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Epithelial-Mesenchymal Transitions in development and disease: old views and new perspectives

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ABSTRACT The epithelial to mesenchymal transition (EMT) is a fascinating phenotypic change that is undertaken by embryonic and adult cells in physiological and pathological conditions, respectively. This change in cell behavior involves the loss of epithelial characteristics and the acquisition of migratory properties. While it has long been established as a fundamental process in the generation of many different embryonic tissues, its significance during tumor progression as an initial determining step in the metastatic cascade has remained a matter of debate. Recent molecular analyses coupled with state-of-the-art imaging technology have helped to define the EMT as an important landmark, not only during tumor progression, but also during the development of other pathologies such as organ fibrosis. Spanish groups have contributed to the analysis of EMT both from the developmental and the pathological point of view, in particular assessing the implication of the *Snail* genes in this process. Interestingly, the contribution of Spanish scientists to the existence of EMT in tumors possibly goes back more than 100 years, when Cajal referred to some "pear-like cells, not attached to each other" in his description of human breast carcinomas.

KEY WORDS: *snail transcription factors, EMT, cell migration*

The EMT in embryonic development

The epithelial to mesenchymal transition (EMT) involves profound changes in the morphology and behavior of epithelial cells. Not only do epithelial cells loose contact with their neighbors but they also become motile and can break through the basement membrane that separates different tissues within the embryo. Decades ago, embryologists rapidly became aware of this transformation (Hay, 1968; Thiery, 1984; Bellairs, 1987; Hay, 1989) in part because EMT occurs repeatedly during embryonic development for the generation of tissues and organs whose precursors originate far from their final destination. EMT is necessary for the embryo to allow epithelial cells to migrate over what may be very long distances. It is important to note that EMT refers to epithelial cells that adopt a mesenchymal cell phenotype and thus, neither the process nor the molecules that trigger it (see below) are generally used to promote the movement of other cell types such as migration of neurons within the developing brain. Another interesting aspect of EMT that must be considered occurs once the cells have reached their destination, where their differentiation into different cell types very often involves the reverse process, a mesenchymal to epithelial transformation (MET). The transient nature of the transformation facilitates the formation of many different embryonic derivatives and explains why the term transition is preferred to that of transformation.

The delamination of the neural crest from the neural tube and that of the mesoderm from the primitive streak in amniotes are considered the prototypical EMTs and these events have contributed much information to understand the cellular and molecular aspects of EMT (Fig. 1A, B). These two tissues give rise to a plethora of derivatives and together, they are the precursors of the peripheral nervous system, the pigment cells, the skeleton, the muscles and components of the dermis. Moreover, the neural crest and the mesoderm are the embryonic tissues in which the *Snail* genes were described, among the principal inducers of EMT. In her excellent review in 1995, Elizabeth Hay proposed the existence of a master mesenchymal regulatory gene(s) that is activated to induce EMT (Hay, 1995). The *Snail* genes, which encode transcription factors of the zinc-finger type, have proven to behave like master genes for EMT, as they are able to induce

Abbreviations used in this paper: EMT, epithelial to mesenchymal transition; MET, mesenchymal to epithelial transition.

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the full process both *in vitro* and *in vivo*. Accordingly, the expression of many molecules that influence both cell morphology and behavior are regulated by the Snail proteins (Fig. 2).

The first indication that *Snail* genes were involved in triggering EMT came form studies in the early chick embryo. Antisense oligonucleotides against Slug (now called Snail2) prevented neural crest and mesoderm delamination (Nieto *et al.,* 1994) and subsequently, it was confirmed that *Snail* genes are crucial for the induction of EMT in different species and tissues (reviewed in Hemavathy *et al.,* 2000; Nieto, 2002; Ip and Gridley, 2002; De Craene *et al.,* 2005; Barrallo-Gimeno and Nieto, 2005). As such, in addition to the neural crest and the mesoderm, *Snail* genes participate in the EMT necessary for the formation of the heart cushions (Romano and Runyan, 2000; Carmona *et al.,* 2000; Timmerman *et al.,* 2004), the parietal endoderm (Velmaat *et al.,* 2000) and the closure of the palate (Martinez-Alvarez *et al.,* 2004; Murray *et al.,* 2007), as well as events in other tissues and organs. Moreover, the *Snail* genes have been the subject of numerous evolutionary studies due to peculiarities such as the evolutionary interchange of the expression patterns of the different *Snail* genes in different species, and their association with the origin of the neural crest (Sefton *et al.,* 1998; Locascio *et al.,* 2002; Manzanares and Nieto, 2003). Likewise the EMT has been studied across evolution given its ancestral role in triggering cell movements in Metazoa (Fritzenwanker *et al.,* 2004). Other developmental genes that are important in EMT include the transcription factors *Twist* (Yang *et al.,* 2004), *E47* (Pérez-Moreno *et al.,* 2001) and *Sip-1* (Comijn *et al.,* 2001). In this review, we will focus our attention on the *Snail* genes since several groups working in Spain have contributed significantly to our understanding of how these genes participate in the EMT process, both during embryonic development and in the adult.

