Snail, ZEB and bHLH factors in tumour progression: an alliance against the epithelial phenotype?

Héctor Peinado, David Olmeda and Amparo Cano

Abstract | The molecular mechanisms that underlie tumour progression are still poorly understood, but recently our knowledge of particular aspects of some of these processes has increased. Specifically, the identification of Snail, ZEB and some basic helix-loop-helix (bHLH) factors as inducers of epithelial–mesenchymal transition (EMT) and potent repressors of E-cadherin expression has opened new avenues of research with potential clinical implications.

Tumour invasion
Inasive tumour cells are able to dissociate and emigrate from primary tumours into adjacent tissues.

Epithelial–mesenchymal transition
The cellular and molecular processes by which epithelial cells lose cell–cell interactions and apico-basal polarity at the same time as acquiring mesenchymal and migratory properties. EMT has a fundamental role in specific developmental stages under strict spatio-temporal regulation.

Intravasation
The process by which tumour cells penetrate the blood or lymphatic vessels, allowing their eventual dissemination to distant organs.

Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Madrid (UAM), Instituto de Investigaciones Biomédicas 'Alberto Sols' CSIC-UAM, Arturo Duperier 4, 28029 Madrid, Spain.
Correspondence to A.C.
e-mail: acano@iib.uam.es
doi:10.1038/nrc2131
Published online 17 May 2007

Local invasion can be considered an initial and essential step in the malignancy of carcinomas, leading to the generation of usually fatal distant metastasis. Tumour invasion appears to be controlled by a coordinated series of cellular and molecular processes that enable tumour cells to dissociate and migrate from the primary tumour. The changes in cell adhesion and migration during tumour invasion are reminiscent of an important developmental process termed epithelial–mesenchymal transition[1,2] (EMT; FIG. 1), a process that also has an active role in other stages of the metastatic cascade such as intravasation[3]. One of the hallmarks of EMT is the functional loss of E-cadherin (encoded by *CDH1*), which is currently thought to be a suppressor of invasion during carcinoma progression[4]. The characterization of E-cadherin regulation during malignant progression has provided important insights into the molecular mechanisms implicated in tumour invasion. In particular, transcriptional repression has recently emerged as a fundamental mechanism for the dynamic silencing of *CDH1* during tumour progression. Indeed, several transcription factors that strongly repress *CDH1* (such as members of the Snail, ZEB and basic helix-loop-helix (bHLH) families) are now thought to be involved in tumour progression, thus having potential clinical interest[5]. Nevertheless, the specific role of these different repressors in tumorigenesis is not fully understood. Despite the increasing evidence that EMT occurs *in vivo*, the influence of EMT in human tumours is currently a matter of controversy[6,7]. In this Review, we will focus our attention on the role of Snail, ZEB and bHLH factors in the regulation of epithelial cell plasticity during tumour progression. We will summarize recently identified signalling pathways, transcriptional mechanisms and target genes, as well as new functions assigned to these factors and their expression profile in human tumours. Finally, we will discuss the potential interplay between these factors during tumour progression, and how the tumour microenvironment governs the action of these factors in suppressing the epithelial phenotype.

Factors that repress E-cadherin

The characterization in 2000 of the zinc-finger factor snail (Snail homologue 1, hereafter called SNAI1 (REF. 8)) as a transcriptional repressor of *CDH1* and an inducer of EMT[9,10] was an important breakthrough, providing new insights into the molecular mechanisms of tumour invasion. Since then, other *CDH1* repressors and EMT inducers have been described. Because of the importance of these factors during tumour progression, a huge effort has been made to understand how these factors behave. SNAI1 and SNAI2 (also known as slug)[11,12] belong to the Snail superfamily of zinc finger transcriptional repressors that participate in developmental EMT (BOX 1) and other processes[8]. ZEB1 (also known as TCF8 and δEF1) and ZEB2 (also known as ZFXH1B and SMAD interacting protein 1 (SIP1)), two members of the ZEB family (BOX 2), have also emerged as key factors that regulate E-cadherin and the induction of EMT[13,14] and are also implicated in the malignancy of different human tumours. Similarly, there is increasing evidence that some bHLH factors, as well as the Id HLH subfamily (BOX 3), are important during tumour cell invasion and metastasis. These transcription factors are members of a large family of proteins that have

At a glance

- Local tumour invasion represents the first step of the metastatic cascade of carcinomas, and requires profound changes in the cell adhesion and migration properties of tumour cells that are reminiscent of developmental epithelial–mesenchymal transition (EMT). EMT is thought to be a dynamic and transient process, and as such is a manifestation of epithelial cell plasticity during tumour progression.
- The loss of functional E-cadherin is a hallmark of EMT and carcinoma cell invasiveness. Transcriptional repression mediated by factors from the Snail (SNAI1 and SNAI2), ZEB (ZEB1 and ZEB2) and basic helix-loop-helix (bHLH: E47 and TWIST) families is a basic mechanism for the dynamic silencing of *CDH1* (the gene that encodes E-cadherin).
- Post-transcriptional modifications are emerging as a meaningful additional level of regulation of various repressors. The protein stability, nuclear localization and functional activity of SNAI1 seem to be controlled by a delicate balance between phosphorylation, zinc transporter proteins and interaction with lysyl-oxidase-like proteins.
- Besides *CDH1*, additional direct and indirect target genes of Snail, ZEB and bHLH factors are being described that encode proteins involved in EMT as well as in cell proliferation, cell survival or angiogenesis, indicating that these factors have additional functions beyond the repression of *CDH1* and the induction of EMT.
- Snail and bHLH factors have recently been implicated in cell-survival and acquired resistance to genotoxic agents by cancer cells, providing new insights into the biological properties conferred by these factors, with potential clinical implications.
- The expression patterns of Snail, ZEB and bHLH factors in different human carcinomas, together with functional studies, indicate that the various factors have different roles during tumour progression, with a more prominent role for SNAI1 in the induction of EMT in primary tumours, whereas the other factors are involved in maintaining the migratory phenotype.
- Complex signalling networks from the tumour microenvironment, including hypoxia and transforming growth factor β (TGFβ), can coordinate the expression and/or function of Snail, ZEB and bHLH factors, and promote their interplay in orchestrating *CDH1* repression and malignant tumour migration.

historically been implicated in lineage determination, of which E47 (encoded by the *E2A* gene, also known as TCF3), TCF4 (also known as E2-2) and TWIST1 act as *CDH1* repressors and inducers of EMT[15,16] (V. Sobrado and A.C., unpublished data). Therefore, it is important to understand the cellular and molecular mechanisms that regulate the expression and function of these factors in both physiological and pathological situations.

Signalling pathways that regulate* CDH1 *repressors. Snail proteins are involved in development through facilitating EMT, so many of the signalling pathways that regulate their expression have been described. During development, EMT is induced both in invertebrate and vertebrate systems by receptor tyrosine kinases (RTKs, which are activated by different signals, such as fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and epidermal growth factor (EGF)), the transforming growth factor β (TGFβ)–bone morphogenetic protein (BMP) pathway and Wnt signalling (see FIG. 2a for an overview)[1,2,8,17]. Studies in cell lines and transgenic mouse models have confirmed that similar signalling pathways regulate SNAI1, and to a lesser extent SNAI2, in EMT associated with carcinogenesis (reviewed in REFS 1,8,18,19). Significantly, there is cross-talk between some of these pathways, such as TGFβ, RTKs–Ras, Notch, hedgehog and/or Wnt or β-catenin pathways[20–24]. In particular, many of these pathways coordinate *CDH1* repression by SNAI1, or collaborate with β-catenin signalling in the induction of EMT[20,25] or SNAI2 expression, both during avian and *Xenopus* development and in tumour cell lines[26–28]. Recently, the high-mobility group protein HMGA2 has been proposed as a major integrator of TGFβ-mediated EMT in human cell lines, coordinating the induction of SNAI1, SNAI2, TWIST and the repression of *ID2* (REF. 29).

