The ID proteins: master regulators of cancer stem cells and tumour aggressiveness

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Abstract | Inhibitor of DNA binding (ID) proteins are transcriptional regulators that control the timing of cell fate determination and differentiation in stem and progenitor cells during normal development and adult life. ID genes are frequently deregulated in many types of human neoplasms, and they endow cancer cells with biological features that are hijacked from normal stem cells. The ability of ID proteins to function as central 'hubs' for the coordination of multiple cancer hallmarks has established these transcriptional regulators as therapeutic targets and biomarkers in specific types of human tumours.

Cancer stem cells
A small population of cells that is found in most types of cancer; these cells have the ability to drive tumour growth and spread through self-renewing cell divisions.

Basic helix–loop–helix (bHLH) transcription factors
Transcription factors that can work in a dimeric state and bind to specific DNA sequences via the stretch of basic amino acids.

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In his efforts in 1890 to define the morphology of tumour cells, David Paul von Hansemann introduced the word 'anaplasia' to designate the process of dedifferentiation: "I have called many cells, perhaps all major cells of epithelial cancer, 'anaplastic', that is, they have passed from a higher state of differentiation to a lesser one. The question now is: is one justified, as happens on many sides, in calling these cells embryonal?" (REF. 1). In this work he identified what we define today as one of the 'hallmarks of cancer' (REFS 2,3). More than a century later, despite the wealth of knowledge on stem cell identity and the increasing understanding of cancer stem-like cells, we only have a weak grasp of the relationship between normal stem and progenitor cells — which are capable of terminal differentiation — and cancer stem cells that maintain self-renewal but have terminal differentiation that is markedly impaired in unperturbed conditions[4–6]. Nevertheless, some of the molecular circuits that preserve stem cells in the undifferentiated state have been identified, and we understand that the state of anaplasia in cancer stem cells requires some of those same circuits[7–11]. The inhibitor of DNA binding (ID) proteins probably bridge the properties of normal and cancer stem cells better than any other family of proteins.

In this Review, we summarize the most recent and somewhat tumultuous developments regarding the ID family in cancer. We briefly present knowledge of the cellular functions of ID proteins and their effectors. Then we review data that show the contribution of ID proteins to tumorigenesis, tumour progression and cancer hallmarks. In addition, we discuss the potential for ID proteins as prognostic markers and drug targets in cancer.

The ID family

Since the cloning of the first ID gene, the mouse *Id1* gene in 1990, ID proteins have been recognized as functional inhibitors of the basic helix–loop–helix (bHLH) transcription factors[12,13]. Genes that encode ID proteins have been isolated from several metazoan species but have been mostly studied in *Drosophila melanogaster* — in which a single ID-like locus, extra macrochaetae (*emc*), encodes an ID-like protein[14,15] — and in mice and humans, which possess four ID family members (ID1–4)[12,16–19]. More than two decades of research has established that inhibition of differentiation is the biological function that is shared by all four members of the ID family (FIG. 1) and that this function is associated with the ability of ID proteins to increase cell proliferation and preserve multipotency[13,20]. The crucial biochemical attribute of each of the four members of the ID family is their binding to the bHLH transcription factors and their inhibition of DNA binding by these factors, which are a family of proteins that control cell fate determination, differentiation and cell proliferation[20]. Tissue-specific and ubiquitously expressed (E protein) bHLH proteins form heterodimers through the HLH dimerization region. In the presence of high levels of ID proteins, ID–bHLH rather than bHLH–bHLH association prevails and, because the ID proteins do not contain a basic region, the ID–bHLH heterodimer is unable to bind to DNA and bHLH-directed transcription is blocked[21] (FIG. 1a). bHLH-mediated transcription generally activates differentiation programmes that are coordinated with cell cycle arrest (FIG. 1b). However, at specific developmental

Key points

- Inhibitor of DNA binding (ID) proteins are a family of highly conserved transcriptional regulators that are pivotal both during developmental processes and in adult tissue homeostasis. ID proteins function by inhibiting basic helix–loop–helix, ETS and paired box (PAX) transcription factors and non-transcription factors of the RB family.
- The major biological effect of ID protein activity is the inhibition of differentiation and maintenance of self-renewal and multipotency in stem cells, and this is coordinated with continuous cell cycling.
- ID proteins are essential components of oncogenic pathways and are activated transcriptionally and post-transcriptionally by oncogenic factors. ID proteins are repressed by tumour suppressors although they have also been shown to function as tumour suppressors in specific tumour types.
- ID proteins are overexpressed in many human cancers and deregulation of ID proteins has a direct role in cancer initiation, maintenance, progression and drug resistance.
- The expression of ID proteins has a prognostic value in many human cancers and interfering with ID activity in tumours that have ID protein activation might provide new avenues for cancer treatment.

stages or in distinct cell types (such as B lymphoid cells) bHLH-mediated transcription promotes cell proliferation and survival, and ID-mediated inhibition of bHLH attenuates these processes[22,23]. These dual and opposing functions of bHLH and ID proteins determine their potential role as tumour suppressors or oncogenes.

Studies have shown that, besides bHLH, ID proteins exert inhibitory activity towards other transcription factors, which belong to the ETS and paired box (PAX) families[24–27]. The negative regulation of ETS transcription factors by IDs has been linked to the inhibition of ETS-mediated induction of INK4A (also known as p16), and this inhibition blocks replicative senescence[24,26,27]. ID proteins also inhibit members of the subfamily of paired-domain transcription factors that comprises PAX2, PAX5 and PAX8 (REF. 26). Members of this subfamily have key functions during the regulation of several developmental processes, including B lymphopoiesis, and they can promote or inhibit oncogenesis in different cellular contexts[28].

The ID family member ID2 (and possibly ID4) interacts with RB, which is a tumour-suppressor protein[29–33]. When ID2 is in stoichiometric excess over active RB — as in cells with genetic inactivation of *Rb1* or in tumour cells with oncogene-mediated accumulation of ID2 — it overrides the tumour-suppressor activity of RB. However, during normal development RB is essential to restrain ID2, as highlighted by the evidence that ID2 contributes to both the developmental defects and the tumorigenic phenotype that are caused by RB deficiency[29,31–33] (FIG. 1b). A similar genetic interaction has recently been described in *D. melanogaster* between *emc* and RB-family protein 1 (*Rbf1*), which encodes one of the two RB proteins[34]. Although we still lack a comprehensive picture of the downstream molecular events that are controlled by the reciprocal regulation of RB and ID2, they are likely to involve the regulation of bHLH and ETS target genes[30,35]. Conversely, it is unlikely that ID2 affects the activity of E2F transcription factors, given that genetic loss of ID2 does not rescue the derepression of E2F target genes in *Rb1*-null cells (A.L. and A.I., unpublished observations).

An important contribution to the understanding of ID protein functions was provided by mouse models of Id gene deletion, especially compound knockout models, which showed the redundant function of ID proteins both during development and tumour genesis (TABLE 1). Although the investigation of ID protein activity in developmental processes remains an important focus in the field, in the past few years there has been increasing interest in the function of ID proteins in cancer biology and particularly cancer stem cell biology. This is not unexpected given the established role of ID proteins in enforcing the undifferentiated state and the unrelenting cell cycling characteristics of embryonic and somatic stem cells[36–42]. Indeed, some of the most exciting but only partially addressed questions in the field include which stem cell properties, cancer stem cell properties and targets are controlled by ID proteins to affect stem cell functions.

Multimodal activation of ID proteins in cancer

ID protein expression is high in stem and progenitor cells, is typically downregulated during differentiation and is re-activated in cancer cells. There are exceptions to this general paradigm, with the remarkable examples of loss-of-function alterations of ID genes that were recently reported by genomic analyses of human cancer samples[43–45]. It is well established that in different cellular contexts ID proteins or distinct ID family members can exert divergent functions and can act as oncoproteins or tumour suppressors. Nonetheless, high expression levels of ID proteins are evident in most tumour types (TABLE 2). The biological relevance of the expression data is substantiated by the evidence that ID proteins are implicated not only in maintaining cancer stem cells and tumour anaplasia but also in many of the cancer-related phenotypes (TABLE 3). Consequently, several studies have experimentally addressed the idea that ID proteins are plausible prognostic markers and therapeutic targets in cancer because specific subsets of human tumours can become addicted to high levels of expression — and consequently high activity — of one or more ID proteins.

ID genes as targets of upstream oncogenic events. Long before genetic alterations were discovered, ID genes could be operationally defined as 'cancer genes' because of the connections between their aberrant expression and several cancer phenotypes. The aberrantly high levels of expression of ID proteins in cancer are often a consequence of transcriptional induction by oncoproteins such as MYC, RAS, SRC, Notch, Ewing's sarcoma (EWS)–Friend leukaemia integration 1 (FLI1) and receptor tyrosine kinases[46–52], and by growth factor-directed signals such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGFβ) and bone morphogenetic proteins (BMPs)[32,42,53–55], which are activated in a constitutive manner in cancer cells and promote processes such as cell proliferation, invasion, angiogenesis and metastasis (FIG. 2). Concordantly, transcriptional regulators that have tumour-suppressor functions (for example, forkhead box protein O3 (FOXO3) and p53) repress ID gene expression[53,54].

Epithelial-to-mesenchymal transition
(EMT). A functional transition in which epithelial cells lose their cell polarity and cell–cell adhesion and assume a mesenchymal cell phenotype, which includes migratory and invasive properties.

Triple-negative breast cancer
(TNBC). A highly aggressive subtype of breast cancer that is defined by the absence of oestrogen receptor, progesterone receptor and *ERBB2* gene amplification.

Burkitt's lymphoma
An aggressive neoplasm that is derived from mature B cells and is cytogenetically characterized by the t(8;14) translocation that deregulates *MYC*.

In many instances, the mechanisms of ID gene activation are unclear; for example, *ID2* deregulation in anaplastic large cell lymphoma seems to be required for nearby chromatin sites to undergo translocation[55]. It is not unusual for multiple genetic and epigenetic events and signalling pathways to cooperate to establish the aberrant levels of ID proteins that underpin cell proliferation and survival, and that are required for tumour maintenance. In non-small-cell lung cancer (NSCLC) ID1 is induced by both epidermal growth factor receptor (EGFR) and α7-nicotine-acetylcholine receptor (α7-nAChR; also known as CHRNA7) signalling[56,57], which are two well-established mechanisms that drive the growth and progression of this tumour type[58]. EGFR and α7-nAChR signalling converge on SRC, which induces the transcriptional activation of *ID1* through the BMP signalling pathway and MYC. By increasing ID1 levels, these oncogenic networks promote NSCLC cell proliferation, epithelial-to-mesenchymal transition (EMT; a process that renders tumour cells more invasive and aggressive), metastasis and chemoresistance *in vitro* and in xenograft models[59]. Similar effects have been reported in pancreatic adenocarcinoma, whereby the nicotine–α7-nAChR–SRC pathway converges on ID1 to promote metastasis and chemoresistance *in vivo*[60]. Accordingly, both in NSCLC and in pancreatic cancer, high levels of ID1 expression are associated with tumour subgroups that are characterized by the highest histological grades and the poorest clinical outcome.

