Dissecting pathways contributing to oncogenic transformation by the tyrosine kinase receptor TrkB

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CHAPTER 4

Epithelial-mesenchymal transition and senescence: two cancer-related processes are crossing paths

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Epithelial-mesenchymal transition and senescence: two cancer-related processes are crossing paths

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The epithelial-mesenchymal transition is involved in several physiological processes. However, it is also believed to contribute to cancer progression. Conversely, cellular senescence constitutes a failsafe program against cancer progression. Interestingly, EMT and senescence seem to cross paths, with several factors playing dominant roles in both settings. Here, we describe recent observations that link these important cellular processes.

Epithelial-mesenchymal transition

Cancer is a complex disease against which mammals have developed multiple protective mechanisms. Late-stage cancer is almost invariably accompanied by metastasis, accounting for the most common cause of death of cancer patients. The metastatic cascade comprises several steps, ultimately leading to the emergence of secondary tumors at distant sites from the primary lesions (Geiger & Peeper, 2009). One process contributing to the first phase of metastasis is the epithelial-mesenchymal transition (EMT). EMT is well known for its important roles in embryogenesis and development, in which epithelial cells acquire properties reminiscent of those of mesenchymal cells. Full differentiation and the establishment of a specific tissue architecture may involve several rounds of EMT, but also of the reverse process, mesenchymal-epithelial transition (MET) (Thiery et al., 2009).

EMT is accompanied by the loss of the cell-cell contacts so typical of epithelial cells, and the acquisition of migratory and motile properties. It is for these reasons that EMT can have adverse effects to the organism, contributing to pathological processes such as fibrosis and cancer (Kalluri & Neilson, 2003). In particular, when adopted by cancer cells, EMT allows for the invasion and intravasation of tumor cells into surrounding tissues, blood and lymphatic circulation (Christofori, 2006; Thiery, 2002). Similar to its physiological role, also in metastasis EMT conceivably corresponds to a transient and dynamic process, which involves several steps. Furthermore, it does not appear to occur in the bulk of the tumor cells but rather locally, at the invasive fronts of a tumor (Brabletz et al., 2005; De Wever et al., 2008; Spaderna et al., 2006).

Epithelia are formed by polarized layers of epithelial cells, which are tightly connected by adherens junctions. These consist of E-cadherin, α-, β- and γ-catenin and are connected to the actin cytoskeleton, thus providing the cells with a rigid structure. During EMT, several epithelial proteins, like E-cadherin, α-, β- and γ-catenin are downregulated, whereas mesenchymal proteins, including vimentin,

fibronectin and smooth muscle actin, can be upregulated (Jechlinger et al., 2003). The most common characteristic of EMT is the downregulation of E-cadherin. This glycoprotein can be repressed by two types of transcription factors, basic helix-loop-helix transcription factors including E12/E47, Twist1 (Twist) and Twist2 (Dermo1), and the zinc finger transcription factors, including the Snail family (Snail or Snai1 and Slug or Snai2), and the Zeb family, comprising Zeb1 and Zeb2 (also called SIP1) (Batlle et al., 2000; Cano et al., 2000; Comijn et al., 2001; Eger et al., 2005; Hajra et al., 2002; Perez-Moreno et al., 2001; Yang et al., 2004). These transcription factors bind to E-boxes within the *CDH1* promoter and thereby suppress its transcription. Most of these factors have been demonstrated to play a critical role in invasion and metastasis (Comijn et al., 2001; Polyak & Weinberg, 2009; Spaderna et al., 2008; Yang et al., 2004; Yin et al., 2007). EMT can be induced by several oncogenes, including RAS^{V12} (Janda et al., 2002), ErbB2 (Jenndahl et al., 2005) and TrkB (Smit et al., 2009; Kupferman et al., 2010). They activate multiple effectors including the PI3 and MAP kinases, but also the Wnt, Notch and NFkB pathways, all of which are involved in EMT regulation (Grille et al., 2003; Huber et al., 2005; Lemieux et al., 2009), can be activated.