In recent years additional signals have emerged that participate in the regulation of *CDH1* repression in mammals (FIG. 2a), such as the induction of SNAI1 and ZEB1 expression by prostaglandin E2 (PGE2)[30,31]. Autocrine or paracrine signals can also induce the expression of the Snail genes in different systems, including endothelin 1, vascular endothelial growth factor (VEGF), autocrine motility factor (AMF), stem cell factor (SCF)–KIT, RAF1, calreticulin, hypoxia, reactive oxygen species or laminin 5 (REFS 32–40). Interestingly, although some of these pathways also induce TWIST (VEGF and Wnt) or ZEB factors (TGFβ–BMP), others appear to be specific to SNAI1 and SNAI2 or ZEB factors (FIG. 2a). The regulation of *CDH1* repressors by steroid and related receptors has been analysed in some hormone-associated cancer cells. For example, in prostate cancer cells, the androgen analogue dihydrotestosterone (DHT) can induce SNAI2 and to a lesser extent SNAI1 expression[41]. Signalling through the oestrogen receptor (ER) negatively regulates SNAI1 expression in breast cancer cells (FIG. 2a) through a mechanism involving metastasis-associated gene 3 (MTA3), a subunit of the nucleosome remodelling and histone deacetylation (NuRD) transcriptional co-repressor complex. Therefore, the loss of MTA3 or ER and SNAI1 upregulation is associated with a poor prognosis in breast cancer[42]. By contrast, ZEB1 can be induced by oestrogen signalling cascades[43], although the biological significance of this finding in tumorigenesis remains unknown.

Comparative analysis of *SNAI1* and *SNAI2* promoters from different species has identified conserved and functional response elements[18], such as AP1 and AP4 sites, SMAD-binding elements, LEF1 binding sites and two conserved E-boxes[21,22,28,44–46]. Recent studies have shown that SNAI1 binds to and represses its own promoter, indicating the existence of an autoregulatory loop[44]. In addition, a negative-feedback mechanism was recently described for the regulation of SNAI1 by scatter factor (also known as hepatocyte growth factor, HGF) involving the mitogen activated protein kinase (MAPK) target protein early growth response 1 (*EGR1*), which is also a SNAI1 target gene[47] (TABLE 1). Surprisingly, avian SNAI2 has the ability to self-activate during neural crest development following inductive signals from a BMP–SOX2–protein kinase A (PKA) pathway[27]. Importantly, this latter work proposes an activator function for SNAI2, in contrast to the assumed function of Snail factors as repressors[48] (BOX1).

E-cadherin
The major calcium-dependent cell–cell adhesion molecule, functionally organized in cadherin–catenin complexes at adherens junctions and essential for the establishment of embryonic epithelium and the homeostasis of adult epithelial tissues. The functional loss of E-cadherin occurs in most carcinomas associated with a high tumour grade and invasiveness.

Epithelial cell plasticity
Progressive changes occurring in the gene-expression programmes that correspond with the diverse phenotypic manifestations that are seen as cells progress from an epithelial cell type to a complete mesenchymal phenotype and *vice versa*.

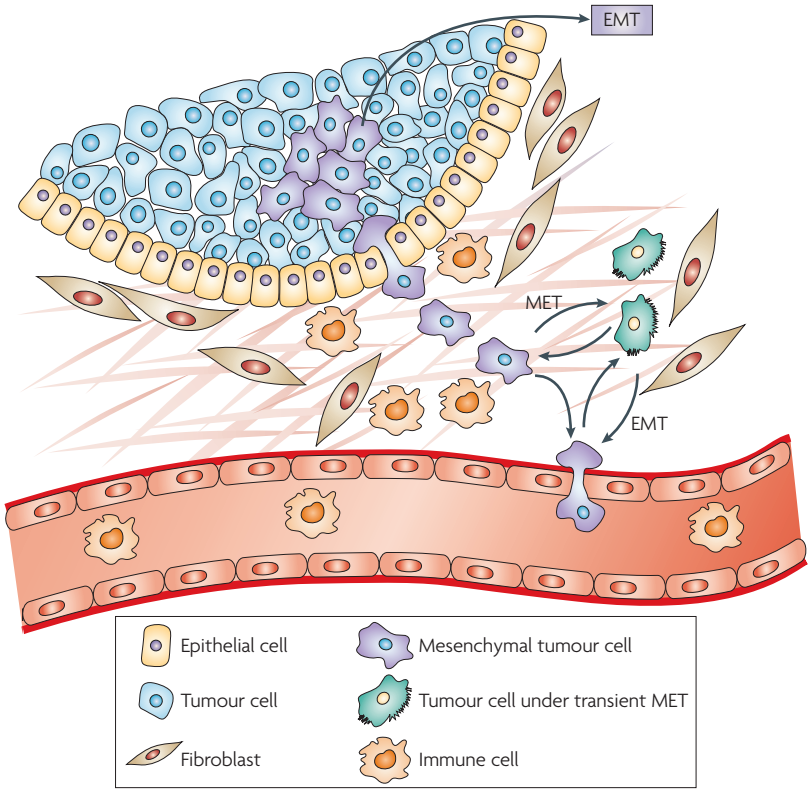


Figure 1 | **EMT as a dynamic process and a manifestation of epithelial plasticity.** Schematic diagram of the main cell properties affected in a transient versus stable epithelial–mesenchymal transition (EMT) process. Cells that underwent EMT during tumour invasion are characterized by the loss of cell–cell adhesion and polarity accompanied by cytoskeleton rearrangements and increased cell motility. Sometimes, cells that had previously undergone EMT could transiently re-acquire an epithelioid phenotype by reverse mesenchymal–epithelial transition (MET) as the result of new interactions with the tumour microenvironment. EMT could also have an active role during metastatic spreading by promoting other malignant properties such as tumour cell intravasation[16], and stable MET can occur at established secondary metastases[2].

Apart from some of the signals indicated above, little information is available regarding the specific regulation of bHLH and ZEB factors during tumour progression (FIG. 2a). The regulation of the expression of Id factors might influence that of bHLH factors, as Id proteins can repress the activities of bHLH class I proteins (BOX 3). Indeed, Id proteins may be induced in response to several inducers of EMT and/or oncogenic pathways in mammalian cell systems, such as TGFβ–BMP, VEGF or insulin-like growth factor 1 (IGF1), as well as by activated Ras, β-catenin or phosphotidylinositol-3 kinase (PI3K) (reviewed in REFS 49,50) (FIG. 2a). Therefore, they may, through their dominant negative action, impinge on the activity of E47 factors. With regards to ZEB factors, ZEB2 can modulate TGFβ signalling and influence TGFβ-mediated EMT[51] in different contexts.

Dynamic repression mechanisms. The mechanisms through which different repressors control *CDH1* and other genes during EMT are being uncovered (FIG. 2b). The modification of chromatin structure has emerged as a major regulatory event promoted by these proteins during EMT[52,53], and pioneering analysis of SNAI2 repression highlighted the involvement of histone deacetylases (HDACs)[54]. Accordingly, we defined a model for SNAI1 repression of *CDH1* involving the direct recruitment of a repressor complex formed by the co-repressor SIN3A, and HDAC1 and HDAC2 (REF. 55) (FIG. 2b). However, other co-repressors can also regulate the activity of the Snail proteins, like the C-terminal binding protein CTBP1, as first characterized in *Drosophila melanogaster*[48]. SNAI2 also represses *BRCA2* through CTBP1 and HDAC1 in human breast cancer cell lines[56], and SNAI2 can cooperate with CTBP1 and CTBP2, and HDAC1 and HDAC3 to repress *CDH1* through a mechanism that remains to be elucidated (V. Bolós and A.C., unpublished data) (FIG. 2b). This interaction seems to be indirect, as the CTBP-binding sequence is not conserved in vertebrate *SNAI2* (REF. 48) and SNAI2 does not directly bind to either CTBP[57]. Studies in several cancer cell lines show that CTBP co-repressor complexes may also collaborate in the repression of *CDH1* by ZEB factors[58], although the ZEB2–CTBP interaction is dispensable for *CDH1* repression[59]. Importantly, CTBP and p300 co-regulator levels seem to be crucial in controlling *CDH1* expression in colorectal tumours because they can regulate ZEB1 repressor or activator activity[60], as has previously been shown for ZEB factors in mammalian cells and during *Xenopus* development[61]. The activity of CTBP co-repressor complexes can be further modified by interaction with other co-factors, such as PNN, that relieve CTBP1-mediated *CDH1* repression[62] (FIG. 2b). Notably, protein acetylation and sumoylation can modulate the efficiency with which CTBP represses *CDH1*, as these protein modifications alter either the nuclear localization of CTBP or its interactions with ZEB2 (REFS 63,64) (FIG. 3c). Furthermore, CTBP activity is regulated by NADH levels[65]. Unfortunately, so far the mechanisms involved in the E47 and TWIST-mediated repression of *CDH1* remain largely unknown. However, although the information available points to heterodimerization with Id regulators[29,66] (E. Cubillo and A.C., unpublished data), other mechanisms cannot be ruled out.