ID regulation by microRNAs. Recent research has shown that ID mRNA levels are regulated by microRNAs (miRNAs). In NSCLC, miR-381 and miR-29b bind to the *ID1* 3′ untranslated region (UTR) and repress *ID1* translation, thereby countering the increase of ID1 levels by SRC[61,62]. Low levels of miR-381 and miR-29b correlate with high levels of ID1 and reduced survival in patients with lung adenocarcinoma, and this supports the hypothesis that these microRNAs can function as tumour suppressors by opposing ID1 accumulation. A similar mechanism has been reported in neuroblastoma, in which the accumulation of ID2 is driven by MYCN and countered by miR-9 and miR-103 during retinoic acid-induced differentiation *in vitro*[63]. A regulatory interplay between miR-335 and ID4 has been described in breast cancer cells in which loss of miR-335 sustains the expression of ID4 (REF. 64). It was reported that ID4 is involved in the transcriptional repression of the tumour suppressor *BRCA1* and in turn BRCA1 represses *ID4* transcription as part of an oestrogen-dependent regulatory loop that balances the expression of these two genes[65–67]. Furthermore, higher expression levels of ID4 were detected in triple-negative breast cancer (TNBC) — a subtype that is associated with loss of BRCA1, highly aggressive clinical behaviour and a stem cell-like gene expression signature — than in non-TNBC[68,69]. It has been reported that ID4 sustains breast cancer stem cell properties[70], so the accumulation of ID4 in TNBC might contribute to both disabling *BRCA1* transcription and driving the stemness phenotype.

Genetic alterations of ID genes in human cancer. The lack of identification of genetic lesions in ID genes during the pre-genomic era left the question of whether ID genes are oncogenes unanswered. Convincing evidence for recurrent gain-of-function genetic events that target ID genes is still not evident, despite recent high-throughput genomic analyses of multiple tumour types. An exception is the finding that *ID4* is amplified in approximately 30% of high-grade serous ovarian cancers[71].

Paradoxically, three recent massively parallel sequencing studies[43–45] of Burkitt's lymphoma[72] provided convincing support for the idea that in some contexts ID genes function as tumour-suppressor genes. Of the Burkitt's lymphoma samples studied, 34%–68% had inactivating biallelic mutations of *ID3* that cause the reduction of both the stability of the ID3 protein and its binding affinity for the bHLH transcription factors E12 and E47; encoded by *TCF3* (also known *E2A*). Interestingly, 11% of Burkitt's lymphoma samples had mutations in *TCF3* only at conserved residues in the bHLH region, and 13% had mutations in both *TCF3* and *ID3*. TCF3-mutant proteins retain their DNA-binding and transcriptional activity but show significant loss of binding affinity for ID3. However, co-occurrence of *ID3* and *TCF3* mutations suggests that mutant TCF3 might also acquire an aberrant transcriptional function or that it is required for halting the inhibitory activity of the other ID proteins. In B cells, TCF3 modulates essential genes for germinal centre function and B cell survival, including genes encoding the B cell receptor, and TCF3 upregulates ID3 and, to a lesser extent, ID1 and ID2; this creates an inhibitory loop that would restrain TCF3 activity in normal cells. Thus, constitutive activation of the TCF3 transcriptional programme caused by gain-of-function *TCF3*

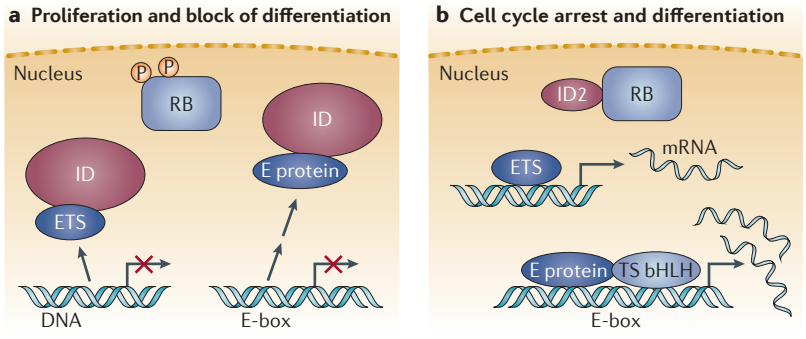

Figure 1 | **ID proteins: proliferation and inhibition of differentiation. a** | High levels of inhibitor of DNA binding (ID) proteins are present in stem and progenitor cells, where they sequester ubiquitously expressed basic helix–loop–helix (bHLH) transcription factors (also known as E proteins) and ETS transcription factors and thereby inhibit the transcription of lineage-specific and cell cycle-inhibitory genes[12,18–20,25,27]. ID2 has been shown to interact with the tumour-suppressor protein RB, but hyperphosphorylated, inactive RB is incapable of binding to ID2 (REFS 29–32). **b** | With the downregulation of ID proteins (indicated by the smaller size of the ID shape), E proteins and tissue-specific bHLH (TS bHLH) transcription factors heterodimerize and bind to E-box sites in the promoters of target genes. ETS transcription factors are also released from the inhibitory activity of ID proteins and activate transcription. Low levels of ID2 are controlled by active hypophosphorylated RB. The transcription of lineage-specific genes and cell cycle-inhibitory genes is activated with a resulting loss of stem cell properties and acquisition of differentiated features and cell cycle arrest[38,104]. P, phosphate.

Table 1 | **Developmental and cancer phenotypes of ID-knockout mice**

Mouse model	Developmental phenotype	Cancer phenotype	Refs
Id1$^{-/-}$	No major abnormalities	Impaired cancer stem cell properties in orthotopic malignant glioma	201
Id2$^{-/-}$	Perinatal lethality; reduced body size; absence of lymph nodes and Peyer's patches; absence of natural killer cells; loss of Langerhans cells; defective lactation; and defective specification of dopaminergic neurons	Spontaneous intestinal adenoma	202–205
Id2$^{-/-}$;*Rb1*$^{+/-}$	None	Reduced pituitary tumour formation, reduced pituitary tumour cell proliferation and reduced angiogenesis in the pituitary tumour	33
Id3$^{-/-}$	Defects in humoral immunity and T cell development	• Impaired cancer stem cell properties in orthotopic malignant glioma • Spontaneous T cell lymphoma	206,207
Id1$^{-/-}$;*Id3*$^{-/-}$ and *Id1*$^{-/-}$;*Id2*$^{-/-}$;*Id3*$^{-/-}$	Lethal embryonic day 13.5; vascular defects in forebrain; premature neuronal differentiation; and cardiac defects	Not applicable	104,208
Id1 and *Id3* loss of single or multiple allele combinations	None	Loss of vascular integrity and reduced growth of genetic models of breast cancer (*MMTV–NeuYD* and *MMTV–Wnt1*) and human tumour cell xenografts	116,118, 123
Id4$^{-/-}$	Perinatal lethality; reduced body size; neural progenitor defects; and defective differentiation of oligodendrocyte lineage	Not applicable	176,178
Id1$^{L/L}$;*Id2*$^{L/L}$;*Id3*$^{-/-}$ and nestin–Cre	Neonatal lethality; and defects in self-renewal and proliferation of neural stem and progenitor cells	Not applicable	38
Id1$^{L/L}$;*Id2*$^{L/L}$;*Id3*$^{-/-}$	Not applicable	Reduced tumour growth and loss of cancer stem cell properties in an orthotopic malignant glioma model*	82

ER, oestrogen receptor; *Id*, inhibitor of DNA binding; *MMTV*, mouse mammary tumour virus; shp53, short hairpin RNAs targeting *Trp53*. *$Hras^{V12}$;*CreER*;shp53 lentiviral particles were injected in the hippocampi of 4-week-old *Id1*$^{L/L}$;*Id2*$^{L/L}$;*Id3*$^{-/-}$ mice.

mutations or loss-of-function *ID3* mutations endows Burkitt's lymphoma cells with a ligand-independent, 'tonic' form of BCR signalling that activates the PI3K pathway, which is essential for Burkitt's lymphoma cell survival. Therefore, the particular tumour-suppressor role of ID3 in B cells is linked to the essential and unique function of TCF3 in B cell development and survival, which explains the oncogenic role of deregulated TCF3 activity in B cells. However, mutations of ID3 are absent in diffuse large B cell lymphoma (DLBCL), suggesting that Burkitt's lymphoma and DLBCL might originate from different cell types of the lymphoid germinal centre with different requirements for the TCF3–ID3 pathway[43–45]. The finding that Burkitt's lymphoma harbours inactivating mutations of ID3 and gain-of-function mutations of TCF3 stands in contrast to the observation that E proteins, which are the primary targets of ID proteins, usually function as tumour suppressors. Indeed, mice in which E protein-encoding genes are knocked out are prone to develop tumours[73–77]. In addition, the expression of E proteins in several types of tumour cells results in the activation of genes that promote differentiation and inhibit progression of the cell cycle[35,78–84]. Another suggestion for a tumour-suppressor role of an ID gene comes from the finding that the *ID4* promoter is hypermethylated and silenced in certain types of leukaemia[85,86]. Although the mutations of *ID3* provide an unquestionable example of loss-of-function events that show a tumour-suppressor function of ID genes, there is overwhelming support for the idea that activation — and not inactivation — of ID genes is predominantly selected for in the vast majority of cancer cell types[13].

Deregulation of ID protein stability. Perturbation of the ubiquitin–proteasome pathway has been linked to the accumulation of ID proteins in cancer. ID proteins are short-lived proteins (normally lasting for 10–20 minutes), and they are targeted for degradation by the ubiquitin–proteasome pathway[87,88]. Proteasome-mediated degradation of proteins is regulated by E3 ubiquitin ligases that link a ubiquitin chain to target substrates, which leads to substrate degradation[89]. Ubiquitin ligase activity is opposed by deubiquitylases (DUBs), which increase substrate stability by removing ubiquitin moieties[90]. Both E3 ligases and DUBs regulate ID protein accumulation (FIG. 3). ID1, ID2 and ID4 are substrates of the anaphase-promoting complex/cyclosome (APC/C)–CDH1 (also known as FZR1) E3 ubiquitin ligase owing to the presence of a canonical CDH1-recognition domain (D-box) in the carboxy-terminal region of these proteins[91].

Table 2 | **ID protein expression in common cancer types**

Cancer type	ID protein	Expression	Phenotype	Prognosis	Refs
Bladder	ID1	Increased	Invasion	Poor	209
Breast	ID1	Increased	Invasion and angiogenesis	Poor	122–124
	ID2	Reduced	Differentiation and reduced invasion	Good	210
	ID4	Reduced	Lymph node metastasis	Poor	211
Brain	ID1	Increased	Proneural	Good	113
	ID2	Increased	Mesenchymal	Poor	82
	ID3	Increased	Mesenchymal	Poor	82
	ID4	Increased	Proliferation, anaplasia and associated with high-grade cancer	Poor	212
Colon and rectal	ID1	Increased	Hyperproliferation and CSC	Poor	83,213
	ID2	Increased	Hyperproliferation	Poor	213
	ID3	Increased	CSC	Poor	83
	ID4	Reduced	Dedifferentiation	Poor	214
Oesophageal	ID1	Increased	Associated with metastasis	Poor	215
Gastric	ID1	Increased	Loss of differentiation	Poor	216
	ID3	Increased	Loss of differentiation	Poor	216
Kidney	ID1	Increased	Lower CR rate	Poor	217
Head and neck	ID1	Increased	Tumour angiogenesis	Poor	218
Leukaemia	ID1	Increased	Myeloid leukaemia	Poor	219
	ID4	Increased	Myelodisplastic syndrome	Leukaemic transformation	220
Hodgkin's lymphoma	ID2	Increased	Loss of B cell properties	Not applicable	221
Non-Hodgkin's lymphoma	ID3	Mutated	Loss of B cell differentiation	Not applicable	43–45
Pancreatic	ID1	Increased	Hyperproliferation and tumour angiogenesis	Poor	222,223
	ID2	Increased	Hyperproliferation	Poor	223,224
	ID3	Increased	Hyperproliferation and metastasis	Not applicable	225
Prostatic	ID1	Increased	Tumour progression	Poor	155,226
	ID3	Increased	Tumour progression	Poor	155
	ID2	Increased	Tumour progression	Poor	226
	ID4	Increased	Associated with metastasis	Poor	227
Thyroid	ID1	Increased	Tumour growth	Not applicable	228
Ovarian	ID4	Increased	Tumour growth	Not applicable	71
	ID1	Increased	Tumour angiogenesis and anaplasia	Poor	229
Nasopharyngeal carcinoma	ID1	Increased	Invasion	Poor	218
Liver	ID1	Increased	Associated with progression	Poor	230,231

CR, complete response; CSC, cancer stem cell; ID, inhibitor of DNA binding.