The EMT in tumor progression

As discussed in the first publication that described a relationship between *Snail* genes and EMT, their pathological activation could contribute to the onset of an invasive or metastatic phenotype during the progress of epithelial cancers (Nieto *et al.,* 1994). At the cellular level, the delamination of malignant cells from the primary tumor is reminiscent of that undertaken by neural crest cells and the mesoderm. Indeed, Snail is activated in the invasive

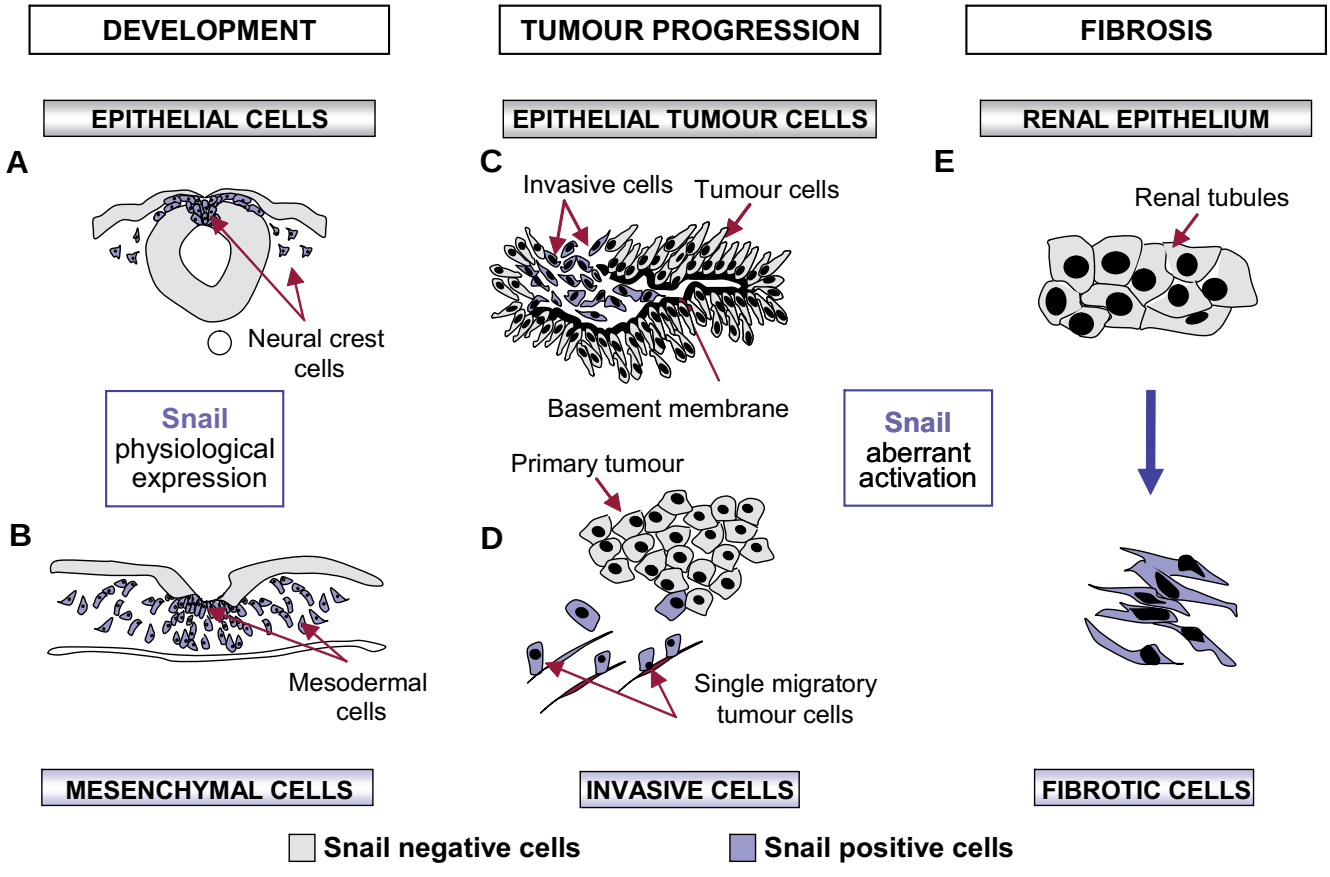

Fig. 1. Snail and the Epithelial-Mesenchymal Transition (EMT) in health and disease. (A,B) *The EMT is fundamental for the development of many tissues and organs, including the neural crest and the mesoderm of amniotes.* **(C)** *Snail is activated* in vivo *at the invasive front of chemically-induced mouse skin tumors and it is present in human carcinomas of different etiologies, where it is inversely correlated with the degree of differentiation and is associated with lymph-node metastasis (see Barrallo-Gimeno and Nieto, 2005, for a review).