The fact that SNAI1, SNAI2, ZEB1 and ZEB2 recruit specific chromatin-remodelling complexes supports a dynamic link between transcriptional repression and the epigenetic gene silencing of *CDH1* during tumour progression and EMT[52]. Whether these factors also control other genes by this mechanism during EMT remains to be established.

Post-transcriptional regulation. Important advances have been made recently in understanding the post-transcriptional regulatory mechanisms that affect EMT regulators such as SNAI1 (FIG. 3a). The subcellular localization of SNAI1 can be modulated by phosphorylation[67] involving the p21-activated kinase 1 (PAK1)[68]. Interestingly, the nuclear localization of SNAI1 in zebrafish embryos can also be controlled by the expression of the zinc transporter LIV1, which lies downstream of signal transducer and activator of transcription 3 (STAT3)[69]. On the other hand, glycogen synthase kinase 3β (GSK3β) phosphorylation of the

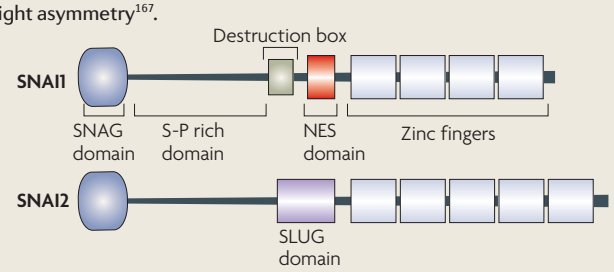
E-boxes
Recognition sequences for basic helix-loop-helix (bHLH) factors and other transcription factors like Snail and ZEB. They are formed by the consensus palindromic sequence CANNTG.

Box 1 | **Snail factors**

The Snail superfamily is divided into the Snail and Scratch families, with three members of the Snail family having been described in vertebrates to date: SNAI1, SNAI2 and SNAI3 (REF. 8). Members of the Snail family are zinc-finger transcription factors that share a common organization: a highly conserved C-terminal region, containing four–six zinc fingers (C_2H_2 type) and a divergent N-terminal region (see figure). The zinc fingers function as the sequence-specific DNA-binding domains that recognize consensus E2-box type elements C/A(CAGGTG)[17]. Snail factors are currently thought to be transcriptional repressors[48]. Their repressor capacity is dependent on the SNAG domain[55]: 7–9 amino acids in the N-terminal part of the protein that are conserved between Snail and growth factor independence (Gfi) proteins.

The central region of the Snail proteins have a serine–proline-rich region that is highly divergent between Snail members. SNAI2 proteins contain the so-called slug domain in this region, the function of which remains elusive. By contrast, two different functional domains have been identified in the central region of SNAI1 proteins: a regulatory domain containing a nuclear export signal (NES)[67] and a destruction box domain[70]. The phosphorylation of proline/serine residues in both regions and potential modification of adjacent lysine residues has been implicated in the subcellular localization of SNAI1, protein stability and repressor activity[67,70,72].

SNAI1 is expressed during mesoderm formation, gastrulation and neural crest development, as well as in most developmental processes in which epithelial–mesenchymal transition (EMT) is required[2,17]. *SNAI2* expression has been associated with mesoderm and migratory neural crest cells, as well as in other tissues not always associated with EMT[8]. SNAI1 is essential for mouse gastrulation[166], and SNAI1 and SNAI2 for neural crest development in frog and avian embryos, respectively[17]. However, they seem not to be essential for mouse neural crest formation, but are instead involved in left–right asymmetry[167].



Comparative scheme of the main structural domains found in mammalian SNAI1 and SNAI2.

SNAI1 nuclear export signal (NES) and destruction box (BOX 1) provokes its cytoplasmic export and ubiquitin-mediated proteasome degradation[70,71]. In addition to phosphorylation, other interactions also appear to influence the stability of SNAI1, such as cooperation with lysyl-oxidase like 2 and 3 (LOXL2 and LOXL3) to repress *CDH1* and induce EMT[72]. Interestingly, LOXL2 stabilizes SNAI1 by regulating its interaction with GSK3β, supporting a model in which SNAI1 activity is balanced by opposing signals[73] (FIG. 3a). Less is known about the post-transcriptional regulation of SNAI2, although a recent study in *Xenopus* indicates that the partner of paired (Ppa) protein modulates its stability and degradation[74] (FIG. 3b). Unfortunately, even less is known about the post-transcriptional regulation of ZEB factors. Although the repressor activity of ZEB2 seems to be impaired by PC2-mediated sumoylation, preventing its interaction with CTBP[63] (FIG. 3c), the functional consequences for tumour progression remain to be established.

Post-translational regulation of bHLH factors is more complex. The specific expression pattern of particular members regulates their capacity to form functionally active or inactive heterodimers in specific cells or tissues (BOX 3). Phosphorylation also influences bHLH activity (FIG. 3d) and the p38 kinase or MAPK-activated protein kinase 2 (MAPKAPK2) regulate the homo and heterodimerization and/or DNA-binding capacity of E47 (REFS 75,76). *E2A* gene products (E12 and E47) are also targets for G1 cyclin-dependent kinases (CDKs) regulating B-cell growth and survival during development[77]. Interestingly, extracellular signal-regulated kinase (ERK)-mediated phosphorylation of E47 controls its degradation in response to Notch signalling during lymphocyte differentiation[78] (FIG. 3d). These different regulatory mechanisms might explain the pleiotropic effects described for E47 in different cell, tissue and tumour contexts.

Gene targets: somewhere beyond E-cadherin. The *CDH1* repressors share several target genes involved in EMT. However, each repressor can also confer certain specificity to the process by differentially regulating a subset of EMT- and/or tumour-related genes[79,80]. Functional and gene-expression studies of *CDH1* repressors have shown that they act as molecular triggers of the EMT programme by repressing a subset of common genes that encode cadherins, claudins, cytokeratins, integrins, mucins, plakophilin, occludin and ZO proteins that could be classed as an epithelial cluster of molecules mainly targeted to

Box 2 | **Zeb family factors**

The ZEB family of transcription factors contains two members (ZEB1, also known as δEF1, and ZEB2, also known as SIP1) encoded by independent genes (*ZFHX1A* and *ZFHX1B*, respectively). They are characterized by the presence of two zinc-finger clusters at each end and a central homeodomain. ZEB1 and ZEB2 contain 3 or 4 zinc fingers of the C2H2 and C3H type in each cluster (see FIG. 2,3). Apart from the homeobox, other domains can be found in members of this family, such as the the CTBP-binding sequence or the SMAD-interacting domains in ZEB2 (REFS 61,102). ZEB factors interact with DNA through the simultaneous binding of the two zinc-finger domains to high-affinity binding sites composed of bipartite E-boxes (CACCT and CACCTG), as found in the *CDH1* promoter, but whose orientation and spacing can vary in different targets[13,14,102]. The specific transactivation domains have not yet been defined. ZEB factors can transactivate through the recruitment of either co-activators (PCAF or p300 for ZEB1) or co-repressors (CTBP for ZEB2)[61].

ZEB factors are expressed during development in the central nervous system, heart, skeletal muscle and haematopoietic cells. In these tissues, a functional deficiency in one of these factors can be partially compensated by the other, indicative of a common role for both factors[168]. However, the *Zeb2* knockout mouse is embryonic lethal with specific defects in neural crest emigration that can not be compensated for by *Zeb1* (REF. 169). Major differences are found in the expression pattern of both factors in lymphocytes, with a predominant expression of ZEB1 in the thymus during T-lymphocyte development and of ZEB2 in spleen B cells[168].