It is unknown whether distinct phosphorylation events are required for the recognition and degradation of ID proteins by APC/C–CDH1. This protein ligase has a key role in regulating entry into G1 phase and the quiescent G0 phase, and it has been implicated in cell cycle-independent functions[92]. Although expression of ID proteins seems to be regulated during the cell cycle[91,93], APC/C–CDH1 induces degradation of ID1, ID2 and ID4 specifically during the withdrawal from the cell cycle of neurons that are undergoing terminal differentiation and axonal growth[91]. Another E3 ubiquitin ligase, SMAD ubiquitylation regulatory factor 2 (SMURF2), which is involved in TGFβ signalling, targets ID1 and ID3 for degradation during cellular senescence[94]. Concordantly, studies in mice and in human cancer indicate that both APC/C–CDH1 and SMURF2 have tumour-suppressor functions[95,96]. An important question is whether alterations in the degradation machineries for ID proteins have any role in ID-mediated tumorigenesis. Genetic alterations in the ubiquitin ligase recognition domain of ID proteins have not been described for human tumours. However, mechanisms that lead to aberrant stabilization and accumulation of ID proteins might come from intrinsic alterations of the APC/C[97]. Given the considerable functions of APC/C–CDH1 and SMURF2 substrates

in cancer[95,96], it is unlikely that ID proteins are the sole effectors of oncogenesis when the enzymatic activity of these ubiquitin ligases is compromised. Understanding the relative contribution of ID proteins and other substrates to tumour development and cancer hallmarks in the context of APC/C–CDH1 and SMURF2 alterations would help to design multimodal cancer therapies.

Another mechanism of ID protein stabilization involves the alteration of ubiquitin-specific protease 1 (USP1). USP1 is the specific DUB for ID1, ID2 and ID3 in osteosarcoma cells and mesenchymal stem cells (MSCs). Overexpression of USP1 in osteosarcoma samples and cell lines sustains the accumulation of ID proteins, which are essential for growth and retention

Table 3 | **ID-dependent targets that are implicated in cancer initiation and progression**

Biological process	Target	Expression in tumours	Refs
Angiogenesis	α6β4 integrin	Upregulated	104,119
	FGFR1	Upregulated	119
	EPHA1 and EPHA2	Upregulated	104,119
	MMP2	Upregulated	119
	IGF2	Upregulated	119
	VEGFA	Upregulated	33
	HIF1α	Upregulated	33
	IL-6 and IL-8	Upregulated	120,121
	GROα	Upregulated	120
Apoptosis	CDKN1A	Downregulated	159
	CDKN1B	Downregulated	155
	BAX	Downregulated	150,157
	BCL-2	Upregulated	157
	BCL-X_L	Upregulated	150
	BIM	Downregulated	158
Cell cycle regulation and proliferation	CDKN1A	Downregulated	154,159
	CDKN1B	Downregulated	153
	CDKN1C	Downregulated	35,38
	CDKN2A	Downregulated	25
	Cyclin D1	Upregulated	33,232
	Cyclin E	Upregulated	48
Cell migration and invasion	MMP2	Upregulated	104
	MMP9	Upregulated	233
	SEMA3F	Downregulated	128
Epithelial-to-mesenchymal transition	N-cadherin	Upregulated	138–140
Signalling	PI3K and AKT	Upregulated	150,155,157
Stem cell renewal	NANOG	Upregulated	39
	Brachyury	Downregulated	39
	HES1	Upregulated	234
	RAP1GAP	Downregulated	38,82
	CDKN1A	Downregulated	83
	CDKN1C	Downregulated	38
	Cyclin E	Upregulated	48
	Notch	Upregulated	48
	SOX2	Upregulated	48,105
	SOX4	Upregulated	105
	LIF	Upregulated	42

CDKN, cyclin-dependent kinase inhibitor; EPHA, ephrin type-A receptor; FGFR1, fibroblast growth factor receptor 1; GROα, growth-regulated-α; HES1, hairy and enhancer of split 1; HIF1α, hypoxia-inducible factor 1α; IGF2, insulin-like growth factor 2; IL, interleukin; LIF, leukaemia inhibitory factor; MMP, matrix metalloproteinase; RAP1GAP, RAS-related protein 1 (RAP1) GTPase-activating protein; SEMA3F, semaphorin 3F; SOX, SRY-box; VEGFA, vascular endothelial growth factor A.

of the stem cell-like properties in osteosarcoma[41]. These findings strengthen the case for the control of ID protein degradation as a mechanism of tumour suppression and strongly support the role of ID proteins in the maintenance of the cancer stem cell phenotype. In future studies it will be important to determine how the E3 ubiquitin ligase and DUB pathway is fine-tuned to regulate ID protein turnover during cell fate determination in embryonic and somatic stem cells, and to determine how important the deregulation of this pathway is for the aberrant accumulation of ID proteins in individual cancers and cancer stem cells.

ID proteins and cancer stem cells

Cancer stem cells are thought to be a subpopulation in the bulk of tumour cells that has the capacity to initiate tumours and recapitulate the lineage heterogeneity of the parental tumour. They are also defined by properties that are characteristic of tissue stem cells, such as self-renewal and multipotency. The positive function of ID proteins in cancer stem cells is best understood in colon cancer and malignant glioma, although it is plausible that other types of cancer stem cells depend on ID proteins: in particular, cancers that originate from tissues in which ID proteins sustain attributes of normal stem cells (such as breast, prostate, muscle, neural and haematopoietic stem cells)[37,38,98–103] (BOX 1). In colon cancer stem cells, the combined expression of ID1 and ID3 increased both self-renewal and tumour initiation[83]. In agreement with these findings, ablation of ID genes *in vitro* increased the proportion of cancer stem cells that underwent symmetric cell division with differentiated cell fate, which caused the loss of stem cell markers in the progeny. Cancer stem cells have increased resistance to chemotherapeutic agents and, in accordance with this, silencing of ID1 and ID3 in culture-based assays sensitized cells to oxaliplatin[83]. In high-grade glioma, ID proteins are co-expressed in the diverse cell populations that constitute the tumour, including glioma stem cells. In an orthotopic model of brain cancer that was driven by the $HRAS^{V12}$ oncogene, deletion of conditional *ID1*, *ID2* and *ID3* alleles in the tumour cells decreased the glioma stem cell population (nestin-positive and stage-specific embryonic antigen 1 (SSEA1)-positive cells), blocked tumour growth and extended mouse survival. Furthermore, cells that were selected *in vitro* for the ability to self-renew were completely devoid of tumorigenic capacity if ID genes were deleted after the implantation of these cells into the mouse brain; cells retained robust tumorigenic potential if ID genes were intact[82]. This finding is in line with the observation that embryonic neural stem cells (NSCs) completely lose self-renewal

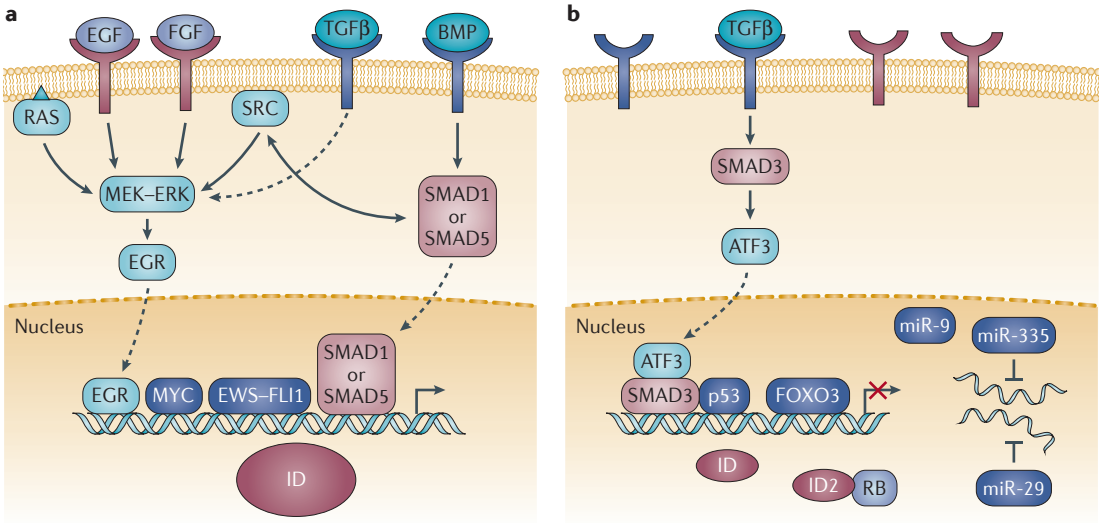

Figure 2 | **Regulation of ID protein expression by oncogenes and tumour-suppressor genes.** **a** | Receptor tyrosine kinases (such as epidermal growth factor receptor (EGFR) and fibroblast growth factor receptor (FGFR)), RAS and SRC converge on MEK–ERK to activate inhibitor of DNA binding (ID) gene transcription via the early growth response (EGR) transcription factors[47,52,121,197,198]. Notch signalling activates ID expression during developmental processes and in cancer cells[46,50,51]. MYC and Ewing's sarcoma (EWS)–Friend leukaemia integration 1 (FLI1) oncoproteins directly bind to the promoters of ID genes at E-boxes and ETS binding sites, respectively[32,199]. Intracellular signalling that is initiated by bone morphogenetic protein (BMP)–SMAD induces expression of ID through SMAD binding to BMP-responsive elements in the promoters of ID genes[200], whereas ID gene transcription that is induced by transforming growth factor-β (TGFβ) in some contexts might occur through non-canonical signalling pathways such as MEK–ERK (indicated by the dashed arrow)[134,135]. Crosstalk between SRC and BMP–SMAD signalling increases ID transcription[47]. **b** | The tumour suppressors p53 and forkhead box protein O3 (FOXO3) exert inhibitory activity on ID transcription[53,54]. Growth inhibitory signals by TGFβ–SMAD in epithelial cells induce the expression of activating transcription factor 3 (ATF3), which forms a repressive complex with SMAD on ID gene promoters[129,131,132]. Post-transcriptional inhibition of ID is executed by growth inhibitory microRNAs (such as miR-335, miR-9 and miR-29)[63,64]. Physical interaction between hypophosphorylated RB and ID2 further limits ID2 function[30–32]. Dashed arrows in part **a** and part **b** indicate intermediate steps before ID promoter activation or repression. EGF, epidermal growth factor; FGF, fibroblast growth factor.