* **(D)** *Multiphoton intravital microscopy has facilitated the visualization of individual primary tumor cells migrating away from the tumor mass (Wang* et al., *2002).* **(E)** *Snail is maintained silent in the adult and its pathological activation in the kidney leads to renal fibrosis (Boutet* et al., *2006).*

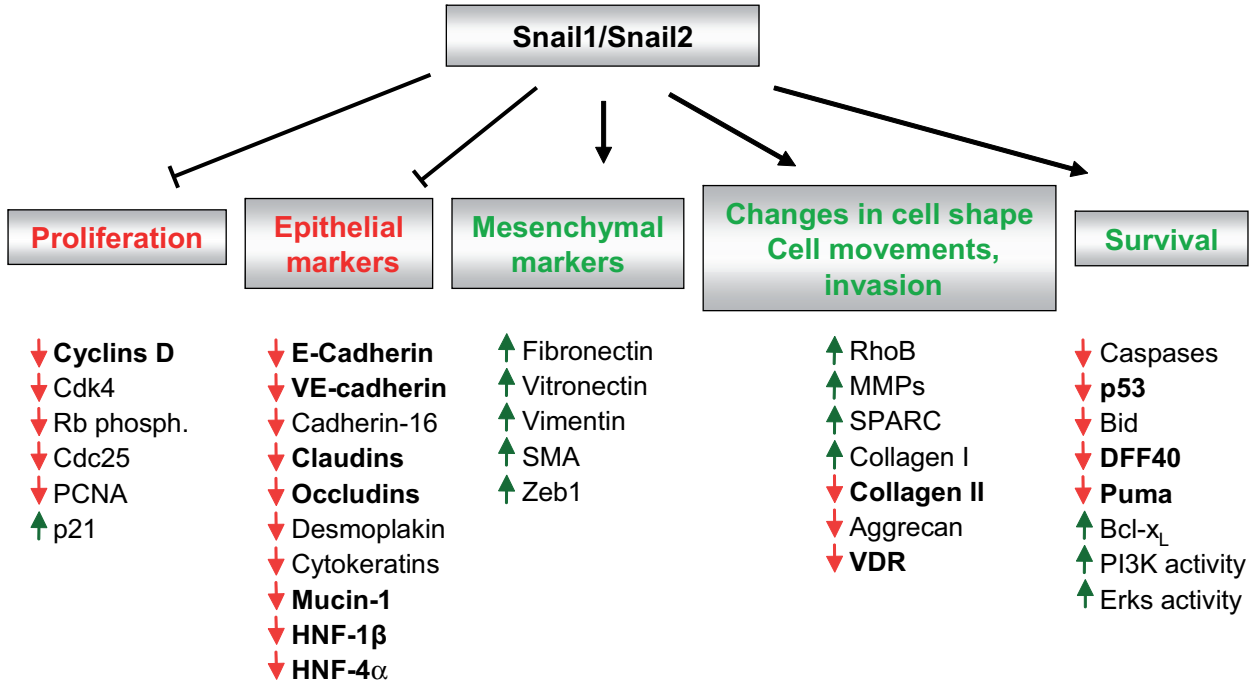

Fig. 2. Downstream targets of Snail. *In addition to molecules involved in morphological changes and the acquisition of motile and invasive properties, the* Snail *genes also regulate cell proliferation and cell death. The targets that are directly regulated by* Snail *genes are shown in bold. Bid, Bcl - interacting death agonist; Cdk, cyclin-dependent kinase; DFF, DNA fragmentation factor; Erks, extracellular signal-regulated kinases; HNF, hepatocyte nuclear factor; MMPs, metalloproteinases; PI3K, phosphoinositide 3-Kinase; p21, cyclin-dependent kinase inhibitor; p53, tumor suppressor; Rb, retinoblastoma; SMA, alpha smooth muscle actin; SPARC, secreted protein, acidic and rich in cysteine; VDR, vitamin D receptor. Updated from a previous version in Barrallo-Gimeno and Nieto, 2005.*