Box 3 | **bHLH family factors**

The basic common structure for all helix loop helix (HLH) family members involves two parallel amphipatic α-helices joined by a loop required for dimerization. This structure can be found alone, as seen in the Id protein family, or accompanied by a basic domain (bHLH; FIGS 2,3). Additional regulatory domains can be found in some family members, such as a leucine zipper domain (MYC) or a PAS domain (bHLH–PAS, hypoxia inducible factor 1α (HIF1α))[170].

bHLH proteins bind to DNA using a consensus E-box (CANNTG) site as homo- or heterodimers[171] (FIGS 2,3).

Transcriptional transactivation activity has been mapped to the AD1 domain, at the N-terminal half of some bHLH proteins, and a second activation domain can also be found in some bHLHs[172]. In some cases, bHLH proteins can act as transcriptional inducers or repressors by the recruitment of histone acetyl transferase (HAT) proteins, such as p300 or the SPT–ADA–GCN5–acetyltransferase (SAGA) complex, or co-repressors such as groucho or SIN3A[170].

The HLH family members have been classified into seven families according to their tissue distribution, dimerization capacities and DNA-binding specificities (reviewed in REF. 170). With regards to epithelial–mesenchymal transition (EMT), the most representative belong to class I, also known as E-proteins, encoded by *TCF3* (E12 and E47 isoforms), *TCF4* (E2-2A and E2-2B isoforms) and *TCF12* (α/β isoforms). They are widely expressed and act as homodimers or heterodimers with class II proteins. Class II factors are tissue-specific bHLH proteins that always act as heterodimers with class I factors, among which TWIST1 and TWIST2 can be found. Class V HLHs, known as Id proteins (Id1–4), lack the basic domain and so are unable to bind DNA. Ids act as class I and class II dominant-negative factors because of their high heterodimerization affinity with class I bHLHs. bHLH heterodimers are involved in cell lineage determination and the control of cell proliferation, whereas Id proteins are key regulators of a wide range of developmental and cellular processes, including cell-cycle regulation, proliferation and angiogenesis[49,50,170].

promote EMT (TABLE 1). More importantly, chromatin immunoprecipitation assays (ChIP) indicate that some of these repressed genes are direct targets of these repressors. For example, in human cancer cells ZEB2 binds directly to plakophilin 2 and ZO3 promoters[81], whereas SNAI1 has been shown to bind cytokeratins 17 and 18, vitamin D receptor (*VDR*), occludin, hepatocyte nuclear factor 4α (HNF4α), HNF1β, *EGR1*[47,82–86], as well as its own promoter[44]. Similarly, SNAI2 directly binds to the occludin promoter[36], and SNAI1 and SNAI2 have also been found to bind promoters that encode proteins related with cell survival or apoptosis, such as p53, BID, DFF40, PUMA and BRCA2 (REFS 56,86,87) (TABLE 1, see below).

Regarding the genes controlled by each repressor, SNAI1 has been shown to regulate several genes potentially implicated in different processes during tumorigenesis, such as cyclin D2, proliferating cell nuclear antigen (PCNA), skeletrophin, prostaglandin dehydrogenase (HPGD), ATPaseβ1 or cadherin 16 (REFS 30,82,88–91). Similarly, SNAI2 controls other genes, such as integrin α3, collagen 2α1 in human cell lines as well as itself, as has been shown during avian neural crest formation[45,92,93]. Specific gene regulation has also been observed in microarray studies showing that Snail and E47 factors have different roles during EMT in epithelial cell lines[79,84]. Nevertheless, in some specific contexts these transcription factors could also share the control of common target genes, albeit in opposing ways[79,94].

Although all the aforementioned factors were initially characterized as *CDH1* repressors, the upregulation of mesenchymal genes observed after their expression indicates that they might also act as gene inducers[95]. Indeed, ZEB factors have been described as transcriptional activators in some specific contexts[43,96]. It is noteworthy that TWIST can induce N-cadherin[97] expression in prostate cancer cells, whereas E47 induces INK4a (encoded by *CDKN2A*), p21 (encoded by *CDKN1A*) and could be involved in the induction of α-smooth muscle actin in different cell systems[94,98,99]. As Snail factors have generally been thought to be transcriptional repressors[48], the mechanisms by which they induce mesenchymal genes remain largely unknown, and seem to be indirect and/or context-dependent. In fact, SNAI1, ZEB2 and TGFβ indirectly upregulate matrix metalloproteinase 2 (MMP2) expression through the ETS1 transcription factor[100], and the induction of MMP9 by mouse SNAI1 involves activated MAPK and the SP1 and ETS factors[101]. Interestingly, we recently detected a cluster of mesenchymal genes similarly upregulated by SNAI1, SNAI2 and E47 factors in Madin-Darby canine kidney (MDCK) cells, including genes that encode several collagens and ECM-related proteins, such as SPARC, plasminogen activator inhibitor 1 (PAI1) and tissue inhibitor of metalloproteinase 1 (TIMP1)[79]. These data suggest that at least in some specific backgrounds, additional markers could be used to track EMT independently of the leading cause, opening the door to future studies to define the 'genetic signature of EMT'.

We can speculate about the hierarchy of Snail, ZEB and bHLH repressors during EMT, irrespective of their expression pattern and interactions[53]. A binding affinity strategy is a possible mechanism through which these factors might target specific promoters. Although all factors bind to highly related E-box elements (BOX 1,2,3), SNAI1 and ZEB2 seem to recognize E-boxes with higher affinity than SNAI2, E47 and ZEB1, respectively, in both cellular and developmental studies[11,13,51]. Interestingly, the requirement of bipartite E-boxes for ZEB2 to bind to DNA suggest that ZEB factors could also use a shield strategy, blocking the access of other transcription factors to some specific promoters in *Xenopus* embryos[102]. Undoubtedly, complete knowledge of both the specific E-box sequences recognized and the binding strategies of each factor will be useful in the future to define new potential therapeutic targets during EMT and tumour progression.

Snail, bHLH and ZEB factors in cancer

Expression of repressors in cancer. Since SNAI1 was originally shown to be expressed in invasive carcinoma cells[9,10], these *CDH1* repressors have increasingly been detected in tumours and derived cell lines (see TABLE 2 for recent data from human tumours). The expression of SNAI1 in breast carcinomas is associated with E-cadherin repression and metastasis[70,103–105], and with tumour recurrence and poor prognosis[106,107]. Similarly, the expression of SNAI2 has been associated with poor clinical outcome in breast and ovarian tumours[106,108], but SNAI2 expression also correlates with a partially differentiated phenotype in breast carcinoma[105], indicating that SNAI1 and SNAI2 might have different effects on the invasive behaviour of breast cancers[105,109].

