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Deregulating EMT and Senescence: Double Impact by a Single Twist

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The acquisition of a fully malignant phenotype is limited by several barriers, including cellular senescence and the requirement to undergo an epithelial-mesenchymal transition (EMT). Deregulation of these processes is believed to occur by largely independent events. In this issue of *Cancer Cell*, Ansieau et al. (2008) challenge this view.

Tissues often contain several small and inconspicuous neoplastic lesions, which rarely progress to full malignancy. It is believed that multiple factors underlie this phenomenon. These include apoptosis, the necessity to acquire invasive competence, and the activation of a cellular senescence program. The last of these is receiving increased attention, moving from being a phenomenon seen exclusively in cultured cells to one constituting a critical tumor suppression function in vivo. It can be triggered by telomere malfunction or unscheduled activation of oncogenic signaling (Prieur and Peeper, 2008). Cellular (oncogene-induced) senescence is characterized by a largely irreversible cell-cycle arrest, often a typical flattened cell morphology, and induction of a tumor suppressor network.

Another factor proposed to limit progression of epithelial tumors is the requirement to undergo epithelial-mesenchymal transition (EMT). While important for embryonic development, EMT is often adopted by cancer cells, endowing them with a migratory and/or invasive phenotype. It is characterized by decreased cell adhesion, which is usually accompanied by the downregulation of E-cadherin. This important epithelial adhesion protein is regulated by several transcription factors, including Twist, Snail, and Zeb1.

Although these tumor-restricting processes are generally seen as largely independent traits, Ansieau et al. (2008) report that, in fact, they may not be distinctly autonomous as previously thought. The authors asked whether Twist1 (overexpression of which is linked to breast cancer infiltration; Yang et al. [2004]) and its close cousin Twist2 are activated in cancer. To address this, they analyzed MMTV-*ErbB2*-driven murine mammary tumors, where *TWIST2* levels were increased in the majority of these lesions. When extending this study to human breast carcinomas, the authors observed instead that *TWIST1* was upregulated in half of the cases.

The notion that Twist1 may play a relatively more important role in human breast cancer was supported when Twist proteins were depleted from T47D human breast cancer cells harboring multiple copies of *ERBB2*. The authors observed that only *TWIST1* knockdown resulted in a moderate increase in senescence-associated β-galactosidase activity (SA-β-gal, a commonly used marker of senescence). This was accompanied by a modest increase in the number of cells that actually underwent cell-cycle arrest, which may be explained by assuming that the genetic wiring implementing senescence-associated proliferative arrest in these cancer cells is disrupted. In human RPMI 7951 melanoma cells harboring a mutant $BRAF^{E600}$ oncoprotein, codepletion of the two Twist proteins resulted in a similar response. An interesting question prompted by these observations is whether in the same setting, Twist is required for the invasive and metastatic capacities of these cells. Previous work has shown this to be the case within the context of murine breast tumor cells (Yang et al., 2004).

A connection between Twist, senescence, and the cell-cycle machinery was initially suggested almost a decade ago (Maestro et al., 1999). Twist1 and Twist2 (also called Dermo1) were identified in a screen for genes antagonizing Myc-induced apoptosis. While Twist1 reversed

the induction of cell-cycle arrest by p53, a key regulator of cellular senescence and proliferation, Twist1 and Twist2 were demonstrated to enhance oncogenic transformation of cells expressing E1A and H-Ras^{V12}. Extending these findings, Ansieau et al. (2008) show here that both Twist proteins cooperate with H-Ras^{V12} to produce tumors in immunodeficient nude mice. Whereas in Myc-driven apoptosis, Twist1 primarily suppresses ARF, Ansieau et al. demonstrate that in a senescence setting, Twist proteins suppress p21^{Cip1} in a p53-independent manner and p16^{Ink4a}, both at the promoter level.