Cellular senescence

Another mechanism involved in cancer progression is cellular senescence. Senescent cells fail to proliferate, but remain metabolically active. Senescence can be triggered by short or malfunctioning telomeres (called replicative senescence), but also prematurely, by a variety of stress signals, including unscheduled oncogenic signaling (Campisi, 2005; Serrano et al., 1997). "Oncogene-induced senescence" (OIS), as the latter phenomenon is called, relies on the activation of tumor suppressor gene networks often comprising RB and p53, mediating cell cycle arrest (Prieur & Peeper, 2008). In addition to elevated tumor suppressor signaling, OIS is associated with several other hallmarks, including increased activity of lysosomal β-galactosidase (SA-β-GAL), chromatin remodeling and induction of DNA damage (Adams, 2009). In many settings, OIS is associated with the secretion of dozens of cytokines, comprising the "Senescence-Messaging Secretome", or SMS, denoting its communicative role (Kuilman & Peeper, 2009). Cellular senescence can be triggered not only by oncogene activation, but also by the loss of tumor suppressor genes, including *PTEN*, *NF1* and *RB* (Collado & Serrano).

Although OIS has long been viewed as an exclusive *in vitro* phenomenon, it is being increasingly recognized as a critical feature of mammalian cells to suppress tumorigenesis, acting alongside cell death programs like apoptosis. For example, human melanocytic nevi (moles), benign tumors that have a low propensity to progress towards melanoma, display several hallmarks of OIS. In addition to harboring an oncogenic mutation (most commonly $BRAF^{E600}$), they display little proliferative activity, a characteristic that is typically maintained for decades. Furthermore, nevi express elevated levels of $p16^{INK4A}$ and have increased SA-β-GAL activity (Michaloglou et al., 2005; Mooi & Peeper, 2006). Similar results have recently been reported for

BRAF^{E600} knockin mice in which the activated kinase is expressed selectively in the melanocytic compartment (Dankort et al., 2009; Dhomen et al., 2009). Several additional mouse models have also shown senescence biomarkers in early neoplastic lesions (Kuilman et al., 2010). For example, lung adenomas expressing oncogenic RAS^{V12} are in a senescent state in contrast to invasive adenocarcinomas that are proliferating (Collado et al., 2005).

EMT players regulating senescence

For almost two decades, the transcription factor Twist has been known for its important role in embryonic development (Bate et al., 1991). More recently, Twist, but also other EMT regulators, have attracted considerable attention for their contribution to cancer progression. For example, Weinberg and co-workers found that Twist induces EMT and, as such, plays a critical role in metastasis (Yang et al., 2004). Other transcription factors from the Snail and Zeb family are endowed with similar capacities (Peinado et al., 2007). Earlier, a role for Twist 1 and 2 in apoptosis was revealed in an expression library screen for cDNAs suppressing the pro-apoptotic effect of the *myc* oncogene in MEFs (Maestro et al., 1999). In that setting, Twist reverts p53-induced cell cycle arrest and suppresses *Arf*, a gene that is highly induced during cellular and oncogene-induced senescence (Kamijo et al., 1997; Zindy et al., 2003). Similarly, in human prostate epithelial cells, Twist bypasses cellular senescence in an p14^{ARF}-dependent fashion (Kwok et al., 2007). Conversely, RNAi depletion of Twist induces cell cycle arrest in gastric cancer cells, which correlates with regulation of the major ARF effector, p53 (Feng et al., 2009).

Further mechanistic insight into how Twist impacts on the cell cycle machinery has evolved. Twist overexpression prevents the upregulation of p21^{CIP1} and p53 upon genotoxic stress in several cell lines (Li et al., 2009; Valsesia-Wittmann et al., 2004; Vichalkovski et al., 2010). In addition to indirect regulation through ARF, Twist may regulate p53 by a direct interaction, thereby preventing it from binding to DNA (Shiota et al., 2008). Consistent with this, adriamycin treatment leads to increased levels of a protein complex comprising p53, MDM2 and Twist (Li et al., 2009). A search for upstream targets revealed that PKB/AKT can phosphorylate Twist at Ser42, inhibiting p53 activity in response to γ-irradiation and promoting cell cycle progression (Vichalkovski et al., 2010). Ansieau *et al.* showed that Twist affects the transcriptional regulation of p16^{INK4A} and p21^{CIP1} (Ansieau et al., 2008), arguing together with previous publications that Twist can simultaneously deregulate the p53 and RB pathways, both of which affect several processes, including senescence. The signaling pathways targeted by Twist may even go beyond this, as suggested by the observation that Twist enhances the transforming activity of N-MYC in *Ink4a-ARF*$^{-/-}$ MEFs (Valsesia-Wittmann et al., 2004). Twist can also augment the transforming effects of E1A and Ras^{V12} (Maestro et al., 1999), although it remains to be seen whether Twist acts in such contexts during human tumor progression (Weinberg, 2008).