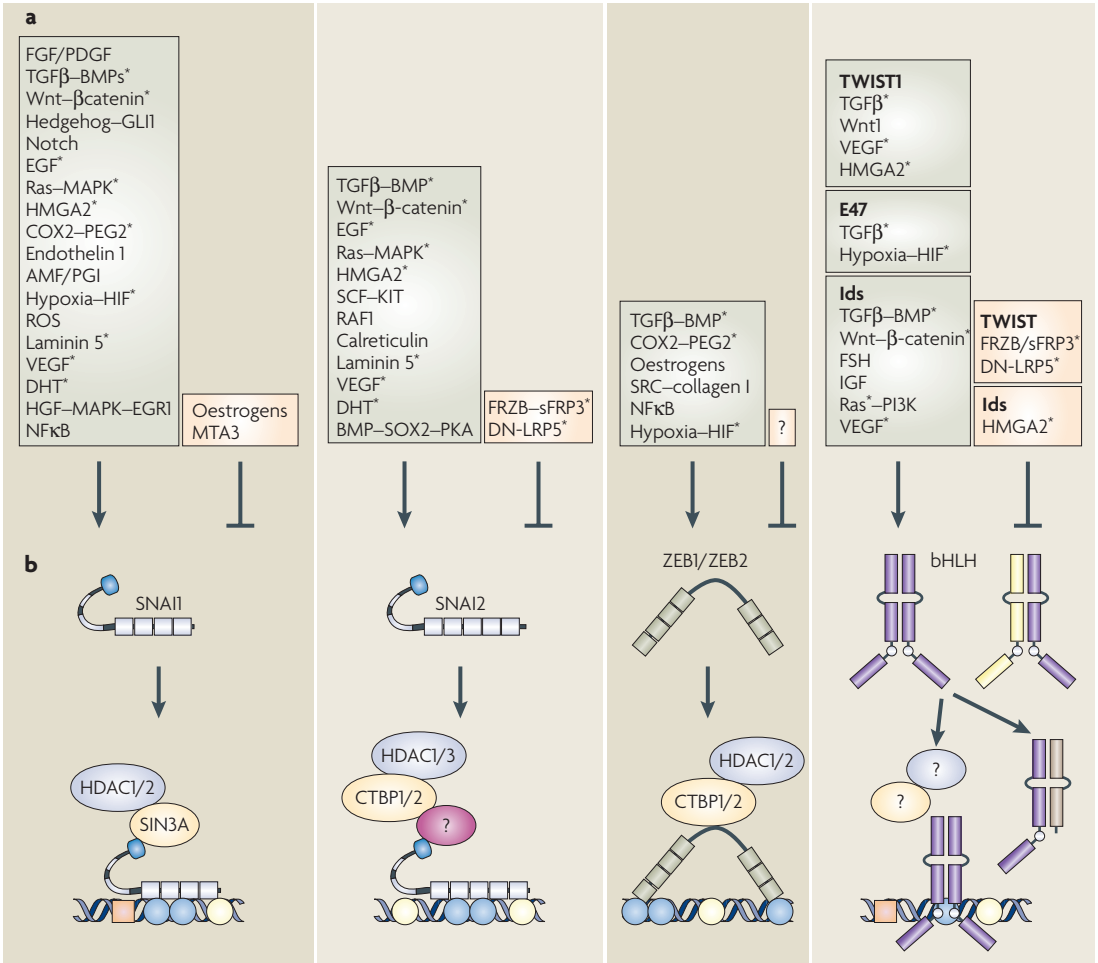


Figure 2 | **Signalling pathways and repressor mechanisms implicated in the regulation and function of Snail, bHLH and ZEB factors. a** | Overview of the main signalling pathways implicated in the regulation of the expression of Snail, bHLH and ZEB factors, underscoring the recently described pathways and the pathways that could be implicated in the regulation of several transcription factors (indicated by an asterisk). **b** | Schematic view of the main repression mechanism of these factors, representing the main co-repressor complexes or proposed interactions in the repressor mechanism of each transcription factor. AMF, autocine mobility factor; DN-LRP5, dominant negative low density lipoprotein receptor-related protein 5; FGF, fibroblast growth factor; FRZB, frizzled-related protein; FSH, follicle stimulating hormone; HMG, high mobility group; PEG2, prostaglandin E2; PGI, phosphoglucose isomerase; PKA, protein kinase A; sFRP3, secreted frizzled-related protein 3.

Therefore, although the expression of both SNAI1 and SNAI2 is associated with lymph node status, SNAI2 might participate in the cohesive migration of invasive semi-differentiated tubules of ductal carcinomas[105]. Studies in colorectal cancer indicated SNAI1 expression was associated with the downregulation of VDR and E-cadherin[60,85,110], with therapeutic implications both for vitamin D treatment in Snail-negative tumours[111] and for the likelihood of distant metastases[112]. The expression of SNAI2 in colorectal cancer is thought to be an independent marker of poor prognosis[113]. Initial studies showed *SNAI1* mRNA transcripts were associated with *CDH1* downregulation in diffuse-type gastric tumours[114], but this was not confirmed in an immunohistochemical analysis of a larger series[115].

SNAI1 expression has been associated with MMP expression and/or invasiveness in squamous-cell carcinomas (SCC) and hepatocarcinomas[116–119]. Moreover, SNAI1 has been associated with distant metastasis, *CDH1* silencing and promoter hypermethylation in oesophageal SCC[116], as well as with E-cadherin-deficient invasive areas in hepatocarcinomas[120]. Another study of oesophageal SCC reported that SNAI2 expression in primary tumours correlates with the reduced expression of E-cadherin, increased invasive behaviour, lymph node status and poor clinical outcome[121]. The expression of SNAI1 was not analysed in this study. Increased *SNAI2* mRNA expression has also been described in primary lung adenocarcinomas and is associated with shorter time to recurrence and reduced overall survival[122,123]. Remarkably, the expression of Snail factors has also been reported in synovial sarcomas as an alternative to inactivating mutations that silence *CDH1* and induce the spindle cell phenotype[124]. SNAI2 expression might also contribute to malignancy in melanomas[125].

Table 1 | **Gene targets of the *CDH1* repression factors***

Transcription factor	EMT-related genes	Other genes	References
SNAI1	*CDH1*‡; *CK17/18*‡; *SNAI1*‡; *VDR*‡; occludin‡; *HNF4α*‡; *HNF1β*‡; claudin-1/-3/-4/-7; collagen 2α1; *MUC1*; *ZEB1*§	*TP53*‡; *BID*‡; *DFF40*‡; *RAB25*‡; *PFKP*‡; *SLC27A2*‡; *EGR1*‡; *CDKN1A*; *CCND2*; *PCNA*; skelethrophin; *PGDH*; *ATPaseβ1*	9,10,30,44,47, 55,82–86,88, 92, 94,95,156
SNAI2	*CDH1*‡; occludin‡; claudin 1; integrin α3; collagen 2α1; *SNAI2*§‖; *CK8*; *CK19*	*PUMA*‡; *BRCA2*‡; *TP53*‡; *BID*‡; *DFF40*‡	11,12,27,36,56, 86, 87,92,93,156
ZEB2	*CDH1*‡; plakophilin 2‡; connexin 26‡; *ZO3*‡	Integrin α4‖	13,81,102,128
ZEB1	*CDH1*‡; *MUC1*	*CCNG2*‡§; *RBL2*‡§ (p130); integrin α4§‖	14,95,102,173
E47	*CDH1*	*CDKN2A*‡§; smooth muscle actin‡§; *CDKN1A*§	15,94,98,99
TWIST1	*CDH1*	*CDKN1A*	16,94

*Gene targets described from promoter assays and/or electrophoretic mobility assays (EMSA). ‡Genes analysed by chromatin immunoprecipitation assays (ChIP). §Upregualated genes. ‖Studies in non-mammalian systems. CCND2, cyclin D2; CDH1, E cadherin; CDKN, cyclin dependent kinase inhibitor; CK, cytokeratin; DFF, DNA fragmentation factor; EGR1, early growth response 1; MUC, mucin; PFKP, phosphofructokinase, platelet type; PGDH, hydroxyprostaglandin dehydrogenase; RBL, retinoblastoma like; SLC27A2, solute carrier family 27, member 2.

These studies indicate that SNAI1 is associated with invasion, secondary metastasis and a poor clinical outcome, as well as occasionally being expressed in distant metastases[117] (TABLE 2). However, no significant association between SNAI1 and SNAI2 and clinical parameters has been observed, and contradictory expression data for the same tumour type have been reported by different groups [103,105,106]. These discrepancies might be due to technical issues, possibly derived from the undefined specificity of most commercial anti-SNAI1 and 2 antibodies and, importantly, from the inappropriate assessment of nuclear staining and/or the discrimination between cytoplasmic and nuclear Snail stain found in most studies. Unfortunately, these drawbacks mean that the data available regarding SNAI1 and SNAI2 expression in tumour samples must be interpreted with care. In addition, although studies on the expression of Snail factors by reverse transcription PCR (RT-PCR) from whole tumour samples might overcome the uncertainties derived from immunohistochemistry, they are not suitable for differential cellular localization or detection in specific tumour regions such as invasive areas. Furthermore, additional problems with interpretation of RT-PCR data may arise from the existence of a *SNAI1P* human retrogrene with a sequence very similar to *SNAI1* but whose expression is not correlated with invasive or metastatic behaviour[126]. The recent characterization of independent anti-SNAI1 antibodies that show clear nuclear staining might more accurately assess the involvement of SNAI1 in human biopsy samples[115,127]. Interestingly, initial studies indicate that SNAI1 expression is weakly associated with SCC tumour cells, but it shows more prominent staining at the tumour–stroma interface[127], indicating that SNAI1 is expressed in restricted areas and perhaps in both tumour and reactive stromal cells, as previously indicated by *in situ* hybridization (ISH)[103,120]. It also seems plausible that stromal-positive cells represent tumour cells that have undergone EMT[127].