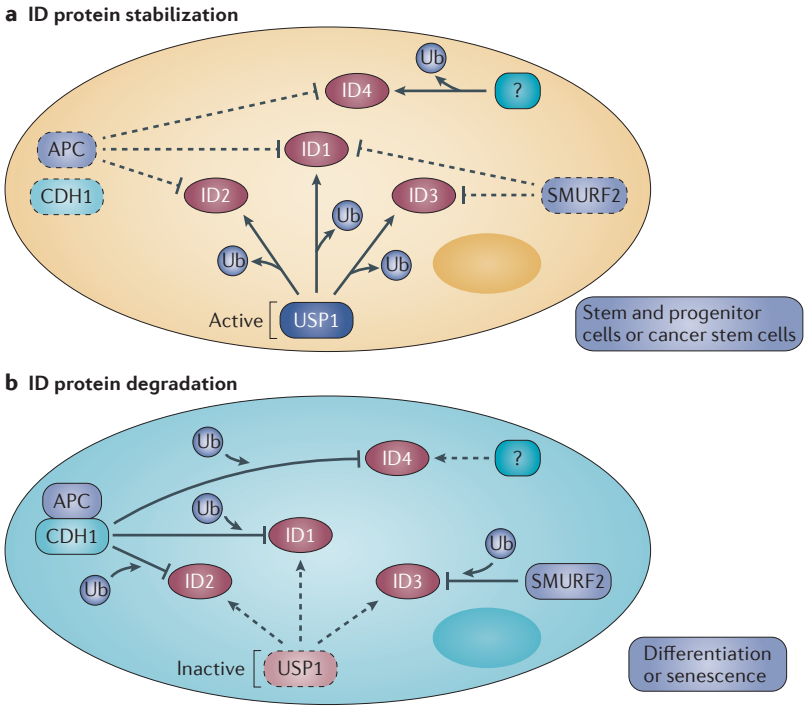

Figure 3 | **Control of ID protein stability by ubiquitin ligases and deubiquitylating enzymes.** **a** | In stem and progenitor cells (and potentially irreversibly in cancer stem cells) the ubiquitin (Ub) ligases that target inhibitor of DNA binding (ID) proteins for ubiquitin-mediated proteasomal degradation — anaphase-promoting complex/cyclosome (APC/C)–CDH1 and SMAD ubiquitylation regulatory factor 2 (SMURF2) — are inactive (shown by dashed outlines and dashed arrows)[92,94–96]. Conversely, the enzyme that deubiquitylates ID (ubiquitin-specific protease 1 (USP1)) is overexpressed. ID proteins are stable and accumulate at high levels to maintain stemness. **b** | During differentiation and senescence the ubiquitin ligases for ID proteins are active and the expression of USP1 is downregulated. ID proteins are ubiquitylated and destabilized, thereby triggering differentiation and cell cycle withdrawal[41].

and multi-potency in the absence of *ID1*, *ID2* and *ID3*. However, stem cell properties can be sustained to a level that is almost normal in cells that retain one *ID2* allele, which indicates that ID proteins function redundantly in NSCs[38]. Similarly, in the developing mouse brain, the inactivation of *Id1* and *Id3* (but neither gene alone) is required to trigger premature differentiation of NSCs[104]. The activation of ID1 and ID3 proteins in glioma stem cells is part of the oncogenic response to TGFβ[105]. Short hairpin RNA-mediated silencing of *ID1* and *ID3*, and treatment of glioma stem cells using inhibitors of the TGFβ receptor abrogated glioma stem cell properties *in vitro* and in orthotopic transplantation experiments. In mouse astrocytes that are deficient in cyclin-dependent kinase inhibitor 2A (*Cdkn2a*$^{-/-}$) the expression of ID4 induces glioma stem cell markers in association with activation of cyclin E and Notch signalling[48]. ID4 has also been shown to derepress miR-9*-mediated suppression of SRY-box 2 (*SOX2*), which leads to increased glioma stem cell potential and chemoresistance[106].

As with somatic stem cells (BOX 2), cancer stem cells are anchored to a niche and derive supportive signals through cell–cell contacts with endothelial cells in the blood vessels. The ability to adhere to the niche is a crucial feature of normal stem cells and cancer stem cells[107–111]. Loss of ID proteins disrupts stem cell adhesion to endothelial cells in the niche both in NSCs and in glioma stem cells[38,82] (FIG. 4). ID-mediated repression of bHLH transcription limits the expression of RAS-related protein 1 (RAP1) GTPase-activating protein (*RAP1GAP*) — a bHLH target gene that encodes an inhibitor of the RAP1 GTPase, which controls cell adhesion via integrin signalling[112]. When ID protein levels decrease, for example, at the time of neural differentiation or in the absence of ID proteins in Id-knockout mice, derepression of *Rap1gap* inhibits RAP1 and drives stem cell detachment from the niche. Thus, whereas in NSCs the ID–bHLH axis dynamically regulates cell intrinsic cues that direct stem cell interaction with the niche, the continuous block of bHLH activity by increased ID activity in glioma stem cells locks adhesion signals in an aberrant 'on' state. This is consistent with *RAP1GAP*, *ID2* and *ID3* being part of a five-gene prognostic signature in patients with high-grade glioma. Overall, in both colorectal cancer and malignant glioma, ID proteins function as 'classic' master regulators of stem cell identity, and their combined loss affects both the self-renewal and the tumour-initiating capacity of cancer stem cells (TABLE 3).

Experimental mouse models of high-grade glioma recently showed a different and intriguing function of ID proteins: cells with high ID1 expression manifested high self-renewal potential *in vitro* and tumorigenic capacity when injected orthotopically, whereas cells with low expression of ID1 had impaired *in vitro* self-renewal but, surprisingly, had more robust tumorigenicity[113]. This cell population might represent the cancer counterpart of the stem cell progeny that has been identified in the neurogenic areas of the brain as transit-amplifying progenitors. These progenitors, which express lower levels of ID proteins than NSCs[37], are capable of limited self-renewal but have high proliferative potential[114] and could be the cell of origin for different brain tumour subtypes[115]. The inverse correlation between ID1 expression and tumour progression might explain the slightly better prognosis for the subgroup of patients with glioblastoma that have a proneural gene expression signature and high ID1 expression compared with the subgroup of patients with low ID1, although both subgroups fare poorly. Thus, in certain tumour types, both stem-like cells and cells with features of committed progenitors might harbour the genetic events that confer the capacity to propagate tumours efficiently. These findings also indicate that targeting both cell populations will be important to effectively treat glioblastoma[113].

The function of ID proteins in cancer stem cell adhesion to the niche and the idea that self-renewal and tumour initiation might be attributes of distinct cell populations (stem cell-like cells and highly proliferative progenitors, respectively) warrant further investigations in other types of cancer. However, the documented instances in which cancer stem cells manifest dependency towards the expression of ID proteins for cancer initiation support the conclusion that the redundant expression of ID proteins as a whole preserves the cancer stem cell state.

Transit-amplifying progenitors
The progeny of stem cells that differentiate after a definite number of cell divisions.

ID proteins and angiogenesis

The first evidence that ID proteins have functions that are important for tumour progression, independently of their expression in cancer cells, came from studies that implicated ID in tumour angiogenesis[104,116,117]. ID1 and ID3 proteins accumulated in vascular endothelial cells of human primary tumours and mouse tumours, and *Id1*;*Id3* double-knockout mice that received xenografts or that were genetically engineered to produce tumours showed a marked loss of vascular integrity and delayed tumour progression. The endothelial cell-intrinsic role of ID proteins has been linked to their ability to increase the mobilization and proliferation of bone marrow-derived endothelial progenitor cells (EPCs) that are recruited to form tumour blood vessels[118]. This essential activity of ID proteins indicated that anti-angiogenic therapies that use ID protein inhibition might be useful in the clinic. Increased expression of ID proteins in cancer cells has also been implicated in the promotion of tumour angiogenesis. In both mouse models of cancer and human tumour cells, ID proteins induce the expression of pro-angiogenic factors — hypoxia-inducible factor-1α (HIF1α), vascular endothelial growth factor A (VEGFA)[33], fibroblast growth factor receptor 1 (FGFR1)[119] and cytokines (such as growth-regulated-α (GROα; also known as CXCL1)), interleukin-6 (IL-6) and IL-8 — that increase endothelial cell proliferation and migration and that might influence the biological properties of other cell types in the tumour microenvironment[120,121] (TABLE 3).

Tumour invasion and metastasis

Evidence for the important role of ID genes as potential drivers of metastasis comes from the presence of *ID1* among the 18 genes that constitute the lung metastasis signature for breast cancer cells[122]. *In vivo*, silencing of *ID1* and *ID3*, both of which are highly expressed in metastatic breast cancer cells, impairs metastasis by human breast cancer cell lines and breast cancer cells that are derived from mouse mammary tumour virus (*MMTV*)–*NeuYD*- and *MMTV–Wnt1*-transgenic mice[123]. These studies confirmed earlier findings that the inhibition of ID1 expression has a significant impact on the ability of breast cancer cells to metastasize in xenograft models[124]. The function of ID proteins in metastatic colonization is twofold: increased invasiveness through induction of an EMT programme at the primary site and increased ability to colonize the secondary site through reversal to an epithelial state (mesenchymal-to-epithelial transition (MET)). It has been suggested that ID proteins can promote invasiveness through the extracellular matrix and the increased production of matrix metalloproteinases (MMPs)[124]. However, other studies reported that knockdown of ID1 and ID3 had little effect on the ability of breast cancer cells to extravasate into the lung parenchyma[123]. ID1 also emerged as a direct target of Krüppel-like factor 17 (KLF17), which is a transcriptional repressor that suppresses metastasis in a mouse model of breast cancer, and upregulation of ID1 was essential for both EMT and the metastatic phenotype when KLF17 was knocked down[125]. This pathway has clinical relevance because low KLF17 and high ID1 expression levels in the primary tumour are characteristic of patients with breast cancer who have lymph node metastasis at diagnosis[125]. These pro-metastatic functions of ID proteins in breast cancer suggest mechanistic links between high expression of ID proteins and tumour-initiating and tumour-colonization competency, which is a key attribute of cancer stem cells[126,127].

Studies on the molecular pathways that are engaged by ID proteins to drive invasion and metastasis are still in their infancy. It has been shown that hyperactivation of ID increases migratory features through the inhibition of bHLH-mediated transcription of anti-metastatic genes such as semaphorin 3F (*SEMA3F*)[128]. In primary epithelial tumours, ID protein expression is associated with EMT[127,129,130], which can be activated by cytokines of the TGFβ superfamily. In normal cells the activity of TGFβ is usually linked to the rapid inhibition of ID gene expression[32,131–132]. Conversely, EMT that is induced by TGFβ in epithelial cancer cells is associated with an increase of ID1 expression[130], and recent evidence suggests that the TGFβ-mediated increase of ID1 in mesenchymal breast cancer cells is mediated through the CBP/p300 co-activator[133]. It is possible, although not experimentally proven, that the degree of activation by TGFβ of the expression of mitogenic cytokines and non-canonical TGFβ signalling pathways (MEK–ERK and PI3K, among others) dictates the readout of ID protein levels[134,135]. When they are ectopically expressed *in vitro*, ID proteins have been shown to inhibit or promote EMT. Suppression of EMT has been linked to the inhibition of E47-mediated repression of E-cadherin (also known as cadherin 1)[136,137]. The molecular mechanisms of induction of EMT by overexpression of ID proteins remain unclear. ID proteins might drive the molecular switch

Box 1 | **ID function in normal stem cells**

In embryonic stem cells, somatic stem cells and progenitors, inhibitor of DNA binding (ID) family members are required to preserve stem cell identity and prevent premature differentiation (FIG. 1). The initial studies that linked the properties of stem cells to ID activity showed that in embryonic stem cells pluripotency is sustained by bone morphogenetic protein 4 (BMP4)-mediated expression of ID genes via SMAD signalling[42]. Moreover, the negative effect of *ID1* deletion on the expression of *NANOG*, which is a key factor in maintaining pluripotency, and the positive effect on *Brachyury*, which is a mesendoderm differentiation factor — but not on pro-neural factors — suggested that ID1 and possibly other ID proteins control self-renewal and cell fate determination through different pathways[39]. Several studies have linked ID proteins to embryonic and adult somatic stem cells in different tissue types. In neural stem cells (NSCs) and progenitors, multiple ID family members preserve stem cell properties[37,38,54,176–178]. High levels of ID1 identify type B1 adult NSCs, and ID1 and ID3 are required to maintain the self-renewal capacity of this cell population[37]. Adhesion of NSCs to the niche microenvironment is a fundamental feature that has recently been attributed to ID protein activity via repression of basic helix–loop–helix (bHLH) transcription[38]. ID proteins have been recognized as key factors for stem cell identity in other somatic stem cells, including in murine models of haematopoiesis, in pancreatic and lung development and in adult muscle[98–103]. Collectively, the contributions of ID proteins to the self-renewal of embryonic and somatic stem cells arise from a combination of integrated transcriptional events, most of which are caused by the sequestration of ubiquitously expressed bHLH proteins. This sustains the expression of master regulators of self-renewal and prevents the activation of differentiation programmes and the expression of cell cycle inhibitors.

that upregulates N-cadherin (also known as cadherin 2), which is a key molecular determinant of EMT[138–140]. Aberrant expression of N-cadherin increases the levels of active RHOA, RAC1 and cell division control protein 42 (CDC42), which are small GTPases that are associated with EMT[141,142] and cell motility and that are regulated by ID proteins[138]. Furthermore, the transcriptional repression of *RAP1GAP* by ID proteins unleashes the activity of RAP1, which is a key regulator of cellular adhesion that triggers integrin-mediated cell–extracellular matrix interactions and promotes invasion[82].

