase, respectively. This results in catalytic activation of ABL. SRC family kinases act downstream to EGFR and phosphorylate Tyr 488 (red dot) in the C-lobe of the ABL kinase domain, which prevents MIG6 from activating ABL. Hence, activation of ABL by MIG6 requires low to absent EGFR activation (visualized by ligand-free monomeric EGFR) coupled to loss of Y488 phosphorylation. Likely, the phosphorylation status of ABL Y488 senses the overall abundance of receptor signals (RTKs, GPCRs, integrins) that promote growth and are capable of activating SRC, although this has not been formally addressed as yet. For the sake of clarity, the graphic representation of ABL and SRC is limited to their TK domain; inactive and active kinase domains are depicted in gray and yellow, respectively, regardless of their identity

expression[1] and, if so, whether ABL is an obligatory MIG6 pro-apoptotic partner. The obvious lingering question is does MIG6 have a pro-apoptotic role also in the course of tumor pathogenesis? A convincing affirmative answer comes from a recent paper that showed ablation of *Errfi1* accelerated the development of endometrial carcinoma in a *Pten* null context by suppressing apoptosis via still undefined mechanism/s.[15]

MIG6 biology was further enriched by a recent paper that provides initial evidence that MIG6 can regulate cellular senescence.[16] Xie *et al.*[16] showed that MIG6 is highly expressed in human embryonic diploid fibroblasts undergoing either replicative or oncogene-induced senescence. Overexpression of wtMIG6, but not a MIG6 mutant unable to bind to EGFR, was sufficient to enhance replicative senescence, a phenotype observed also on pharmacological suppression of EGFR. Conversely, reducing MIG6 expression limited RASV12-induced senescence. This analysis provides *prima facie* evidence that EGFR inhibition by MIG6 contributes to cellular senescence (Figure 1) and is in keeping with a report that pharmacological suppression of EGFR drives senescence of epithelial cervical cells infected with HPV-16.[17] At variance with growing cells, accumulation of *ERRFI1* mRNA in senescent fibroblasts was independent of RAS-ERK signaling and instead required recruitment of FOXO3A, an ascertained driver of cell senescence,[18] onto the *ERRFI1* promoter.[16] Thus, MIG6 joins the company of feedback inhibitors (some of which are themselves FOXO targets) whose expression is induced and maintained in

senescent cells in order to implement a state of unresponsiveness to growth stimuli.[19]

It is apparent from the above discussion that MIG6 may operate in the maintenance of cell and tissue homeostasis not only through growth inhibition, but also via the induction of cell senescence and apoptosis. Thus, going back to our initial questions, it is possible that, besides TEB filling, other hyperplastic phenotypes observed in *Errfi1* null mice are caused, at least in part, by mitigated apoptosis. Escape from senescence could instead contribute to the malignant transformation of adenomas that develop in *Errfi1* null mice.[8,12] Addressing these issues will require an expansion of studies focused on tissue-specific ablation of *Errfi1*. Genetic studies in the mouse will be extremely valuable also for fostering our understanding of the pathogenic significance and biologic liabilities attached to reduced MIG6 expression in human tumors.[8] We have also discussed evidence that mouse phenotypes caused by Mig6 loss may not be caused by excess ErbB function. ABL is a bona fide pro-apoptotic MIG6 target and, plausibly, there could be more just awaiting to be discovered.

As MIG6 biology expands, we need to gain insights into the regulation of context-dependent MIG6 functions. We already have some hints. For instance, *ERRFI1* transcription in response to growth stimuli and cellular stress is induced by distinct signaling pathways that, in turn, drive occupancy of the *ERRFI1* promoter by different transcription factors.[1,16,20] Interestingly, *ERRFI1* expression appears to be much higher in senescent rather than growing human fibroblasts.[16] Plausibly, the high-level *ERRFI1* transcription driven by FOXOs could reflect the need for *tout court* cytostatic ErbB repression in senescent and, more in general, stressed cells, whereas lower levels of *ERRFI1* expression in growing cells would provide for fine tuning of the ErbB signal output. As discussed above, growth signals funneled via SRC prevent MIG6 from activating pro-apoptotic signals;[9] stress signals could likewise modulate MIG6 function by impacting on its subcellular localization and/or interaction with effector/target proteins involved in cell death regulation. Ultimately, in stressed cells, regulatory inputs must dictate to MIG6 the right course of action, namely imposition of a transient proliferation blockade to allow recovery from damage as opposed to promotion of either apoptosis or premature senescence, should damage remain unresolved.

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