areas of tumors generated in the skin of mice (Cano *et al.,* 2000; Fig. 1C), in undifferentiated breast tumors (Blanco *et al.,* 2002) and in other carcinomas from different etiologies (see for instance Rosivatz *et al.,* 2002; Saito *et al.,* 2004; Miyoshi *et al.,* 2005; Kuphal *et al.,* 2005; Franci *et al.,* 2006; Boutet *et al.,* 2007). Thus, Snail is now regarded as a marker of tumor malignancy and a target of anti-invasive drugs. Furthermore, Snail has also been implicated in the promotion of tumor recurrence (Moody *et al.,* 2005) and regulates the expression of other molecules unrelated to EMT, such as the vitamin D receptor, with implications in cancer therapy (Palmer *et al.,* 2004).

The molecular analysis of Snail-induced EMT showed that Snail is a strong repressor of *E-cadherin* transcription (Batlle *et al.,* 2000; Cano *et al.,* 2000), which very much influences cell behavior both in embryonic development and tumor progression. Indeed, the loss of E-cadherin expression is clinically regarded as poor prognostic sign, since it is associated with the transition to an invasive phenotype (Perl *et al.,* 1998). In addition to Snail, other E-cadherin repressors that contribute to EMT and tumor progression have subsequently been described. These include members of the ZEB and HLH families that are differentially distributed in tumors of different origins (see Peinado *et al.,* 2007 for a review).

It is important to note that E-cadherin repression is not sufficient to induce EMT or invasive properties, as its re-expression in mesenchymal cells does not induce the reversion to the epithelial phenotype (Navarro *et al.,* 1993). Indeed, E-cadherin repressors, and in particular Snail, directly or indirectly regulate the expression of many additional target genes (Fig. 2) in order to repress the epithelial character and provoke the mesenchymal transition. Moreover, the different Snail family members may be functionally equivalent (Del Barrio and Nieto, 2002) and as well as their many common targets, each may also have specific targets (Moreno-Bueno *et al.,* 2006).

Snail can also promote tumor progression by activating angiogenesis (Peinado *et al.,* 2004b) and there are indications to suggest that silencing Snail can revert invasion (Olmeda *et al.,* 2007). Recent studies are contributing to our knowledge of the mechanisms that control Snail activity as a transcription factor (Peinado *et al.,* 2004a; Peinado *et al.,* 2005).

Animals models generated to study the role of Snail in tumorigenesis are now available and should prove very useful to further study these processes (Perez-Mancera *et al.,* 2005a; 2005b). However, it is important to bear in mind that these models must take into account the fundamental roles played by Snail during embryonic development, which can jeopardize such studies. In summary, EMT is an important step in the acquisition of the invasive phenotype of tumors, providing an example of an important developmental process that adopts a sinister role in the adult, as discussed by Jean Paul Thiery (Thiery, 2002).

The EMT in organ fibrosis

The sinister role of EMT in the adult is not restricted to tumor progression and indeed, adult non-transformed epithelial cells exposed to Snail undergo EMT, disrupting tissue homeostasis. Their aberrant activation in the adult kidney is sufficient to induce