Importantly, the essential role of ID proteins in reinitiating proliferative programmes that are necessary for formation of secondary tumours[123] and the observation that MET is necessary for metastatic seeding[143,144] suggested that ID activity in disseminated tumour cells in the lung might also have a role in MET. Indeed, recent evidence indicates that ID proteins are required for early metastatic colonization of breast cancer cells in the lung owing to the inhibition of the mesenchymal factor TWIST and to the induction of MET. Conversely, ID proteins cannot inhibit SNAIL in the primary tumour, and this preserves EMT[133].

In addition to their tumour cell-intrinsic pro-metastatic function, ID proteins also increase metastatic progression when aberrantly expressed in bone marrow-derived EPCs[145]. Besides their contribution to tumour angiogenesis, which can nevertheless influence the formation of macrometastasis, EPCs also seem to participate in the early stages of metastatic colonization, probably through the production of pro-angiogenic growth factors that facilitate the growth of micrometastatic lesions to macrometastasis[146]. Knockdown of ID1 in bone marrow-derived EPCs but not in tumour cells leads to a significant reduction of pulmonary macrometastasis. Thus, similar to the role of ID proteins in tumour angiogenesis, a dual function of ID proteins — in tumour cells and EPCs — seems to promote tumour invasion and metastasis.

Box 2 | **The stem cell niche**

Stem cells are capable of long-term self-renewal and differentiation and are maintained in appropriate numbers at defined locations. In these locations a specialized extracellular environment defines a supportive stem cell niche and regulates stem cell functions. This complex environment is composed of cells, stem cells and supportive cells (such as stromal cells and endothelial cells in the blood vessels), extracellular matrix (ECM), as well as the signalling molecules that are associated with each population of stem cells. In its simplest form, the niche hypothesis describes a heterologous cell interaction, which fosters the preservation of the stem cell state[179–182]. However, more functions of the niche have been identified as our understanding of how stem cells behave has evolved in parallel with the identification of an increasingly diverse array of participating elements of the microenvironment that regulates these cells[183–186]. Stem cell niches have been identified and characterized in many tissues, including the germ line, bone marrow, digestive and respiratory systems, skeletal muscle, skin, hair follicle, mammary gland, liver and the central and peripheral nervous systems[186–192]. Extensive studies have begun to elucidate the crucial components of many stem cell niches, which include specific mesenchymal, vascular, neuronal, glial and inflammatory cell types, diffusible and cell-surface-associated signalling molecules, and physical parameters such as matrix rigidity, oxygen tension and temperature[183–186,193–196]. In adults, an important function of the niche is to maintain stem cells in the quiescent state and to control the balance between self-renewal and differentiation. The cancer stem cell hypothesis has changed the perspective on cancer. It is yet to be determined whether, similar to normal stem cells, cancer stem cells are dependent on niche signalling and whether genetic lesions in cancer target niche-signalling factors.

Escape from senescence and cell death

ID proteins also function in tumour progression by inhibiting programmed cell death and promoting tumour cell survival. The anti-apoptotic function of ID proteins has been reported in various cell and cancer types, including endothelial and pancreatic β-cells, oesophageal and nasopharyngeal carcinoma, as well as prostate, breast and ovarian cancer[60,138,147–156]. Aberrant levels of ID proteins have been associated with the upregulation of anti-apoptotic and pro-survival factors (BCL-2, BCL-X_L, PI3K–AKT and nuclear factor-κB (NF-κB))[150,157], and the inhibition of pro-apoptotic signals (CDKN1A (also known as p21), CDKN2A and BIM (also known as BCL2L11))[155,158] (TABLE 3). Targeting ID proteins in cancer cells using genetic knockdown, small interfering RNA (siRNA) or specific peptide aptamers induces programmed cell death[60,152–154]. In prostate, breast, lung and oesophageal cancer, reduction of ID protein expression also restored cell death that was induced by chemotherapeutic agents or cytokines[59,60,83,106].

The oncogenic role of ID proteins in cancers of epithelial origin might relate to their ability to overcome the barrier of RAS-induced senescence, which inhibits the oncogenic activity of RAS. In particular, the essential role of ID1 in this process is underscored by the observation that a substantial proportion of HRAS-V12-driven breast tumours regressed when senescence was restored after ablation of ID1 (REF. 159). The molecular determinants that are recruited by ID proteins to release cells from senescence are unclear. They may or may not coincide with the downregulation of the cyclin-dependent kinase inhibitors CDKN2A and CDKN1A, which are known inducers of senescence[25,155,158] (TABLE 3). Nevertheless, breast cancer cells that are transformed by activated HRAS and ID1 show addiction to ID1 expression, thereby supporting the idea that ID1 is an attractive therapeutic target in breast cancer[159]. Although ID1 cooperates with HRAS-V12 to transform mammary epithelial cells, *ID1* alone fails to promote neoplastic transformation after transgenic expression *in vivo* in both the mouse mammary gland and the prostate[160,161]. It is conceivable that ID proteins have context-dependent transforming activity because transgenic ID1 or ID2 expression in lymphoid cells generates tumours[162,163]. Although we have a deeper understanding of the functions of ID proteins in tumour maintenance and progression, the transforming role of ID proteins is postulated on the basis of their capacity to inhibit a powerful and multimodal tumour-suppressive pathway, the RB pathway, via the direct inactivation of RB by ID2 and possibly ID4 (REFS 29–33) or indirectly through the blocking of ETS-mediated expression of INK4A by ID1 (REF. 27).

ID proteins: therapeutic targets in cancer

Several studies have indicated that individual or multiple ID proteins are valid therapeutic targets in mouse models of cancer and human tumour cell lines (reviewed in REF. 13).

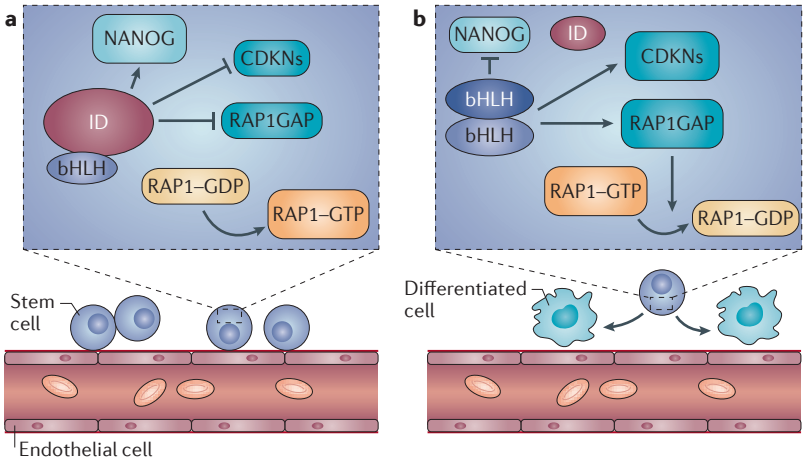

Figure 4 | **ID proteins and the perivascular niche.** **a** | In the presence of high amounts of inhibitor of DNA binding (ID) proteins, transcription that is mediated by basic helix–loop–helix (bHLH) transcription factors is repressed in normal and cancer stem cells, and this results in the induction of stem cell factors such as NANOG[39] and the transcriptional inhibition of cyclin-dependent kinase inhibitors (CDKNs) and RAS-related protein 1 (RAP1) GTPase-activating protein (RAP1GAP)[38]. In the absence of RAP1GAP, RAP1 is preserved in its active state (RAP1–GTP), which results in the activation of integrin signalling and the anchorage of normal stem cells and cancer stem cells to the perivascular niche. **b** | Loss of ID proteins derepresses bHLH transcription, which leads to downregulation of NANOG and induction of CDKNs and RAP1GAP. Loss of RAP1–GTP triggers the release of normal stem cells and cancer stem cells from the perivascular niche, with loss of stem cell characteristics and tumour-initiating capacity.

The anti-ID approaches that have been tested so far can be summarized into two categories, those that aim to extinguish ID gene expression through *in vivo* delivery of ID-specific siRNA molecules and those that use specific peptides to target the protein–protein interaction properties of ID proteins. Several studies have successfully silenced ID genes by transducing siRNAs into cancer cells *in vitro* and have reported a significant effect on tumour growth in xenograft experiments using immunodeficient mice. In a more clinically meaningful system, siRNAs that targeted *ID1* were fused to a peptide (ID1–peptide-conjugated antisense oligonucleotide (ID1–PCAO)) that was known to specifically localize to the tumour neovasculature[164]. In two different mouse models (breast cancer and lung carcinoma) that are characterized by abundant expression of ID1 in the tumour vasculature, systemic delivery of ID1–PCAO using an osmotic pump resulted in successful targeting of the tumour vasculature that ultimately led to increased intra-tumour haemorrhage, hypoxia and the inhibition of growth of primary tumours and metastases compared with controls. The antitumour effects of ID1–PCAO were further potentiated by a combined treatment with the heat shock protein 90 (HSP90) inhibitor 17-allyamino-17-demethoxygeldanamycin (17-AAG), which is a compound that was previously shown to reduce tumour burden by cooperating with genetic ablation of ID genes[165]. Another example of successful delivery of an ID-specific siRNA *in vivo* involved the incorporation of siRNAs into neutral liposomes. Intra-peritoneal administration of a liposome–ID2 siRNA complex into nude mice with subcutaneous xenografts and liver metastasis of human colorectal carcinoma cells reduced tumour burden[148]. A similar but more technologically innovative approach has recently been reported[71]. From a large-scale screening effort, *ID4* was shown to be an important oncogene in ovarian cancer: it was amplified in more than 30% of the cases analysed. Tumour-penetrating nanocomplexes (TPNs) that contained siRNAs against *ID4* limited the growth of established ovarian tumours in mice and extended survival, thereby indicating that ID4 is a potential therapeutic target in ovarian cancer. Although limited by a short-term evaluation, these studies seem to indicate that inhibiting the activity of a specific ID protein does not result in deleterious general or organ-specific effects. Systemic treatment with ID1–PCAO did not affect weight or wound healing, and kidneys were histologically normal 3 weeks after the completion of the treatment. Furthermore, animals that were treated with TPN–ID4 lacked macroscopic or histological signs of organ (bladder, spleen, heart, kidney, liver and ovary) toxicity after a 40-day intraperitoneal administration.