Extending these findings, increasing evidence suggests that the two processes that seem to operate independently, EMT and senescence, are in fact intertwined. For example, while RAS^{V12} induces EMT in epithelial cells, often in a cooperative fashion with TGFβ (Janda et al., 2002), it also induces senescence in human diploid fibroblasts (Serrano et al., 1997). Puisieux and co-workers showed that whereas ectopically expressed ErbB2 induces senescence, overexpression of both Twist and ErbB2 triggers EMT and allows for senescence bypass, both in MEFs and human epithelial cells (Ansieau et al., 2008). This is consistent with the prevailing view that a single oncogene is insufficient to drive cancer progression: it commonly acts cytostatically or induces a death program and requires a collaborating oncogene to convert this into a pro-survival and mitogenic process (Kuilman et al., 2010). But more importantly, the studies mentioned above, and several that will be discussed below, have provided a link between EMT and cellular senescence.

The premise that activation of EMT is linked to suppression of cellular senescence has been proposed also in the context of another EMT regulator, Zeb1 (Liu et al., 2008). Specifically, depletion of *Zeb1* in MEFs causes MET, which is characterized by increased expression of epithelial proteins such as E-cadherin and decreased expression of mesenchymal proteins. Zeb1 loss triggers premature senescence by binding directly to the *CDKN1A* and *INK4B* promoters, thereby simultaneously affecting p53 and RB signaling. In turn, Zeb1 is regulated via the Zeb1/miR200 feedback loop, which is thought to drive cancer progression via promoting EMT and inhibiting senescence (Brabletz & Brabletz, 2010).

These findings prompt several interesting questions. Probably most importantly, does the coordinated deregulation of EMT and senescence reflect only a “collateral effect”, or instead, are there mechanistic links that tie these two processes together (Smit & Peeper, 2008)? A recent study suggests the latter possibility (Ohashi et al., 2010). Esophageal squamous cell carcinoma cells expressing activated EGFR were shown to undergo premature senescence. A subpopulation of cells emerged from this pool, which expressed elevated levels of Zeb1 and 2, as if these factors suppressed the senescence program. Interestingly, when cells were locked in a senescent state by activation of p53, the cytokine TGFβ was no longer able to induce EMT, raising the possibility that senescent cells cannot undergo EMT. This is in line with the findings of Ansieau *et al*. that senescence abrogation is accompanied by an EMT (Ansieau et al., 2010).

Although a general role for other EMT-associated transcription factors in senescence remains to be elucidated, some can regulate several cell cycle regulators that have also been implicated in senescence. For example, in MDCK canine epithelial cells, Snail deregulates the cell cycle machinery by induction of $p21^{CIP1}$ and reduction of cyclin D1 and D2, thereby effectively inducing cell cycle arrest (Vega et al., 2004). Also in HEPG2 cells, Snail induces cell cycle arrest via regulation of $p15^{INK4B}$ (Hu et al., 2008), a relative of $p16^{INK4A}$ that has also been associated with senes-

cence (Michaloglou et al., 2008). Conversely, in combination with an oncogene, Snail can have the opposite effect, for example by inhibiting E2A-induced $p21^{CIP}$ induction (Takahashi et al., 2004). Furthermore, Snail can suppress p53 by direct binding, at least in certain settings (Lee et al., 2009; Lee et al., 2010). Overexpression of another transcription factor, Zeb2, reduces the proliferative capacity of cells, involving inhibition of cyclin D1 (Mejlvang et al., 2007). However, although from these results it appears that EMT-associated transcription factors can regulate the cell cycle through proteins that are also involved in senescence, it remains to be established whether these modes of communication, indeed, have an impact on senescence signaling.

Senescence players regulating EMT

Whereas several prototypic EMT regulators have been implicated in senescence, conversely, a number of key senescence players have been found to affect EMT. For example, the viral oncoprotein SV40 LT, which simultaneously deregulates the cell cycle regulators p53 and RB, can suppress E-cadherin to induce a mesenchymal-like morphology. This is dependent on the downregulation of RB (Martel et al., 1997). As RB was recently demonstrated to play a unique role, among the pocket protein family, in cellular senescence (Chicas et al., 2010), these findings may link RB, senescence and EMT. Also in another setting, when EMT is induced by TGFβ/TNFα in MCF10A cells, RB is downregulated. Furthermore, whereas overexpression of RB blocks the morphological effect and the suppression of E-cadherin expression (Batsche et al., 1998), downregulation of RB induces EMT (Arima et al., 2008). In both cases this is regulated by binding of RB to the *CDH1* promoter, thereby suppressing its transcription (Batsche et al., 1998; Arima et al., 2008). A link between RB and E-cadherin was also described in a slightly different setting: E-cadherin-mediated aggregation prevents cell death induced by active PKC. This is accompanied by RB activation, cell cycle arrest and survival (Day et al., 1999).