The expression of the ZEB and bHLH factors has also been reported in different tumour series, sometimes in parallel with analyses of Snail expression. Following initial studies on ZEB1 and ZEB2 expression[13,95], most studies in human tumours have focused on one of the two factors. Although ZEB2 expression has been analysed in ovarian, SCC, gastric and pancreatic tumours[106,109,114,128,129], ZEB1 expression has mainly been studied in colorectal tumours[60,110] and uterine cancers, where it has been associated with aggressive behaviour[130] (TABLE 2). In contrast to the behaviour of SNAI1 in breast tumours, some of these studies indicate a differential pattern of expression for ZEB and Snail factors in specific tumour types, with higher expression levels of ZEB2 in ovarian carcinoma effusions than in solid metastases[109]. Alternatively, ZEB1 seems to be involved in *CDH1* repression in colon carcinomas that lack SNAI1 (REF. 60). Although the expression of TWIST was originally associated with lobular breast carcinomas[16], this observation has not been confirmed in other series[131]. Indeed, *CDH1* is irreversibly inactivated by genetic and epigenetic alterations in most lobular breast carcinomas[132]. Nevertheless, increased TWIST expression has been associated with high-grade invasive ductal carcinomas[131] and a fatal clinical outcome[108]. The association of TWIST expression with solid metastasis has been reported in prostate cancer, oesophageal SCC and hepatocarcinomas[133–135], and is associated with poor survival in patients with endometrial tumours[136] or melanomas[137] (TABLE 2). No information is currently available on the expression of E47 in human tumour biopsy samples. Nevertheless, recent statistical microarray data analyses of breast carcinomas suggest that *E47* mRNA expression is associated with the basal subtype of breast carcinomas (E. Cubillo, G. Moreno, J. Palacios and A.C., unpublished data). In contrast to the paucity of data on bHLH factors in human samples, several studies have assessed the expression of Id factors in different tumour types. An association was made

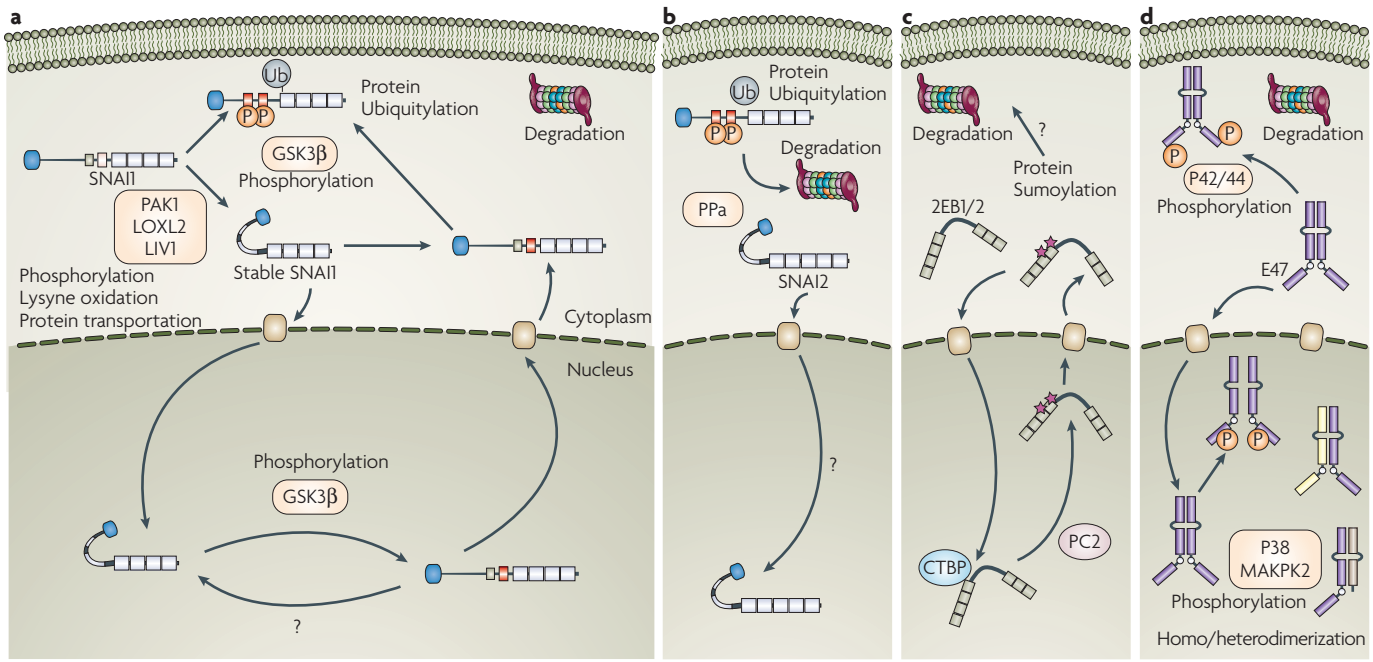


Figure 3 | **Post-transcriptional regulatory mechanisms of Snail, bHLH and ZEB factors. a** | Snail homologue 1 (SNAI1) stability and nuclear translocation can be positively modulated by p21-activated kinase 1 (PAK1), LIV1 or LOXL2. Glycogen synthase kinase 3β (GSK3β) phosphorylation promotes SNAI1 cytoplasmic export and ubiquitin-mediated proteasome degradation. The potential interconnection between the different pathways or modifications is unknown. **b** | SNAI2 stability and degradation is modulated by paired (Ppa), but import and export mechanisms remain unknown. **c** | PC2-mediated sumoylation of ZEB2 prevents its interaction with CTBP, promoting its cytoplasmic localization; the functional consequences of this for protein stability remain to be established. **d** | The expression patterns of the bHLH proteins regulates their capacity to form functionally active or inactive heterodimers in specific cells or tissues. Phosphorylation by p38 kinase and MAPKAPK2 regulates the homo/heterodimerization and/or DNA binding capacity, and the p42 and p44 kinases are implicated in bHLH degradation in response to Notch signalling.

between Id1 expression, metastasis and poor prognosis in several carcinomas (reviewed in REF. 49). However, the lack of specificity of available anti-Id1 antibodies has brought into question some of the previous data that indicates Id1 expression only increases in a subset of highly aggressive metaplasic breast carcinomas and in transitional bladder carcinomas[138]. This latter study also highlights the current difficulties in accurately assessing the expression of different EMT regulators in tumour biopsy samples.

New functions in tumour progression. Recent information on the function of Snail factors indicates that, in addition to inducing EMT, they also alter cell proliferation and cell survival, with important implications for tumour progression[8,82]. SNAI1-expressing cells that have undergone EMT have a low proliferative potential[22,91], showing partial G1/S cell-cycle arrest that is at least in part due to the repression of *CCND2* (the gene that encodes cyclin D2). However, it seems that this behaviour might be cell- or tissue-type dependent[8,139,140]. The active participation of E47 and Id members in cell cycle and proliferation control has also been reported[49] (TABLE 1).

Perhaps the most fascinating newly discovered function of Snail factors is their capacity to protect against cell death, an essential characteristic of tumour cells that might be crucial for the metastatic process. SNAI1 has recently been described as a survival factor in the response to tumour necrosis factor-α (TNFα) and as a potent cell-survival mediator during the emigration of neural crest cells[91]. This effect of Snail factors on cell survival was initially described in *Caenorhabditis elegans* where CES1 (the Scratch homologue; BOX 1) blocks the programmed cell death of specific neurons[141]. The aberrant upregulation of SNAI2 as a result of *E2A–HLF* translocation in human leukaemias is also associated with cell survival[142]. Moreover, SNAI2 is involved in the radioresistance of haematological progenitor cells *in vivo*[143,144]. Recent insights indicate that SNAI2 antagonizes p53-mediated apoptosis in haematological precursors by repressing the p53-mediated transcription of PUMA, a BH3-only antagonist of the anti-apoptotic BCL2 protein[87]. Interestingly, this survival mechanism seems to be evolutionary conserved in *C. elegans*[87], supporting the pro-survival property of all members of the Snail superfamily[8]. The pro-survival action of SNAI1 and SNAI2 has been shown in human carcinoma cells under genotoxic stress induced by chemotherapeutic agents such as doxorubicin[86]. Accordingly, both p53-dependent and p53-independent pro-apoptotic genes are directly downregulated by SNAI1 and SNAI2, and interestingly,