The challenge of targeting protein–protein interactions using small-molecule inhibitors has considerably limited progress towards the identification of bona fide molecules against ID that could be introduced into the clinic[166,167]. A potential solution to the complexity of identifying compounds that have specific ID-inhibitory properties came from the identification of a peptide named 13I, which was found using a phage-display library of HLH domains that harbour amino acid substitutions in residues that are crucial for dimerization[168]. 13I can bind to ID proteins and function as a dominant-interfering inhibitor[169]. Exposure of human neuroblastoma cells to 13I increased TCF3-mediated transcriptional activity, decreased the production of VEGFA and inhibited cell proliferation, invasion and colony formation in soft agar. The value of inhibiting the ID–bHLH heterodimerization is supported by the targeting of MYC using a mutant bHLH–leucine zipper domain called Omomyc[170]. ID-binding peptides were also designed on the basis of the amino acid sequence of the bHLH protein myoblast determination protein 1 (MYOD1)[171]. These peptides have a high affinity for ID1, interfere with ID1 binding to MYOD1 and other bHLH proteins, and cause a proliferative block in cancer cells. In another study, the peptide aptamer ID1/3-PA7 was identified using a yeast two-hybrid screen as a specific interactor with ID1 and ID3, as well as an activator of bHLH-mediated transcription[153,154]. Treatment of breast and ovarian cancer cells with ID1/3-PA7 induced cell cycle arrest and apoptosis *in vitro*. The above studies validate the idea that peptides can be used to interfere with the ability of ID proteins to form complexes with their cellular partners, but therapeutically effective delivery of peptides to cancer cells *in vivo* will require substantial technological developments. Besides technical obstacles to ID-specific therapy, important unanswered questions remain. Can ID inhibition eradicate an advanced disease? Can tumours develop resistance to ID inhibition and, given the redundancy of ID protein activity, how much will resistance depend on compensation by other ID family members? It is important to mention that, besides the therapeutic effects of direct ID targeting,

Osmotic pump
A device that uses the principles of osmotic pressure for the controlled delivery of drugs.

different types of antitumour agents indirectly modulate ID protein levels[105,172–175]. It is possible that downregulation of ID proteins is necessary for the antitumour efficacy of some drugs and deregulated ID protein activity could contribute to drug resistance[59,60,83,106].

Future directions

Several controversies about the functions of ID proteins in cancer have been settled, and it is established that in most human tumours the deregulated expression of ID proteins can be detected both in cancer cells and in the various cell types that facilitate tumour growth. ID deregulation in cancer promotes several tumour-specific phenotypes and thereby promotes tumour progression. By implementing a stem cell-like programme in cancer cells, ID proteins can be considered to be an important target for disabling the many hallmarks of malignancy that are sustained by cancer stem cells. In addition, after more than 20 years of research, high levels of ID protein expression in cancer cells and cells in the tumour microenvironment have emerged as important potential prognostic and diagnostic markers in several tumour types. Technological advances for the reliable and specific targeting of protein–protein interactions are being used to develop drugs that target ID proteins. However, in order for this to be successful, a detailed understanding of the mechanistic complexities that govern the role of individual and multiple ID proteins in each tumour type will be needed. For example, it seems plausible that when co-expressed in the same or different cell types of individual tumours, ID proteins function redundantly to increase tumour aggressiveness. However, the opposing functions that are implemented by different ID family members in specific tumour types mandate a cautionary note regarding simple generalizations. Furthermore, although individual ID genes are mostly dispensable for adult tissues, whether manipulation of potentially redundant ID functions by ID-specific therapeutics will affect the homeostasis of normal adult human tissues remains an important question that will have to be addressed.

The past few years have seen the development and acceptance of the concept that ID proteins are master regulators of a multitude of phenotypes, which collectively create the normal stem cell state. Therefore, it is understandable why increased ID protein expression is required for cancer cells to revert to an embryonic-like phenotype. ID proteins are the molecular proof that David Paul von Hansemann was correct when he referred to tumour anaplasia as an 'embryonic' state. Since then, we have also assigned fundamental roles in progression and maintenance of the most lethal types of human tumours to these 'embryonic cancer cells'. With the current wealth of knowledge of ID protein function and regulation, the pharmacological manipulation of these proteins is undoubtedly a promising venture on the horizon.

1. von Hansemann, D. P. On the asymmetrical cell division in epithelial cancers and its biological significance. *Virchows Arch.* **119**, 299–326 (1890).
2. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
3. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
4. Driessens, G., Beck, B., Caauwe, A., Simons, B. D. & Blanpain, C. Defining the mode of tumour growth by clonal analysis. *Nature* **488**, 527–530 (2012).
5. Hu, J. *et al.* Neutralization of terminal differentiation in gliomagenesis. *Proc. Natl Acad. Sci. USA* **110**, 14520–14527 (2013).
6. Schonberg, D. L., Venere, M. & Rich, J. N. Changing the fate of cancer, one splice at a time. *Proc. Natl Acad. Sci. USA* **110**, 14510–14511 (2013).
7. Barker, N., Bartfeld, S. & Clevers, H. Tissue-resident adult stem cell populations of rapidly self-renewing organs. *Cell Stem Cell* **7**, 656–670 (2010).
8. Chambers, I. & Tomlinson, S. R. The transcriptional foundation of pluripotency. *Development* **136**, 2311–2322 (2009).
9. Holmberg, J. & Perlmann, T. Maintaining differentiated cellular identity. *Nature Rev. Genet.* **13**, 429–439 (2012).
10. Kim, J. *et al.* A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. *Cell* **143**, 313–324 (2010).
11. Suva, M. L., Riggi, N. & Bernstein, B. E. Epigenetic reprogramming in cancer. *Science* **339**, 1567–1570 (2013).
12. Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. & Weintraub, H. The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. *Cell* **61**, 49–59 (1990).
In this study, the first ID gene, *Id1*, was cloned and the ID1 protein was identified as an inhibitor of DNA binding of bHLH transcription factors.
13. Perk, J., Iavarone, A. & Benezra, R. Id family of helix-loop-helix proteins in cancer. *Nature Rev. Cancer* **5**, 603–614 (2005).
14. Ellis, H. M., Spann, D. R. & Posakony, J. W. *extramacrochaetae*, a negative regulator of sensory organ development in *Drosophila*, defines a new class of helix-loop-helix proteins. *Cell* **61**, 27–38 (1990).
15. Garrell, J. & Modolell, J. The *Drosophila extramacrochaetae* locus, an antagonist of proneural genes that, like these genes, encodes a helix-loop-helix protein. *Cell* **61**, 39–48 (1990).
16. Biggs, J., Murphy, E. V. & Israel, M. A. A human Id-like helix-loop-helix protein expressed during early development. *Proc. Natl Acad. Sci. USA* **89**, 1512–1516 (1992).
17. Christy, B. A. *et al.* An Id-related helix-loop-helix protein encoded by a growth factor-inducible gene. *Proc. Natl Acad. Sci. USA* **88**, 1815–1819 (1991).
18. Riechmann, V., van Cruchten, I. & Sablitzky, F. The expression pattern of Id4, a novel dominant negative helix-loop-helix protein, is distinct from Id1, Id2 and Id3. *Nucleic Acids Res.* **22**, 749–755 (1994).
19. Sun, X. H., Copeland, N. G., Jenkins, N. A. & Baltimore, D. Id proteins Id1 and Id2 selectively inhibit DNA binding by one class of helix-loop-helix proteins. *Mol. Cell Biol.* **11**, 5603–5611 (1991).
20. Massari, M. E. & Murre, C. Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. *Mol. Cell Biol.* **20**, 429–440 (2000).
21. Norton, J. D., Deed, R. W., Craggs, G. & Sablitzky, F. Id helix-loop-helix proteins in cell growth and differentiation. *Trends Cell Biol.* **8**, 58–65 (1998).
22. Quong, M. W., Romanow, W. J. & Murre, C. E protein function in lymphocyte development. *Annu. Rev. Immunol.* **20**, 301–322 (2002).
23. Engel, I. & Murre, C. The function of E– and Id proteins in lymphocyte development. *Nature Rev. Immunol.* **1**, 193–199 (2001).
24. Gonda, H. *et al.* The balance between Pax5 and Id2 activities is the key to AID gene expression. *J. Exp. Med.* **198**, 1427–1437 (2003).
25. Ohtani, N. *et al.* Opposing effects of Ets and Id proteins on p16INK4a expression during cellular senescence. *Nature* **409**, 1067–1070 (2001).
26. Roberts, E. C., Deed, R. W., Inoue, T., Norton, J. D. & Sharrocks, A. D. Id helix-loop-helix proteins antagonize pax transcription factor activity by inhibiting DNA binding. *Mol. Cell Biol.* **21**, 524–533 (2001).
27. Yates, P. R., Atherton, G. T., Deed, R. W., Norton, J. D. & Sharrocks, A. D. Id helix-loop-helix proteins inhibit nucleoprotein complex formation by the TCF ETS-domain transcription factors. *EMBO J.* **18**, 968–976 (1999).
28. O'Brien, P., Morin, P. Jr., Ouellette, R. J. & Robichaud, G. A. The *Pax-5* gene: a pluripotent regulator of B-cell differentiation and cancer disease. *Cancer Res.* **71**, 7345–7350 (2011).
29. Iavarone, A., Garg, P., Lasorella, A., Hsu, J. & Israel, M. A. The helix-loop-helix protein Id-2 enhances cell proliferation and binds to the retinoblastoma protein. *Genes Dev.* **8**, 1270–1284 (1994).
30. Iavarone, A. *et al.* Retinoblastoma promotes definitive erythropoiesis by repressing Id2 in fetal liver macrophages. *Nature* **432**, 1040–1045 (2004).
31. Lasorella, A., Iavarone, A. & Israel, M. A. Id2 specifically alters regulation of the cell cycle by tumor suppressor proteins. *Mol. Cell Biol.* **16**, 2570–2578 (1996).
32. Lasorella, A., Noseda, M., Beyna, M., Yokota, Y. & Iavarone, A. Id2 is a retinoblastoma protein target and mediates signalling by Myc oncoproteins. *Nature* **407**, 592–598 (2000).
This study uses mouse models to establish that ID2 is controlled by the retinoblastoma tumour suppressor and is activated by MYC.
33. Lasorella, A., Rothschild, G., Yokota, Y., Russell, R. G. & Iavarone, A. Id2 mediates tumor initiation, proliferation, and angiogenesis in *Rb* mutant mice. *Mol. Cell Biol.* **25**, 3563–3574 (2005).
34. Popova, M. K., He, W., Korenjak, M., Dyson, N. J. & Moon, N. S. Rb deficiency during *Drosophila* eye development deregulates EMC, causing defects in the development of photoreceptors and cone cells. *J. Cell Sci.* **124**, 4203–4212 (2011).
35. Rothschild, G., Zhao, X., Iavarone, A. & Lasorella, A. E proteins and Id2 converge on $p57^{Kip2}$ to regulate cell cycle in neural cells. *Mol. Cell Biol.* **26**, 4351–4361 (2006).
36. Hirai, S. *et al.* RP58 controls neuron and astrocyte differentiation by downregulating the expression of *Id1–4* genes in the developing cortex. *EMBO J.* **31**, 1190–1202 (2012).
37. Nam, H. S. & Benezra, R. High levels of Id1 expression define B1 type adult neural stem cells. *Cell Stem Cell* **5**, 515–526 (2009).