Wild type p53, a key senescence player (Campisi, 2005), inhibits the transcription factor Slug (or Snai2) via MDM2, whereas mutant p53 stabilizes Slug, thereby increasing its levels and enhancing cancer cell invasiveness (Wang et al., 2009). In non-small-cell lung cancer, where p53 is often mutated, Slug expression is high, correlating with low MDM2 levels and lower overall survival.

Another factor that can be associated with cellular senescence and has also been implicated in EMT is $p21^{CIP1}$. It was shown that $p21^{CIP1}$ is inhibited during Ras^{V12}-induced EMT in MCF10A cells. Furthermore, whereas transgenic mice expressing Ras^{V12} display only some features of EMT, compound transgenic mice with a deficiency for $p21^{CIP1}$ show accelerated mammary tumor formation, which may result from the induction of more extensive EMT features (Liu et al., 2009). This suggests that $p21^{CIP1}$ plays a role in EMT both not only *in vitro* but also *in vivo*. But again, to what extent this CKI causally connects senescence and EMT remains to be seen.

The inflammatory protein network seems to represent yet another link between EMT and senescence. It has recently been shown that OIS is accompanied by the induction of several interleukins, revealing that senescence is commonly associated with a robust inflammatory response (Acosta et al., 2008; Coppe et al., 2008; Kuilman et al., 2008; Kuilman & Peeper, 2009). Unexpectedly, OIS can be abrogated (or even reverted) by downregulation of either interleukin 6 (IL-6) or IL-8 (Kuilman et al., 2008). The latter is specifically co-expressed with $p16^{INK4A}$-positive non-proliferating cells in colon adenomas, suggesting that some interleukins contribute to tumor suppression in benign human lesions (Kuilman et al., 2008). Similarly, silencing of a chemokine receptor, CXCR2, results in bypass from senescence (Acosta et al., 2008). On the other hand, IL-6 is able to induce EMT in epithelial cells, suggesting that interleukins also participate in the regulation of EMT (Sullivan et al., 2009). Furthermore, EGF-induced EMT in ovarian carcinoma cells induces both IL-6 expression and secretion (Colomiere et al., 2009). Also IL-8 is associated with EMT: it is induced in cells undergoing TGFβ-induced EMT (Bates et al., 2004). Interestingly, CXCR1 (but not CXCR2) is also induced in these cells. The interleukin-related protein ILEI was shown to be important for EMT and metastasis (Waerner et al., 2006). These observations suggest that the inflammatory network is important for both senescence and EMT.

Concluding remarks

Recent observations suggest that two important processes involved in cancer progression, senescence and EMT, are crossing paths. For example, several transcription factors can both inhibit senescence and induce EMT (Figure 1). In doing so, they seem to have a double role in promoting cancer: while an override of the senescence program contributes to the acquisition of indefinite proliferative

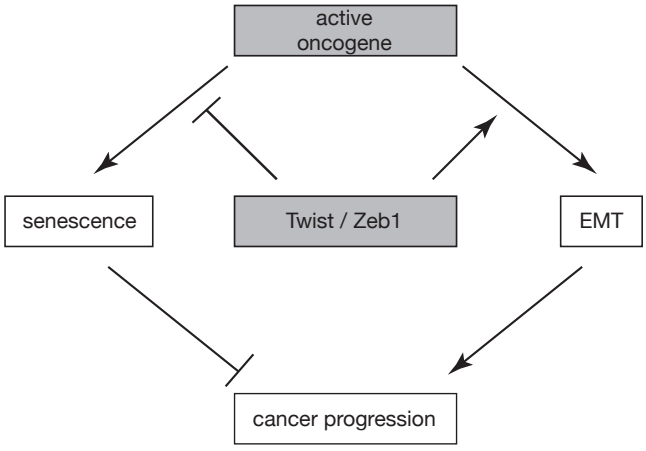

Figure 1: Working model schematically depicting how EMT and senescence are linked and contribute to cancer progression. An active oncogene can either induce senescence or EMT, dependent on the cellular context. Conversely, transcription factors like Twist and Zeb1 can have a double impact on cancer progression by simultaneously inhibiting oncogene-induced senescence and promoting EMT.