Table 2 | **Expression of Snail, ZEB and bHLH factors in human tumours**

Gene	Cancer factor type	Associated clinico-pathological features
SNAI1	Breast carcinoma	Lymph node metastasis[103,104]; effusions[106,108]; distant metastasis[70,105]; tumour recurrence[107]*
	Ovarian carcinoma	Hypoxia[163]; distant metastasis[106]
	Colon carcinoma	VDR downregulation[85,110]; distant metastasis[112]
	Squamous cell carcinoma	Increased MMP expression[118]; invasion and distant metastasis[116,117]; expression at the tumour–stroma interface[127]
	Hepatocarcinoma	Invasive front[120]; poor prognosis[119]
	Synovial sarcoma	Spindle phenotype[124]
SNAI2	Breast carcinoma	Effusions[106,109]; metastasis and recurrence[108]; partial differentiation[105]
	Ovarian carcinoma	Overall survival[106]
	Colon carcinoma	Poor survival (independent prognosis factor)[113]
	Squamous cell carcinoma	Lymph node metastasis; invasion and poor prognosis[121]
	Lung adenocarcinoma and mesothelioma	Invasion[122]; overall survival[123]
	Melanomas*	Metastasis[125]
ZEB2	Ovarian carcinoma	Effusions[106,109]
	Gastric tumours	Histological type[114]
	Pancreatic tumours	Differentiation grade[129]
	Squamous cell carcinoma	Overall survival[128]
ZEB1	Uterine carcinoma	Poor prognosis[130]
	Colon carcinoma	E-cadherin downregulation (Snail-negative tumours)[60,110]
TWIST1	Ductal breast carcinoma	Invasion and angiogenesis[131]; poor prognosis[108]
	Uterine carcinoma; prostate tumours	Poor survival[136]; metastasis[133]
	Gastric tumours	Histological type[114]
	Squamous cell carcinomas	Distant metastasis[134]
	Hepatocarcinomas	Distant metastasis[135]
	Melanomas	Poor survival[137]

*Data from cell lines, tumour models or microarray data. MMP, matrix metalloproteinase; VDR, vitamin D receptor.

the expression of *TP53* was itself moderately repressed by SNAI1 and SNAI2 binding (TABLE 1). Other studies have shown the participation of the SCF–KIT signalling pathway in the acquired multidrug resistance of malignant mesothelioma cells mediated by SNAI2 induction[145]. Moreover, the recent identification of *BRCA2* as a direct target of SNAI2 in breast carcinoma cells[56] supports the implication of Snail factors in regulating the response to different genotoxic insults. Interestingly, a pro-survival influence might not be exclusive to Snail factors. An important pro-survival function has been described for TWIST in neuroblastoma, antagonizing the pro-apoptotic function of MYC by downregulating the ARF–p53 pathway[146]. Furthermore, some studies indicate that TWIST expression confers resistance to different chemotherapeutic agents, such as vincristine and taxol, in carcinoma cells[133,147], and multidrug resistance conferred by E47 expression has also been reported[148]. Nevertheless, whether this resistance is associated with the ability of these factors to induce EMT remains to be resolved. The recent association of aberrant SNAI1 and/or SNAI2 expression with decreased disease-free and overall patient survival in breast, ovarian, hepatocellular, SCC, colorectal cancer and lung adenocarcinoma (TABLE 2), makes the implication of Snail factors in tumour recurrence a very attractive hypothesis that might be related to their pro-survival activity, an avenue that deserves further study.

Recently, it has been suggested that Snail-mediated tumour recurrence might be associated with the maintenance of self renewal in cancer stem-cell-like cells[149]. Indeed, this was initially proposed for SNAI2 in relation to the SCF–KIT pathway in haematological tumours[35]. Importantly, cell survival properties might also be linked to tumour invasion, EMT and stem-cell properties, as suggested by the recent description of 'migratory cancer stem cells' (MSCs) in colorectal cancer[150]. It will be interesting to see if MSCs exist in different tumour types, and the potential influence of Snail and/or bHLH factors on these cells.

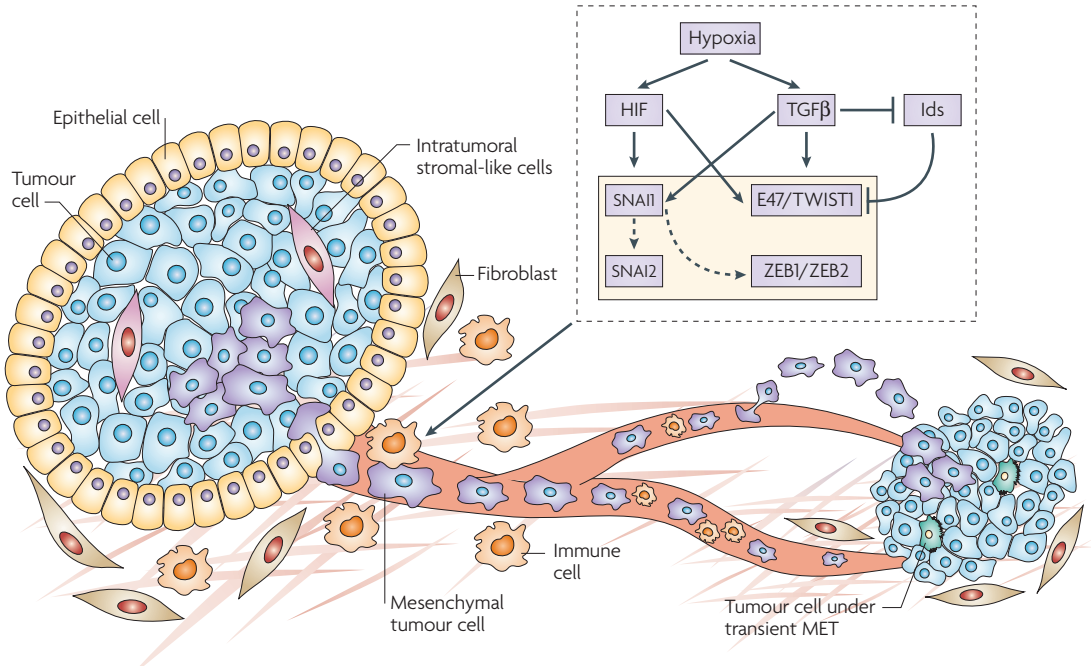


Figure 4 | **Interplay of different factors in cancer.** Schematic of the potential interplay of *CDH1* repressors and the microenvironment during tumour progression. Hypoxia- and TGFβ-derived signals could promote the interplay of Snail, ZEB and bHLH factors that orchestrate *CDH1* repression and epithelial–mesenchymal transition (EMT) during tumour progression. SNAI1 could be implicated in the initial migratory phenotype and considered as an early marker of EMT that sometimes contributes to the induction of other factors. By contrast, SNAI2, ZEB1, ZEB2 and/or TWIST could be responsible for the maintenance of migratory cell behaviour, malignancy and other tumorigenic properties. Pink cells indicate intra-tumoral stromal-like cells.

Influence of the microenvironment

The influence of the microenvironment in tumour development is now well established[151]. Indeed, the full metastatic process might be envisaged as an evolutionary process in which genetically heterogeneous cell populations are driven to evolve by changing environmental pressures[3]. This concept has renewed interest in the influence of the microenvironment on tumour progression. Here, we shall focus only on recent data related to the influence of the microenvironment on EMT and the expression of Snail, bHLH and/or ZEB factors and their interactions.

A plethora of signalling factors influence the expression of Snail, ZEB and bHLH factors (FIG. 2a). Although some such signals function in autocrine loops in tumour cells[32,34], many others are likely to be derived from the tumour microenvironment. Among these, Wnt and TGFβ signalling are being extensively studied. Indeed, recent data show that the Wnt antagonist frizzled-related protein (FRZB), or a dominant-negative low density lipoprotein related protein 5 (LRP5) co-receptor, induce the reversion of EMT associated with the strong downregulation of SNAI2 and TWIST[152] (FIG. 2a). In conjunction with the transcriptional and post-transcriptional regulation of SNAI1 by GSK3β[46,70,71], these data support an active role of the microenvironment in the control of Wnt signals, the dynamic regulation of EMT mediators and epithelial plasticity.