38. Niola, F. *et al.* Id proteins synchronize stemness and anchorage to the niche of neural stem cells. *Nature Cell Biol.* **14**, 477–487 (2012).
This paper shows how ID genes control a neural stem cell-intrinsic transcriptional programme that preserves stem cell adhesion to the niche through regulation of RAP1GAP, which is a repressor of RAP1 GTPase activity.
39. Romero-Lanman, E. E., Pavlovic, S., Amlani, B., Chin, Y. & Benezra, R. Id1 maintains embryonic stem cell self-renewal by up-regulation of Nanog and repression of Brachyury expression. *Stem Cells Dev.* **21**, 384–393 (2012).
40. Suh, H. C. *et al.* Id1 immortalizes hematopoietic progenitors *in vitro* and promotes a myeloproliferative disease *in vivo*. *Oncogene* **27**, 5612–5623 (2008).
41. Williams, S. A. *et al.* USP1 deubiquitinates ID proteins to preserve a mesenchymal stem cell program in osteosarcoma. *Cell* **146**, 918–930 (2011).
In this study USP1 is identified as the deubiquitylating enzyme for ID proteins. USP1 stabilizes ID and preserves the mesenchymal stem cell state in osteosarcoma.
42. Ying, Q. L., Nichols, J., Chambers, I. & Smith, A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* **115**, 281–292 (2003).
43. Love, C. *et al.* The genetic landscape of mutations in Burkitt lymphoma. *Nature Genet.* **44**, 1321–1325 (2012).
44. Richter, J. *et al.* Recurrent mutation of the *ID3* gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. *Nature Genet.* **44**, 1316–1320 (2012).
45. Schmitz, R. *et al.* Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature* **490**, 116–120 (2012).
References 43, 44 and 45 implicate *ID3* as a tumour suppressor gene in sporadic cases of Burkitt's lymphoma.
46. Chadwick, N. *et al.* Identification of novel Notch target genes in T cell leukaemia. *Mol. Cancer* **8**, 35 (2009).
47. Gautschi, O. *et al.* Regulation of Id1 expression by SRC: implications for targeting of the bone morphogenetic protein pathway in cancer. *Cancer Res.* **68**, 2250–2258 (2008).
48. Jeon, H. M. *et al.* Inhibitor of differentiation 4 drives brain tumor-initiating cell genesis through cyclin E and notch signaling. *Genes Dev.* **22**, 2028–2033 (2008).
49. Lasorella, A. *et al.* Id2 is critical for cellular proliferation and is the oncogenic effector of N-myc in human neuroblastoma. *Cancer Res.* **62**, 301–306 (2002).
50. Meier-Stiegen, F. *et al.* Activated Notch1 target genes during embryonic cell differentiation depend on the cellular context and include lineage determinants and inhibitors. *PLoS ONE* **5**, e11481 (2010).
51. Reynaud-Deonauth, S. *et al.* Notch signaling is involved in the regulation of *Id3* gene transcription during *Xenopus* embryogenesis. *Differentiation* **69**, 198–208 (2002).
52. Tam, W. F. *et al.* Id1 is a common downstream target of oncogenic tyrosine kinases in leukemic cells. *Blood* **112**, 1981–1992 (2008).
53. Birkenkamp, K. U. *et al.* FOXO3a induces differentiation of Bcr-Abl-transformed cells through transcriptional down-regulation of Id1. *J. Biol. Chem.* **282**, 2211–2220 (2007).
54. Paolella, B. R. *et al.* p53 directly represses Id2 to inhibit the proliferation of neural progenitor cells. *Stem Cells* **29**, 1090–1101 (2011).
55. Mathas, S. *et al.* Gene deregulation and spatial genome reorganization near breakpoints prior to formation of translocations in anaplastic large cell lymphoma. *Proc. Natl Acad. Sci. USA* **106**, 5831–5836 (2009).
56. Schuller, H. M. Is cancer triggered by altered signalling of nicotinic acetylcholine receptors? *Nature Rev. Cancer* **9**, 195–205 (2009).
57. Improgo, M. R., Tapper, A. R. & Gardner, P. D. Nicotinic acetylcholine receptor-mediated mechanisms in lung cancer. *Biochem. Pharmacol.* **82**, 1015–1021 (2011).
58. Pillai, S. *et al.* ID1 facilitates the growth and metastasis of non-small cell lung cancer in response to nicotinic acetylcholine receptor and epidermal growth factor receptor signaling. *Mol. Cell Biol.* **31**, 3052–3067 (2011).
59. Ponz-Sarvise, M. *et al.* Inhibitor of differentiation-1 as a novel prognostic factor in NSCLC patients with adenocarcinoma histology and its potential contribution to therapy resistance. *Clin. Cancer Res.* **17**, 4155–4166 (2011).
60. Trevino, J. G. *et al.* Nicotine induces inhibitor of differentiation-1 in a Src-dependent pathway promoting metastasis and chemoresistance in pancreatic adenocarcinoma. *Neoplasia* **14**, 1102–1114 (2012).
61. Rothschild, S. I. *et al.* MicroRNA-29b is involved in the Src-ID1 signaling pathway and is dysregulated in human lung adenocarcinoma. *Oncogene* **31**, 4221–4232 (2012).
62. Rothschild, S. I. *et al.* MicroRNA-381 represses ID1 and is deregulated in lung adenocarcinoma. *J. Thorac Oncol.* **7**, 1069–1077 (2012).
63. Annibali, D. *et al.* A new module in neural differentiation control: two microRNAs upregulated by retinoic acid, miR-9 and -103, target the differentiation inhibitor ID2. *PLoS ONE* **7**, e40269 (2012).
64. Heyn, H. *et al.* MicroRNA miR-335 is crucial for the BRCA1 regulatory cascade in breast cancer development. *Int. J. Cancer* **129**, 2797–2806 (2011).
65. Beger, C. *et al.* Identification of Id4 as a regulator of BRCA1 expression by using a ribozyme-library-based inverse genomics approach. *Proc. Natl Acad. Sci. USA* **98**, 130–135 (2001).
66. Welcsh, P. L. *et al.* BRCA1 transcriptionally regulates genes involved in breast tumorigenesis. *Proc. Natl Acad. Sci. USA* **99**, 7560–7565 (2002).
67. de Candia, P., Benera, R. & Solit, D. B. A role for Id proteins in mammary gland physiology and tumorigenesis. *Adv. Cancer Res.* **92**, 81–94 (2004).
68. Roldan, G., Delgado, L. & Muse, I. M. Tumoral expression of BRCA1, estrogen receptor α and ID4 protein in patients with sporadic breast cancer. *Cancer Biol. Ther.* **5**, 505–510 (2006).
69. Wen, Y. H. *et al.* Id4 protein is highly expressed in triple-negative breast carcinomas: possible implications for BRCA1 downregulation. *Breast Cancer Res. Treat.* **135**, 93–102 (2012).
70. Park, S. J., Kim, R. J. & Nam, J. S. Inhibitor of DNA-binding 4 contributes to the maintenance and expansion of cancer stem cells in 4T1 mouse mammary cancer cell line. *Lab. Anim. Res.* **27**, 333–338 (2011).
71. Ren, Y. *et al.* Targeted tumor-penetrating siRNA nanocomplexes for credentialing the ovarian cancer oncogene *ID4*. *Sci. Transl Med.* **4**,147ra112 (2012).
This paper shows that the *ID4* gene is amplified in ovarian cancer and that *ID4*-specific siRNA in a tumour-penetrating nanocomplex suppresses tumour growth and improves the survival of tumour-bearing mice.
72. Campo, E. *et al.* The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood* **117**, 5019–5032 (2011).
73. Bain, G. *et al.* E2A deficiency leads to abnormalities in αβ T-cell development and to rapid development of T-cell lymphomas. *Mol. Cell Biol.* **17**, 4782–4791 (1997).
74. Engel, I. & Murre, C. Disruption of pre-TCR expression accelerates lymphomagenesis in E2A-deficient mice. *Proc. Natl Acad. Sci. USA* **99**, 11322–11327 (2002).
75. Engel, I. & Murre, C. E2A proteins enforce a proliferation checkpoint in developing thymocytes. *EMBO J.* **23**, 202–211 (2004).
76. Herblot, S., Aplan, P. D. & Hoang, T. Gradient of E2A activity in B-cell development. *Mol. Cell Biol.* **22**, 886–900 (2002).
77. Li, J. *et al.* Mutation of inhibitory helix-loop-helix protein Id3 causes γδ T-cell lymphoma in mice. *Blood* **116**, 5615–5621 (2010).
78. Ciarrocchi, A. *et al.* Id1 restrains p21 expression to control endothelial progenitor cell formation. *PLoS ONE* **2**, e1338 (2007).
79. Funato, N., Ohtani, K., Ohyama, K., Kuroda, T. & Nakamura, M. Common regulation of growth arrest and differentiation of osteoblasts by helix-loop-helix factors. *Mol. Cell Biol.* **21**, 7416–7428 (2001).
80. Hertel, C. B., Zhou, X. G., Hamilton-Dutoit, S. J. & Junker, S. Loss of B cell identity correlates with loss of B cell-specific transcription factors in Hodgkin/Reed-Sternberg cells of classical Hodgkin lymphoma. *Oncogene* **21**, 4908–4920 (2002).
81. Liu, Y., Encinas, M., Comella, J. X., Aldea, M. & Gallego, C. Basic helix-loop-helix proteins bind to TrkB and p21(Cip1) promoters linking differentiation and cell cycle arrest in neuroblastoma cells. *Mol. Cell Biol.* **24**, 2662–2672 (2004).
82. Niola, F. *et al.* Mesenchymal high-grade glioma is maintained by the ID-RAP1 axis. *J. Clin. Invest.* **123**, 405–417 (2013).
This study uses a mouse model of malignant glioma to show that ID proteins are necessary for glioma genesis and maintenance, and it identifies a five-gene prognostic signature in human samples, including ID, bHLH, *RAP1GAP* and *CDKN1C*, which are involved in stem cell adhesion and cell cycle control.
83. O'Brien, C. A. *et al.* ID1 and ID3 regulate the self-renewal capacity of human colon cancer-initiating cells through p21. *Cancer Cell* **21**, 777–792 (2012).
This paper shows that ID genes sustain cancer stem cell and tumour-initiating phenotypes in colon cancer by preserving the expression of p21.
84. Pagliuca, A., Gallo, P., De Luca, P. & Lania, L. Class A helix-loop-helix proteins are positive regulators of several cyclin-dependent kinase inhibitors' promoter activity and negatively affect cell growth. *Cancer Res.* **60**, 1376–1382 (2000).
85. Chen, S. S. *et al.* Silencing of the inhibitor of DNA binding protein 4 (ID4) contributes to the pathogenesis of mouse and human CLL. *Blood* **117**, 862–871 (2011).
86. Yu, L. *et al.* Global assessment of promoter methylation in a mouse model of cancer identifies *ID4* as a putative tumor-suppressor gene in human leukemia. *Nature Genet.* **37**, 265–274 (2005).
87. Bounpheng, M. A., Dimas, J. J., Dodds, S. G. & Christy, B. A. Degradation of Id proteins by the ubiquitin-proteasome pathway. *FASEB J.* **13**, 2257–2264 (1999).
88. Trausch-Azar, J. S., Lingbeck, J., Ciechanover, A. & Schwartz, A. L. Ubiquitin-Proteasome-mediated degradation of Id1 is modulated by MyoD. *J. Biol. Chem.* **279**, 32614–32619 (2004).
89. Nakayama, K. I. & Nakayama, K. Ubiquitin ligases: cell-cycle control and cancer. *Nature Rev. Cancer* **6**, 369–381 (2006).
90. Amerik, A. Y. & Hochstrasser, M. Mechanism and function of deubiquitinating enzymes. *Biochim. Biophys. Acta* **1695**, 189–207 (2004).
91. Lasorella, A. *et al.* Degradation of Id2 by the anaphase-promoting complex couples cell cycle exit and axonal growth. *Nature* **442**, 471–474 (2006).
This paper shows that the APC/C–CDH1 ubiquitin ligase regulates axonal elongation by targeting ID1, ID2 and ID4 for proteasomal-mediated degradation.
92. Manchado, E., Eguren, M. & Malumbres, M. The anaphase-promoting complex/cyclosome (APC/C): cell-cycle-dependent and -independent functions. *Biochem. Soc. Trans.* **38**, 65–71 (2010).
93. Deed, R. W., Hara, E., Atherton, G. T., Peters, G. & Norton, J. D. Regulation of Id3 cell cycle function by Cdk-2-dependent phosphorylation. *Mol. Cell Biol.* **17**, 6815–6821 (1997).
94. Kong, Y., Cui, H. & Zhang, H. Smurf2-mediated ubiquitination and degradation of Id1 regulates p16 expression during senescence. *Aging Cell* **10**, 1038–1046 (2011).
95. Blank, M. *et al.* A tumor suppressor function of Smurf2 associated with controlling chromatin landscape and genome stability through RNF20. *Nature Med.* **18**, 227–234 (2012).
96. Wasch, R., Robbins, J. A. & Cross, F. R. The emerging role of APC/CCdh1 in controlling differentiation, genomic stability and tumor suppression. *Oncogene* **29**, 1–10 (2010).
97. Wang, Q. *et al.* Alterations of anaphase-promoting complex genes in human colon cancer cells. *Oncogene* **22**, 1486–1490 (2003).
98. Hua, H. *et al.* BMP4 regulates pancreatic progenitor cell expansion through Id2. *J. Biol. Chem.* **281**, 13574–13580 (2006).
99. James, D. *et al.* Expansion and maintenance of human embryonic stem cell-derived endothelial cells by TGFβ inhibition is Id1 dependent. *Nature Biotech.* **28**, 161–166 (2010).
100. Jankovic, V. *et al.* Id1 restrains myeloid commitment, maintaining the self-renewal capacity of hematopoietic stem cells. *Proc. Natl Acad. Sci. USA* **104**, 1260–1265 (2007).
101. Perry, S. S. *et al.* Id1, but not Id3, directs long-term repopulating hematopoietic stem-cell maintenance. *Blood* **110**, 2351–2360 (2007).
102. Rawlins, E. L., Clark, C. P., Xue, Y. & Hogan, B. L. The Id^{2+} distal tip lung epithelium contains individual multipotent embryonic progenitor cells. *Development* **136**, 3741–3745 (2009).