capacity, induction of EMT bypasses rate-limiting aspects of the metastatic cascade. Conversely, at least in theory, this may open new strategic therapeutic avenues that, by targeting the major players promoting EMT and senescence, have a double impact. This potential perspective not withstanding, there are several important questions remaining. Although senescence bypass can be accompanied by EMT, it will be of interest to determine whether, in fact, EMT contributes to the override of senescence. Indeed, although several observations have revealed features of EMT in association with (abrogation of) cellular senescence, or vice versa, in many cases it remains to be established whether the processes are mechanistically tied together. For example, is there a direct role for E-cadherin in the regulation of senescence? While Twist and other transcription factors that can promote EMT have multiple effector genes, also several senescence players, including *CDKN2A*, harbor E-boxes in their promoters (Zheng et al., 2004), suggesting that the EMT-associated transcription factors can also regulate the cell cycle without direct regulation of E-cadherin. Finally, it is interesting to note that EMT and senescence share the phenomenon that the biomarkers that accompany these processes are regulated differentially in different (genetic and cellular) settings, for reasons that are as yet largely unclear. Answers to these and related questions will not only increase our understanding of these two mechanisms driving cancer progression, but eventually also help to improve strategies for therapeutic intervention.

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References

Acosta JC, O'Loghlen A, Banito A, Guijarro MV, Augert A, Raguz S, Fumagalli M, Da Costa M, Brown C, Popov N, Takatsu Y, Melamed J, d'Adda di Fagagna F, Bernard D, Hernando E and Gil J. (2008). Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* **133:** 1006-18.

Adams PD. (2009). Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. *Mol Cell* **36:** 2-14.

Ansieau S, Bastid J, Doreau A, Morel AP, Bouchet BP, Thomas C, Fauvet F, Puisieux I, Doglioni C, Piccinin S, Maestro R, Voeltzel T, Selmi A, Valsesia-Wittmann S, Caron de Fromentel C and Puisieux A. (2008). Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. *Cancer Cell* **14:** 79-89.

Ansieau S, Morel AP, Hinkal G, Bastid J and Puisieux A. (2010). TWISTing an embryonic transcription factor into an oncoprotein. *Oncogene* **29:** 3173-84.

Arima Y, Inoue Y, Shibata T, Hayashi H, Nagano O, Saya H and Taya Y. (2008). Rb depletion results in deregulation of E-cadherin and induction of cellular phenotypic changes that are characteristic of the epithelial-to-mesenchymal transition. *Cancer Res* **68:** 5104-12.

Bate M, Rushton E and Currie DA. (1991). Cells with persistent twist expression are the embryonic precursors of adult muscles in Drosophila. *Development* **113:** 79-89.

Bates RC, DeLeo MJ, 3rd and Mercurio AM. (2004). The epithelial-mesenchymal transition of colon carcinoma involves expression of IL-8 and CXCR-1-mediated chemotaxis. *Exp Cell Res* **299:** 315-24.

Batlle E, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J and Garcia De Herreros A. (2000). The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* **2:** 84-9.

Batsche E, Muchardt C, Behrens J, Hurst HC and Cremisi C. (1998). RB and c-Myc activate expression of the E-cadherin gene in epithelial cells through interaction with transcription factor AP-2. *Mol Cell Biol* **18:** 3647-58.

Brabletz S and Brabletz T. (2010). The ZEB/miR-200 feedback loop--a motor of cellular plasticity in development and cancer? *EMBO Rep* **11:** 670-7.

Brabletz T, Hlubek F, Spaderna S, Schmalhofer O, Hiendlmeyer E, Jung A and Kirchner T. (2005). Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. *Cells Tissues Organs* **179:** 56-65.

Campisi J. (2005). Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* **120:** 513-22.

Cano A, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F and Nieto MA. (2000). The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* **2:** 76-83.

Chicas A, Wang X, Zhang C, McCurrach M, Zhao Z, Mert O, Dickins RA, Narita M, Zhang M and Lowe SW. (2010). Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence. *Cancer Cell* **17:** 376-87.

Christofori G. (2006). New signals from the invasive front. *Nature* **441:** 444-50.

Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguria A, Zaballos A, Flores JM, Barbacid M, Beach D and Serrano M. (2005). Tumour biology: senescence in premalignant tumours. *Nature* **436:** 642.

Collado M and Serrano M. Senescence in tumours: evidence from mice and humans. *Nat Rev Cancer* **10:** 51-7.

Colomiere M, Ward AC, Riley C, Trenerry MK, Cameron-Smith D, Findlay J, Ackland L and Ahmed N. (2009). Cross talk of signals between EGFR and IL-6R through JAK2/STAT3 mediate epithelial-mesenchymal transition in ovarian carcinomas. *Br J Cancer* **100:** 134-44.