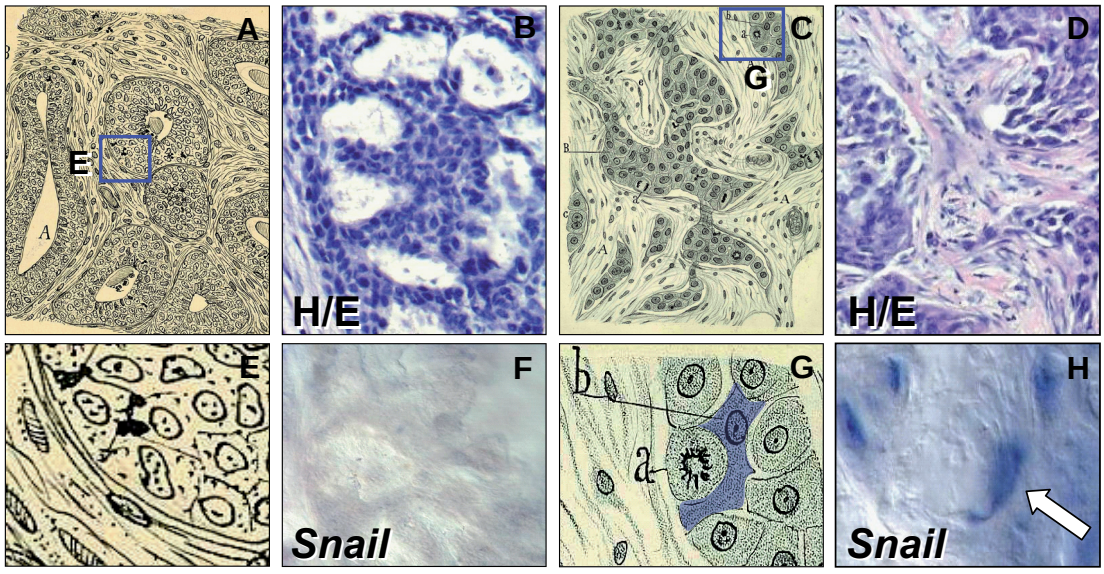

Fig. 3. From Cajal's "pear-like" cells to the *Snail*-expressing cells in tumors. *Cajal's drawing of human breast carcinoma biopsies.* **(A,E)** *Differentiated and undifferentiated ductal breast carcinomas, respectively.* **(C,G)** *Details of these drawings to better visualize the morphology of cells. The letters in* **(G)** *indicate a mitotic cell (labeled "a" by Cajal) and a fusiform or pear-like cell, described as invasive and capable of migrating due to the lack of the cellular cement (labeled "b" and shaded in blue). Adapted from Figs. 68 and 62 in (Ramón y Cajal, 1900). Histological staining* **(B,D)** *and* in situ *hybridization* **(F,H)** *showing that* Snail *is expressed in dedifferentiated breast carcinomas. Note that the labeled cells closely resemble those described by Cajal. Pictures adapted from Blanco* et al., *2002.*

tubular EMT and the development of fibrosis in transgenic mice (Fig. 1E) and pathological Snail expression is observed in the fibrotic areas of human kidneys (Boutet *et al.,* 2006). Until recently, renal fibrosis was thought to be provoked by the activation of interstitial fibroblasts that deposit an excess of collagen fibers. However, recent studies have shown that renal tubular epithelial cells also undergo EMT (Iwano *et al.,* 2002). Furthermore, Snail is upregulated during the EMT suffered by hepatocytes (Valdés *et al.,* 2002) and mesothelial cells in patients treated with peritoneal dialysis (Yañez-Mo *et al.,* 2003). Thus, it appears that *Snail* genes must be maintained silent in the adult. Snail activity is not only regulated at the level of transcription but its subcellular localization is also subject to strict control (Dominguez *et al.,* 2003; Zhou *et al.,* 2004). Epigenetic mechanisms are likely to be fundamental in silencing Snail since an increase in its expression can be attributed to promoter demethylation, and such increases have been correlated with the invasive properties of carcinoma cell lines (Fraga *et al.,* 2004). Interestingly, this reactivation of Snail can be considered—as a return to the embryonic cellular state since it fulfills the same function in the adult as in the embryo, the induction of EMT.

The EMT in tumor progression: A hundred years later?

Although the magnificent contribution of Santiago Ramón y Cajal to modern Neurobiology has been well acknowledged, not that many scientists are aware that he also made significant contributions in other fields. As well as his scientific publications he also wrote some extremely successful books, the best known of which is the *"Textura del sistema nervioso del hombre y los vertebrados"* first published in Spanish in 1899. However, he also wrote an excellent and comprehensive Manual of Pathological Anatomy for which he generated all the histological slides and drawings. It was in this book that he described the histopathology of many diseases in detail including an extremely interesting chapter on carcinomas. His description of the cells in mammary tumors is not only extremely detailed but also, it is illuminating and far ahead of its time in terms of his ideas about tumor malignancy. When describing the characteristics of an invasive breast tumor he mentions: "*The epithelial islands are not surrounded by a basement membrane.. We must mention the fusiform, pear-like and star-like forms.. These cells are not attached to each other.. This explains their invasive ability*" (Ramon y Cajal, 1900). Even today, it would be difficult to better describe the epithelial cells that acquire invasive properties. These cells were superbly illustrated by Cajal and Fig. 3 includes some drawings adapted from Figures 62 and 68 of the third edition of his Manual, published in 1900. It is fascinating to see how accurately he illustrated both a differentiated and an undifferentiated and invasive human breast carcinoma (Fig. 3A and C, respectively). Interestingly enough, in infiltrating ductal breast carcinomas (IDC) Snail expression is inversely correlated with the degree of differentiation (Blanco *et al.,* 2002). What is more, the Snail-expressing cells in these tumors are very reminiscent in location and shape to those described by Cajal more than 100 years ago (Fig. 3G-H). Thus, it seems that Cajal "understood" the process of EMT many years before its significance in tumor malignancy was established.