The exciting premise that activation of EMT might be linked to suppression of cellular senescence has been proposed in the context of another EMT-regulating transcription factor, Zeb1 (Liu et al., 2008). It was shown that mutation of *Zeb1* in mice causes a mesenchymal-epithelial transition (MET), characterized by increased expression of epithelial proteins such as E-cadherin and decreased expression of mesenchymal proteins. In addition, Zeb1-deficient murine embryonic fibroblasts accumulated the cell-cycle-inhibitory protein p21^{Cip1} and underwent premature senescence in culture. Ansieau et al. (2008) show that Twist, too, inhibits the accumulation of p21^{Cip1}, raising the possibility that this cyclin-dependent kinase inhibitor acts as a point of signal convergence.

The findings currently reported show that a link between EMT and senescence is not specific for Zeb1 but is seen also for Twist. Exploiting the finding that ectopic expression of ErbB2 induces cellular senescence (Trost et al., 2005), Ansieau et al. (2008) show that coexpression with either Twist protein suppresses the increase of SA-β-gal activity and the senescence-associated flat cell morphology. This observation is in keeping with the previous finding that Twist suppresses senescence of immortalized human prostate epithelial cell lines (Kwok et al., 2007).

Ansieau et al. (2008) find that the effect of Twist (in the presence of oncogenic signals) on cellular senescence is accompanied, in the same experimental cell system, by EMT. This was illustrated by the cooperative suppression of epithelial proteins including E-cadherin and claudin, upregulation of the mesenchymal protein vimentin, and concomitant morphological transformation to a fibroblast-like phenotype. Therefore, the full manifestation of both senescence bypass and activation of EMT seems to require the collaboration of Twist with oncogenic signals like ErbB2 or Ras. The fact that simultaneous effects on EMT and senescence have now been observed for at least three transcription factors, Twist1, Twist2, and Zeb1, suggests the emergence of a more universal biological concept, in which key EMT-regulating transcription factors simultaneously regulate invasion, motility, and senescence (Figure 1).

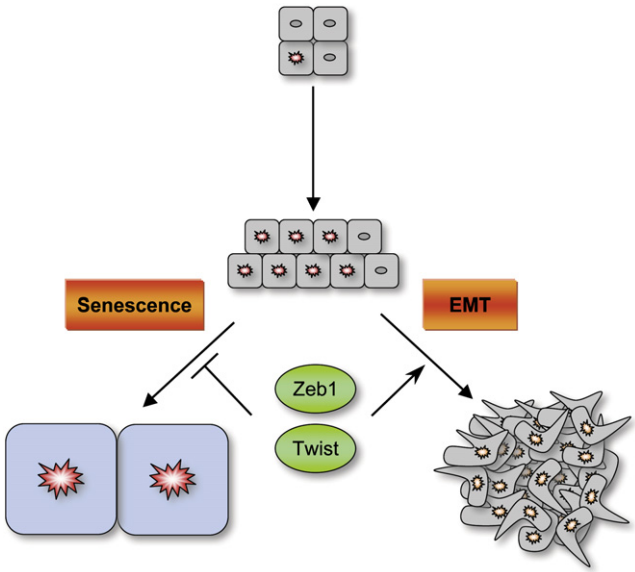

Figure 1. Role of Twist and Zeb1 in Cancer Progression
Upon the acquisition of one or more oncogenic mutations, incipient cancer cells may expand and form a premalignant lesion. Tumor expansion is limited by several constraints, including (oncogene-induced) cellular senescence and the need to acquire an invasive phenotype. In the context of oncogenic mutations, transcription factors like Twist and Zeb1 can simultaneously suppress the senescence response and induce an epithelial-mesenchymal transition (EMT), both contributing to malignant progression.

The findings by Ansieau et al. (2008) prompt several interesting questions, such as whether the coordinated deregulation of EMT and senescence reflects only a "collateral effect," as the authors propose. Or are there instead mechanistic links tying together these two processes? For example, could an EMT-like process contribute to senescence bypass? Senescent cells can adopt a "flat cell phenotype," in which they grow to an abnormally large size, which is often lost when they bypass senescence, raising the possibility that senescence is tightly coupled to cellular morphology. And conversely, can cells with an intact senescence program undergo a full EMT? Indeed, is it only coincidental that Twist suppresses the *INK4a/ARF* locus (encoding p16^{Ink4a} and ARF) and *CDKN1A* (encoding p21^{Cip1}), genes that are so central in senescence signaling?