The regulation of Snail and ZEB factors by signalling pathways related to inflammatory and extracellular matrix components has added further insight into the participation of the microenvironment in the regulation of EMT. The upregulation of ZEB2 expression associated with E-cadherin repression in pancreatic cells grown in the presence of type I collagen is in agreement with the inverse correlation between ZEB2 and E-cadherin in pancreatic tumours[129]. The expression of SNAI1 and SNAI2 may be induced in hepatocarcinoma cells by exposure to laminin 5 through a mechanism dependent on active integrin α3 and TGFβ[40]. These data are consistent with the upregulation of laminin 5 and SNAI1, and the downregulation of E-cadherin at the invasive front of hepatocarcinomas[40,120]. The influence of inflammatory components or the inflammatory-related response of the tumour microenvironment on the regulation of EMT-modulators has recently been highlighted through the induction of SNAI1 by the cyclooxygenase 2 (COX2)–PGE2 pathway, and the induction of SNAI1 and ZEB through nuclear factor κB (NFκB) and some interleukins[21,153]. Interestingly, NFκB can be inhibited by active GSK3β resulting in the repression of SNAI1 expression[46], thus potentially linking Wnt antagonist and inflammatory-derived signals in SNAI1 regulation. The complex interactions between the microenvironment and the regulation of the diverse activities of 'EMT regulators' are highlighted in other studies that linked vascular epithelial growth factor receptor 1 (VEGFR1)

overexpression with the upregulation of SNAI1, SNAI2, E47 and TWIST[33], and the active participation of these factors and Ids in angiogenesis[49,131,154]. Together, these data support the mutual influence of the tumour and components of its microenvironment on the dynamic control of EMT and the angiogenic response during tumour progression.

Strategy: the interplay of different factors in cancer. The data obtained in recent years indicate that *CDH1* repressors interact during tumour progression. The localization of SNAI1 at the tumour–stroma interface and the observation that SNAI1 promotes the expression of ZEB factors in oesophageal SCC cells is evidence that SNAI1 may be implicated in the initial stages of invasion. At the same time, ZEB1 and ZEB2 could be considered to be additional predictive markers[127,155]. Together with functional studies in other malignant epithelial cells[95,154,156], these data also support a differential and hierarchical role for these repressors during EMT in epithelial carcinogenesis[53,79,155], as has previously been indicated in developmental studies. Recently, a model was proposed in which SNAI1 and SNAI2 were expressed in the nuclei of primary tumour cells in ovarian carcinomas, whereas after tumour effusion, they were located and weakly expressed in the cytoplasm (and were inactive), with ZEB2 upregulated[109]. These studies suggest that these repressors could be differentially and/or sequentially expressed at distinct anatomical sites during tumour progression, supporting their differential participation in ovarian tumours. Importantly, these studies also shed light on the implication of these repressors in tumour models that do not follow the classical EMT model, such as ovarian cancer, in which E-cadherin is not downregulated in ovarian carcinoma effusions. Therefore, partial E-cadherin downregulation, together with the expression of Snail or other repressors could be a metastatic strategy for some tumours at the same time as promoting cell viability. Co-expression of these factors with E-cadherin could also have an important role in specific cell migration during metastasis, as in 'cohort migration'[157]. This behaviour has been observed for SNAI2 in certain situations, such as during keratinocyte re-epithelialization[158], and together with SNAI1 in several cancer cells and effusions[105,106,109]. Importantly, the signalling pathways that control SNAI1 and SNAI2 stability and localization (FIG. 3) could also have a key role in E-cadherin maintenance after the detachment of cancer cells from primary tumours, regardless of *SNAI1* and *SNAI2* expression levels. Together, these functional and clinical studies indicate that under specific situations, SNAI1 could be implicated in the initial migratory phenotype of primary tumours and considered as an early marker of EMT (FIG. 4). By contrast, SNAI2, ZEB1, ZEB2 and/or TWIST could be responsible for the maintenance of migratory cell behaviour, malignancy and other tumorigenic properties. However, this model might only operate in certain tumour types owing to the specific and independent roles of the different factors during tumour progression (such as TWIST in intravasation[16]), and it should be subjected to more detailed analysis.

A key molecule that might promote the interplay of these repressors during EMT is TGFβ (FIG. 4). Recent studies have pointed to a dual role for TGFβ during EMT mediated by *CDH1* repressors. On one hand, TGFβ promotes Id1 and 2 downregulation[29,66], relieving its negative action on the *CDH1* repressor E47. On the other hand, TGFβ also promotes the expression of SNAI1, SNAI2, ZEB1, ZEB2 and TWIST in cell- and/or tissue-dependent contexts[29,159]. These findings provide a new link between the coordinated activity of TGFβ on different *CDH1* repressors to induce EMT (FIG. 4). Hypoxia is also emerging as a potentially important event that coordinates the activity of *CDH1* repressors. The loss of Von Hippel Lindau (VHL) protein in renal carcinoma cells results in the loss of *CDH1* expression, indicative of the hypoxia inducible factor-dependent induction of E47, SNAI1 and ZEB[38,160,161]. Interestingly, the potential cooperation of TGFβ signalling in the effects promoted by VHL loss, indicate that TGFβ and hypoxia may orchestrate the action of these repressors during EMT and tumour progression[38]. Studies in human ovarian cancer cell lines also showed that both SNAI1 and SNAI2 are associated with *CDH1* repression after HIF1 expression induced by hypoxia[162]. These data support previous suggestions that SNAI1 could repress *CDH1* in conditions of hypoxia, promoting tumour malignancy in ovarian carcinomas[163]. Notably, the recent observation that the LOX protein is essential for hypoxia-induced metastasis[164], and that LOXL2 promotes SNAI1 stabilization and repressor activity[72], is supportive of a potential cooperation between hypoxia, SNAI1 and LOX, LOXL2 and LOXL3 proteins in tumour malignancy[165]. Together, these data suggest that hypoxia and TGFβ-derived signals could promote the interplay of Snail, ZEB and bHLH factors that orchestrate *CDH1* repression and EMT during tumour progression (FIG. 4), as well as increasing the malignant, migratory and angiogenic behaviour of tumour cells.

Concluding remarks

The past 6 years have produced significant advances in our understanding of the molecular mechanisms of *CDH1* downregulation and tumour progression, particularly in relation to the role of Snail, ZEB and some bHLH factors. From the modification of the classical 'two-hit hypothesis' for *CDH1* silencing proposed by Cheng and colleagues[104], whereby mutational inactivation works with transcriptional repression, we have learned that additional regulatory mechanisms have a crucial role in E-cadherin regulation and tumour progression. These include post-translational modifications, protein–protein interactions and the effects of the tumour microenvironment. Furthermore, the recent recognition of additional functions and gene targets of Snail, ZEB and bHLH factors has opened new avenues to fully understand their function and potential clinical and therapeutic implications in cancer. A full understanding of the contribution of different extracellular factors in the tumour microenvironment, together with the full development of specific reagents, will help elucidate the specific and collaborative implications of the different factors that regulate the epithelial phenotype during carcinogenesis.

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Acknowledgements
The authors wish to thank F. Portillo, G. Moreno-Bueno, E. Cubillo and all the members of AC's laboratory for their critical feedback and thoughtful suggestions. We apologize to those investigators whose experimental work has not been cited or cited indirectly owing to space limitations. Experimental work at AC's laboratory was supported by grants from the Ministerio de Educación y Ciencia (MEC) and the European Union. HP and DO are supported by postdoctoral contracts from the Fundación Científica de la Asociación Española Contra el Cáncer and MEC, respectively.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
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AMF | *BRCA2* | calreticulin | β-catenin | cadherin 16 | *CDH1* | *CDKN1A* | *CDKN2A* | collagen 2α1 | COX2 | CTBP | cyclin D2 | *E2A* | EGF | endothelin 1 | ETS1 | FRZB | GSK3β | HDAC1 | HDAC2 | HDAC3 | HGF | HIF1 | HMGA2 | *ID2* | IGF | KIT | LOXL2 | LOXL3 | LRP5 | MAPKAPK2 | MMP2 | MMP9 | MTA3 | MYC | NFκB | occludin | PAI1 | PAK1 | PC2 | PCNA | PGE2 | PI3K | plakophilin 2 | PNN | RAF1 | SCF | skeletrophin | SNAI1 | SNAI2 | SP1 | SPARC | STAT3 | TCF4 | TGFβ | TIMP1 | VEGF | VEGFR1 | VHL | ZEB1 | ZEB2

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