Comijn J, Berx G, Vermassen P, Verschueren K, van Grunsven L, Bruyneel E, Mareel M, Huylebroeck D and van Roy F. (2001). The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. *Mol Cell* **7:** 1267-78.

Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, Nelson PS, Desprez PY and Campisi J. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* **6:** 2853-68.

Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky WE, Jr., You MJ, DePinho RA, McMahon M and Bosenberg M. (2009). Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nat Genet* **41:** 544-52.

Day ML, Zhao X, Vallorosi CJ, Putzi M, Powell CT, Lin C and Day KC. (1999). E-cadherin mediates aggregation-dependent survival of prostate and mammary epithelial cells through the retinoblastoma cell cycle control pathway. *J Biol Chem* **274:** 9656-64.

De Wever O, Pauwels P, De Craene B, Sabbah M, Emami S, Redeuilh G, Gespach C, Bracke M and Berx G. (2008). Molecular and pathological signatures of epithelial-mesenchymal transitions at the cancer invasion front. *Histochem Cell Biol* **130:** 481-94.

Dhomen N, Reis-Filho JS, da Rocha Dias S, Hayward R, Savage K, Delmas V, Larue L, Pritchard C and Marais R. (2009). Oncogenic Braf induces melanocyte senescence and melanoma in mice. *Cancer Cell* **15:** 294-303.

Eger A, Aigner K, Sonderegger S, Dampier B, Oehler S, Schreiber M, Berx G, Cano A, Beug H and Foisner R. (2005). DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. *Oncogene* **24:** 2375-85.

Feng MY, Wang K, Song HT, Yu HW, Qin Y, Shi QT and Geng JS. (2009). Metastasis-induction and apoptosis-protection by TWIST in gastric cancer cells. *Clin Exp Metastasis* **26:** 1013-23.

Geiger TR and Peeper DS. (2009). Metastasis mechanisms. *Biochim Biophys Acta* **1796:** 293-308.

Grille SJ, Bellacosa A, Upson J, Klein-Szanto AJ, van Roy F, Lee-Kwon W, Donowitz M, Tsichlis PN and Larue L. (2003). The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. *Cancer Res* **63:** 2172-8.

Hajra KM, Chen DY and Fearon ER. (2002). The SLUG zinc-finger protein represses E-cadherin in breast cancer. *Cancer Res* **62:** 1613-8.

Hu CT, Wu JR, Chang TY, Cheng CC and Wu WS. (2008). The transcriptional factor Snail simultaneously triggers cell cycle arrest and migration of human hepatoma HepG2. *J Biomed Sci* **15:** 343-55.

Huber MA, Kraut N and Beug H. (2005). Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr Opin Cell Biol* **17:** 548-58.

Janda E, Lehmann K, Killisch I, Jechlinger M, Herzig M, Downward J, Beug H and Grunert S. (2002). Ras and TGF[beta] cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. *J Cell Biol* **156:** 299-313.

Jechlinger M, Grunert S, Tamir IH, Janda E, Ludemann S, Waerner T, Seither P, Weith A, Beug H and Kraut N. (2003). Expression profiling of epithelial plasticity in tumor progression. *Oncogene* **22:** 7155-69.

Jenndahl LE, Isakson P and Baeckstrom D. (2005). c-erbB2-induced epithelial-mesenchymal transition in mammary epithelial cells is suppressed by cell-cell contact and initiated prior to E-cadherin downregulation. *Int J Oncol* **27:** 439-48.

Kalluri R and Neilson EG. (2003). Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* **112:** 1776-84.

Kamijo T, Zindy F, Roussel MF, Quelle DE, Downing JR, Ashmun RA, Grosveld G and Sherr CJ. (1997). Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* **91:** 649-59.

Kuilman T, Michaloglou C, Mooi WJ and Peeper DS. (2010). The essence of senescence. *Genes Dev* **in press**.

Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, Aarden LA, Mooi WJ and Peeper DS. (2008). Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* **133:** 1019-31.

Kuilman T and Peeper DS. (2009). Senescence-messaging secretome: SMS-ing cellular stress. *Nat Rev Cancer* **9:** 81-94.

Kupferman ME, Jiffar T, El-Naggar A, Yilmaz T, Zhou G, Xie T, Feng L, Wang J, Holsinger FC, Yu D and Myers JN. (2010). TrkB induces EMT and has a key role in invasion of head and neck squamous cell carcinoma. *Oncogene* **29:** 2047-59.