Like Twist and Zeb1, the EMT-regulating transcription factor SIP1 (which also communicates with the cell-cycle machinery; Mejlvang et al. [2007]) has E-cadherin as a transcriptional target; it will therefore be interesting to determine whether E-cadherin has a direct role in cellular senescence. Furthermore, as Twist has been implicated in regulating proinflammatory cytokine gene expression (Sosic et al., 2003) and as it has recently been shown that interleukins play a crucial role in oncogene-induced senescence (Kuilman et al., 2008; Acosta et al., 2008), further investigations into the role of cytokines in Twist-dependent cellular senescence are warranted.

So it seems that, by deregulating both cellular senescence and EMT, proteins like Twist and Zeb1 can deliver a doubly damaging effect contributing to tumor progression. Based on their findings, Ansieau et al. (2008) challenge the concept that metastasis corresponds to the final step of the tumorigenic cascade. Instead, they propose that the dissemination of primary tumor cells residing at the invasive edge may commence early in response to environmental cues, coupling metastatic spread to the bypass of senescence. A link between EMT and senescence is sufficiently fascinating to justify tackling these questions.

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Inflaming Gastrointestinal Oncogenic Programming

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The etiology of gastrointestinal tumors implicates a role for chronic inflammation in response to pathogenic microflora as a promoting force for full neoplastic progression. Recently, Oguma and coworkers (2008) demonstrated that TNFα, derived from recruited macrophages, potentiates Wnt/β-catenin signaling and gastric carcinogenesis by activating Akt signaling and GSK3β phosphorylation independent of the NF-κB pathway in initiated epithelial cells. These observations provide a missing link in the mechanism whereby chronic inflammation, in response to *Helicobacter*, regulates the "penetrance" of initiating oncogenic mutations in the gastrointestinal tract leading to gastrointestinal tumorigenesis.

Epidemiologic studies have long supported a link between chronic inflammation and development of solid tumors (Coussens and Werb, 2002; Thun et al., 2004). More recently, through utilization of immunocompetent mouse models of multistage carcinogenesis, the molecular mechanisms whereby chronic engagement of the immune system (inflammation) potentiates development of epithelial cancers have begun to be elucidated (Balkwill et al., 2005; de Visser et al., 2006; Karin et al., 2006). Missing, however, has been insight into which soluble mediators derived from chronically activated immune cells are significant for regulating the penetrance of neoplastic cells harboring initiating mutations. For example, in the gastrointestinal tract, malignancy is frequently preceded by chronic inflammation sometimes associated with *Helicobacter pylori* infection (Blaser, 2000) or in individuals harboring activating mutations in the *APC* or *CTNNB1* genes or enhanced activation of Wnt/β-catenin signaling (Clements et al., 2002).

APC is a multifunctional cytoplasmic protein whose gene is frequently mutated in several types of gastrointestinal cancers. APC regulates both genomic instability and hyperactivation of the Wnt/β-catenin signaling pathway, and nuclear β-catenin accumulation is found in colon carcinoma cells at invasive fronts (Fodde and Brabletz, 2007). Recent experimental studies have revealed that malignant conversion of initiated *APC* mutant cells is potentiated by chronic activation of the Wnt/β-catenin signaling pathway (Fodde and Brabletz, 2007); however, links between these molecular mediators and chronic inflammation have not been previously identified.

Oguma and coworkers (2008) investigated this link and found that both enhanced Wnt expression and infection by gastric microflora induce submucosal infiltration by macrophages secreting high levels of tumor necrosis factor-alpha (TNFα). Binding of TNFα to TNF receptors on gastric epithelial cells enhances Akt phosphorylation that in turn induces glycogen synthase kinase 3β (GSK3β) phosphorylation, resulting in stabilization and nuclear accumulation of β-catenin that potentiates gastric carcinogenesis (Figure 1). To reveal this pathway, the authors utilized a mouse model of gastric tumorigenesis in which Wnt1 is expressed in gastrointestinal mucosal epithelia, K19-Wnt1 transgenic mice, which develop sporadic dysplastic lesions in the glandular stomach, mimicking hyperactivation of Wnt/β-catenin signaling commonly observed in patients with gastric carcinoma