Old views and new perspectives

Contrary to the long established concept of the role of EMT in the formation of embryonic tissues, the significance of EMT in metastasis remains a matter of debate (Tarin, 2005). As a matter of fact, there is still little convincing evidence that this process

occurs in human tumors. In the first place, EMT is likely to be a focal event at the initial stages of tumorigenesis and in addition, it is a dynamic process making the visualization of malignant cells with a migratory mesenchymal phenotype extremely difficult. It is very clear that for a metastasis to form, cells must colonize a distant site. During embryonic development, migratory mesenchymal cells cease their migration and differentiate when they have reached their destination (Sefton *et al.,* 1998). They concomitantly downregulate *Snail* expression and undergo MET, losing their mesenchymal phenotype. This makes it also very difficult to see them in the mesenchymal state, but it is in agreement with the re-expression of E-cadherin observed in axillary lymph node metastases (Bukholm *et al.,* 2000) and in some experimentally generated metastases (Mareel *et al.,* 1991).

The recent development of intravital multiphoton microscopy together with novel approaches in the use of fluorescent cell markers has enabled the initial steps of tumour dissemination to be analysed *in vivo* in animal models. Accordingly, single carcinoma cells that have lost their epithelial polarity can be seen to migrate out of primary tumors (Wang *et al.,* 2002; Condeelis and Segal, 2003; Fig. 1D). These data provide the first direct evidence of EMT at the initial stages of the metastatic cascade *in vivo*.

Another interesting concept that should be addressed is the proliferative state of the malignant cells that delaminate from the primary tumor. At first sight, one always thinks of cancer being associated with high rates of proliferation. However, there is little proliferation at the invasive front of carcinomas (Jung *et al.,* 2001) and the dramatic cytoskeletal changes that take place during EMT are probably incompatible with cell division (Barrallo-Gimeno and Nieto, 2005). Indeed, cells transiently stop dividing before undergoing migration during embryonic development. Thus, although unregulated proliferation is fundamental for tumors to form and grow, this is not the case during their malignant phase. Interestingly, Snail blocks cell cycle progression by repressing the expression of the *Cyclin D* gene and increasing the expression of the cell cycle inhibitor p21 (Vega *et al.,* 2004). The capacity to visualize fluorescent cells disseminating from tumors has also enabled them to be isolated and purified, permitting their molecular signature to be defined. Significantly, such analyses have demonstrated that invasive cells are not proliferative (Condeelis *et al.,* 2005) indicating that the proliferation of tumor cells can be dissociated from malignancy.

Finally, another property associated to Snail-induced EMT is the resistance of cells to apoptosis or cell death. Indeed, these cells become resistant to the loss of survival factors, the action of direct apoptotic stimuli and to genotoxic stress (Inoue *et al.,* 2002; Perez-Losada *et al.,* 2003; Vega *et al.,* 2004, Kajita *et al.,* 2004). Thus, Snail confers a selective advantage to embryonic cells migrating towards their final destination and to invasive malignant cells in their attempts to disseminate and form metastasis. Again, the *in vivo* purification of disseminating cells has confirmed that these cells are resistant to conventional chemotherapy (Goswami *et al.,* 2004).

In summary, we have significantly advanced our knowledge in the last decade about the cellular processes that have been hijacked from normal developmental networks and aberrantly employed in adult pathologies. The combination of sophisticated animal models and the development of fluorescent probes and nanodevices that can be incorporated into state-of-the-art intravital microscopes seem to offer promise in our fight against one of the most devastating aspects of cancer, the metastatic process, as well as against degenerative organ diseases.

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