Kwok WK, Ling MT, Yuen HF, Wong YC and Wang X. (2007). Role of p14ARF in TWIST-mediated senescence in prostate epithelial cells. *Carcinogenesis* **28:** 2467-75.

Lee SH, Lee SJ, Jung YS, Xu Y, Kang HS, Ha NC and Park BJ. (2009). Blocking of p53-Snail binding, promoted by oncogenic K-Ras, recovers p53 expression and function. *Neoplasia* **11:** 22-31, 6p following 31.

Lee SH, Shen GN, Jung YS, Lee SJ, Chung JY, Kim HS, Xu Y, Choi Y, Lee JW, Ha NC, Song GY and Park BJ. (2010). Antitumor effect of novel small chemical inhibitors of Snail-p53 binding in K-Ras-mutated cancer cells. *Oncogene* **29:** 4576-87.

Lemieux E, Bergeron S, Durand V, Asselin C, Saucier C and Rivard N. (2009). Constitutively active MEK1 is sufficient to induce epithelial-to-mesenchymal transition in intestinal epithelial cells and to promote tumor invasion and metastasis. *Int J Cancer* **125**: 1575-86.

Li QQ, Xu JD, Wang WJ, Cao XX, Chen Q, Tang F, Chen ZQ, Liu XP and Xu ZD. (2009). Twist1-mediated adriamycin-induced epithelial-mesenchymal transition relates to multidrug resistance and invasive potential in breast cancer cells. *Clin Cancer Res* **15:** 2657-65.

Liu M, Casimiro MC, Wang C, Shirley LA, Jiao X, Katiyar S, Ju X, Li Z, Yu Z, Zhou J, Johnson M, Fortina P, Hyslop T, Windle JJ and Pestell RG. (2009). p21CIP1 attenuates Ras- and c-Myc-dependent breast tumor epithelial mesenchymal transition and cancer stem cell-like gene expression in vivo. *Proc Natl Acad Sci U S A* **106:** 19035-9.

Liu Y, El-Naggar S, Darling DS, Higashi Y and Dean DC. (2008). Zeb1 links epithelial-mesenchymal transition and cellular senescence. *Development* **135:** 579-88.

Maestro R, Dei Tos AP, Hamamori Y, Krasnokutsky S, Sartorelli V, Kedes L, Doglioni C, Beach DH and Hannon GJ. (1999). Twist is a potential oncogene that inhibits apoptosis. *Genes Dev* **13:** 2207-17.

Martel C, Harper F, Cereghini S, Noe V, Mareel M and Cremisi C. (1997). Inactivation of retinoblastoma family proteins by SV40 T antigen results in creation of a hepatocyte growth factor/scatter factor autocrine loop associated with an epithelial-fibroblastoid conversion and invasiveness. *Cell Growth Differ* **8:** 165-78.

Mejlvang J, Kriajevska M, Vandewalle C, Chernova T, Sayan AE, Berx G, Mellon JK and Tulchinsky E. (2007). Direct repression of cyclin D1 by SIP1 attenuates cell cycle progression in cells undergoing an epithelial mesenchymal transition. *Mol Biol Cell* **18:** 4615-24.

Michaloglou C, Vredeveld LC, Mooi WJ and Peeper DS. (2008). BRAF(E600) in benign and malignant human tumours. *Oncogene* **27:** 877-95.

Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ and Peeper DS. (2005). BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* **436:** 720-4.

Mooi WJ and Peeper DS. (2006). Oncogene-induced cell senescence--halting on the road to cancer. *N Engl J Med* **355:** 1037-46.

Ohashi S, Natsuizaka M, Wong GS, Michaylira CZ, Grugan KD, Stairs DB, Kalabis J, Vega ME, Kalman RA, Nakagawa M, Klein-Szanto AJ, Herlyn M, Diehl JA, Rustgi AK and Nakagawa H. (2010). Epidermal growth factor receptor and mutant p53 expand an esophageal cellular subpopulation capable of epithelial-to-mesenchymal transition through ZEB transcription factors. *Cancer Res* **70:** 4174-84.

Peinado H, Olmeda D and Cano A. (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer* **7:** 415-28.

Perez-Moreno MA, Locascio A, Rodrigo I, Dhondt G, Portillo F, Nieto MA and Cano A. (2001). A new role for E12/E47 in the repression of E-cadherin expression and epithelial-mesenchymal transitions. *J Biol Chem* **276:** 27424-31.

Polyak K and Weinberg RA. (2009). Transitions between epithelial and mesenchymal states:

acquisition of malignant and stem cell traits. *Nat Rev Cancer* **9:** 265-73.
Prieur A and Peeper DS. (2008). Cellular senescence in vivo: a barrier to tumorigenesis. *Curr Opin Cell Biol* **20:** 150-5.
Serrano M, Lin AW, McCurrach ME, Beach D and Lowe SW. (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* **88:** 593-602.
Shiota M, Izumi H, Onitsuka T, Miyamoto N, Kashiwagi E, Kidani A, Hirano G, Takahashi M, Naito S and Kohno K. (2008). Twist and p53 reciprocally regulate target genes via direct interaction. *Oncogene* **27:** 5543-53.
Smit MA, Geiger TR, Song JY, Gitelman I and Peeper DS. (2009). A Twist-Snail axis critical for TrkB-induced epithelial-mesenchymal transition-like transformation, anoikis resistance, and metastasis. *Mol Cell Biol* **29:** 3722-37.
Smit MA and Peeper DS. (2008). Deregulating EMT and senescence: double impact by a single twist. *Cancer Cell* **14:** 5-7.
Spaderna S, Schmalhofer O, Hlubek F, Berx G, Eger A, Merkel S, Jung A, Kirchner T and Brabletz T. (2006). A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal cancer. *Gastroenterology* **131:** 830-40.
Spaderna S, Schmalhofer O, Wahlbuhl M, Dimmler A, Bauer K, Sultan A, Hlubek F, Jung A, Strand D, Eger A, Kirchner T, Behrens J and Brabletz T. (2008). The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer. *Cancer Res* **68:** 537-44.
Sullivan NJ, Sasser AK, Axel AE, Vesuna F, Raman V, Ramirez N, Oberyszyn TM and Hall BM. (2009). Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells. *Oncogene* **28:** 2940-7.
Takahashi E, Funato N, Higashihori N, Hata Y, Gridley T and Nakamura M. (2004). Snail regulates p21(WAF/CIP1) expression in cooperation with E2A and Twist. *Biochem Biophys Res Commun* **325:** 1136-44.
Thiery JP. (2002). Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* **2:** 442-54.
Thiery JP, Acloque H, Huang RY and Nieto MA. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell* **139:** 871-90.
Valsesia-Wittmann S, Magdeleine M, Dupasquier S, Garin E, Jallas AC, Combaret V, Krause A, Leissner P and Puisieux A. (2004). Oncogenic cooperation between H-Twist and N-Myc overrides failsafe programs in cancer cells. *Cancer Cell* **6:** 625-30.
Vega S, Morales AV, Ocana OH, Valdes F, Fabregat I and Nieto MA. (2004). Snail blocks the cell cycle and confers resistance to cell death. *Genes Dev* **18:** 1131-43.
Vichalkovski A, Gresko E, Hess D, Restuccia DF and Hemmings BA. (2010). PKB/AKT phosphorylation of the transcription factor Twist-1 at Ser42 inhibits p53 activity in response to DNA damage. *Oncogene* **29:** 3554-65.
Waerner T, Alacakaptan M, Tamir I, Oberauer R, Gal A, Brabletz T, Schreiber M, Jechlinger M and Beug H. (2006). ILEI: a cytokine essential for EMT, tumor formation, and late events in metastasis in epithelial cells. *Cancer Cell* **10:** 227-39.
Wang SP, Wang WL, Chang YL, Wu CT, Chao YC, Kao SH, Yuan A, Lin CW, Yang SC, Chan WK, Li KC, Hong TM and Yang PC. (2009). p53 controls cancer cell invasion by inducing the MDM2-mediated degradation of Slug. *Nat Cell Biol* **11:** 694-704.
Weinberg RA. (2008). Twisted epithelial-mesenchymal transition blocks senescence. *Nat Cell Biol* **10:** 1021-3.

Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A and Weinberg RA. (2004). Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* **117:** 927-39.

Yin T, Wang C, Liu T, Zhao G, Zha Y and Yang M. (2007). Expression of snail in pancreatic cancer promotes metastasis and chemoresistance. *J Surg Res* **141:** 196-203.

Zheng W, Wang H, Xue L, Zhang Z and Tong T. (2004). Regulation of cellular senescence and p16(INK4a) expression by Id1 and E47 proteins in human diploid fibroblast. *J Biol Chem* **279:** 31524-32.

Zindy F, Williams RT, Baudino TA, Rehg JE, Skapek SX, Cleveland JL, Roussel MF and Sherr CJ. (2003). Arf tumor suppressor promoter monitors latent oncogenic signals in vivo. *Proc Natl Acad Sci U S A* **100:** 15930-5.