103. Suh, H. C. *et al.* Cell-nonautonomous function of Id1 in the hematopoietic progenitor cell niche. *Blood* **114**, 1186–1195 (2009).
104. Lyden, D. *et al.* Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts. *Nature* **401**, 670–677 (1999). **This paper shows that loss of ID genes confers resistance to tumour growth by affecting the integrity of tumour blood vessels.**
105. Anido, J. *et al.* TGF-β receptor inhibitors target the $CD44^{high}/Id1^{high}$ glioma-initiating cell population in human glioblastoma. *Cancer Cell* **18**, 655–668 (2010).
106. Jeon, H. M. *et al.* ID4 imparts chemoresistance and cancer stemness to glioma cells by derepressing miR-9*-mediated suppression of SOX2. *Cancer Res.* **71**, 3410–3421 (2011).
107. Calabrese, C. *et al.* A perivascular niche for brain tumor stem cells. *Cancer Cell* **11**, 69–82 (2007).
108. Chen, S., Lewallen, M. & Xie, T. Adhesion in the stem cell niche: biological roles and regulation. *Development* **140**, 255–265 (2013).
109. Fietz, S. A. & Huttner, W. B. Cortical progenitor expansion, self-renewal and neurogenesis-a polarized perspective. *Curr. Opin. Neurobiol.* **21**, 23–35 (2011).
110. Lathia, J. D. *et al.* Integrin α 6 regulates glioblastoma stem cells. *Cell Stem Cell* **6**, 421–432 (2010).
111. Park, D. M. & Rich, J. N. Biology of glioma cancer stem cells. *Mol. Cells* **28**, 7–12 (2009).
112. Boettner, B. & Van Aelst, L. Control of cell adhesion dynamics by Rap1 signaling. *Curr. Opin. Cell Biol.* **21**, 684–693 (2009).
113. Barrett, L. E. *et al.* Self-renewal does not predict tumor growth potential in mouse models of high-grade glioma. *Cancer Cell* **21**, 11–24 (2012). **This paper shows that glioma cells with low levels of ID1 have limited self-renewal capacity *in vitro* but retain efficient tumour-initiating capacity *in vivo*.**
114. Diaz-Flores, L. Jr. *et al.* Adult stem and transit-amplifying cell location. *Histol. Histopathol.* **21**, 995–1027 (2006).
115. Liu, C. *et al.* Mosaic analysis with double markers reveals tumor cell of origin in glioma. *Cell* **146**, 209–221 (2011).
116. Benezra, R., Rafii, S. & Lyden, D. The Id proteins and angiogenesis. *Oncogene* **20**, 8334–8341 (2001).
117. Benezra, R. Role of Id proteins in embryonic and tumor angiogenesis. *Trends Cardiovasc. Med.* **11**, 237–241 (2001).
118. Lyden, D. *et al.* Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nature Med.* **7**, 1194–1201 (2001).
119. Ruzinova, M. B. *et al.* Effect of angiogenesis inhibition by Id loss and the contribution of bone-marrow-derived endothelial cells in spontaneous murine tumors. *Cancer Cell* **4**, 277–289 (2003).
120. Fontemaggi, G. *et al.* The execution of the transcriptional axis mutant p53, E2F1 and ID4 promotes tumor neo-angiogenesis. *Nature Struct. Mol. Biol.* **16**, 1086–1093 (2009).
121. Jin, X. *et al.* EGFR-AKT-Smad signaling promotes formation of glioma stem-like cells and tumor angiogenesis by ID3-driven cytokine induction. *Cancer Res.* **71**, 7125–7134 (2011).
122. Minn, A. J. *et al.* Genes that mediate breast cancer metastasis to lung. *Nature* **436**, 518–524 (2005).
123. Gupta, G. P. *et al.* ID genes mediate tumor reinitiation during breast cancer lung metastasis. *Proc. Natl Acad. Sci. USA* **104**, 19506–19511 (2007). **This study describes how ID1 and ID3 facilitate sustained proliferation during the early stages of metastatic colonization, subsequent to extravasation into the lung.**
124. Fong, S. *et al.* Id-1 as a molecular target in therapy for breast cancer cell invasion and metastasis. *Proc. Natl Acad. Sci. USA* **100**, 13543–13548 (2003).
125. Gumireddy, K. *et al.* KLF17 is a negative regulator of epithelial-mesenchymal transition and metastasis in breast cancer. *Nature Cell Biol.* **11**, 1297–1304 (2009).
126. Brabletz, T., Jung, A., Spaderna, S., Hlubek, F. & Kirchner, T. Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. *Nature Rev. Cancer* **5**, 744–749 (2005).
127. Scheel, C. & Weinberg, R. A. Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. *Semin. Cancer Biol.* **22**, 396–403 (2012).
128. Coma, S. *et al.* Id2 promotes tumor cell migration and invasion through transcriptional repression of semaphorin 3F. *Cancer Res.* **70**, 3823–3832 (2010).
129. Kang, Y., Chen, C. R. & Massague, J. A self-enabling TGFβ response coupled to stress signaling: Smad engages stress response factor ATF3 for Id1 repression in epithelial cells. *Mol. Cell* **11**, 915–926 (2003).
130. Padua, D. *et al.* TGFβ primes breast tumors for lung metastasis seeding through angiopoietin-like 4. *Cell* **133**, 66–77 (2008).
131. Chang, C., Yang, X., Pursell, B. & Mercurio, A. M. Id2 complexes with the SNAG domain of Snai1 inhibiting Snai1-mediated repression of integrin β4. *Mol. Cell Biol.* **33**, 3795–3804 (2013).
132. Gervasi, M. *et al.* JunB contributes to Id2 repression and the epithelial-mesenchymal transition in response to transforming growth factor-β. *J. Cell Biol.* **196**, 589–603 (2012).
133. Stankic, M. *et al.* TGFβ-Id1 signaling opposes Twist and promotes breast cancer lung colonization via a mesenchymal-to-epithelial transition. *Cell Rep.* **5**,1228–1242 (2013). **This paper shows that, by opposing TWIST, ID1 promotes metastasis by facilitating MET.**
134. Jorda, M. *et al.* Id-1 is induced in MDCK epithelial cells by activated Erk/MAPK pathway in response to expression of the Snail and E47 transcription factors. *Exp. Cell Res.* **313**, 2389–2403 (2007).
135. Xu, J., Lamouille, S. & Derynck, R. TGF-β-induced epithelial to mesenchymal transition. *Cell Res.* **19**, 156–172 (2009).
136. Bolos, V. *et al.* The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. *J. Cell Sci.* **116**, 499–511 (2003).
137. Peinado, H. *et al.* Snail and E47 repressors of E-cadherin induce distinct invasive and angiogenic properties *in vivo*. *J. Cell Sci.* **117**, 2827–2839 (2004).
138. Cheung, P. Y., Yip, Y. L., Tsao, S. W., Ching, Y. P. & Cheung, A. L. Id-1 induces cell invasiveness in immortalized epithelial cells by regulating cadherin switching and Rho GTPases. *J. Cell Biochem.* **112**, 157–168 (2011).
139. Cubillo, E. *et al.* E47 and Id1 interplay in epithelial-mesenchymal transition. *PLoS ONE* **8**, e59948 (2013).
140. Di, K., Wong, Y. C. & Wang, X. Id-1 promotes TGF-β1-induced cell motility through HSP27 activation and disassembly of adherens junction in prostate epithelial cells. *Exp. Cell Res.* **313**, 3983–3999 (2007).
141. Gulhati, P. *et al.* mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. *Cancer Res.* **71**, 3246–3256 (2011).
142. Lamouille, S., Connolly, E., Smyth, J. W., Akhurst, R. J. & Derynck, R. TGF-β-induced activation of mTOR complex 2 drives epithelial-mesenchymal transition and cell invasion. *J. Cell Sci.* **125**, 1259–1273 (2012).
143. Tsai, J. H., Donaher, J. L., Murphy, D. A., Chau, S. & Yang, J. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* **22**, 725–736 (2012).
144. Ocana, O. H. *et al.* Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. *Cancer Cell* **22**, 709–724 (2012).
145. Gao, D. *et al.* Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. *Science* **319**, 195–198 (2008).
146. Rafii, S. & Lyden, D. Cancer. A few to flip the angiogenic switch. *Science* **319**, 163–164 (2008).
147. Ahlqvist, K., Saamarthy, K., Syed Khaja, A. S., Bjartell, A. & Massoumi, R. Expression of Id proteins is regulated by the Bcl-3 proto-oncogene in prostate cancer. *Oncogene* **32**, 1601–1608 (2013).
148. Gray, M. J. *et al.* Therapeutic targeting of Id2 reduces growth of human colorectal carcinoma in the murine liver. *Oncogene* **27**, 7192–7200 (2008).
149. Hui, C. M. *et al.* Id-1 promotes proliferation of p53-deficient esophageal cancer cells. *Int. J. Cancer* **119**, 508–514 (2006).
150. Kim, H. *et al.* Id-1 regulates Bcl-2 and Bax expression through p53 and NF-κB in MCF-7 breast cancer cells. *Breast Cancer Res. Treat.* **112**, 287–296 (2008).
151. Li, B. *et al.* Id-1 promotes tumorigenicity and metastasis of human esophageal cancer cells through activation of PI3K/AKT signaling pathway. *Int. J. Cancer* **125**, 2576–2585 (2009).
152. Ling, M. T., Kwok, W. K., Fung, M. K., Xianghong, W. & Wong, Y. C. Proteasome mediated degradation of Id-1 is associated with TNFα-induced apoptosis in prostate cancer cells. *Carcinogenesis* **27**, 205–215 (2006).
153. Mern, D. S., Hasskarl, J. & Burwinkel, B. Inhibition of Id proteins by a peptide aptamer induces cell-cycle arrest and apoptosis in ovarian cancer cells. *Br. J. Cancer* **103**, 1237–1244 (2010).
154. Mern, D. S., Hoppe-Seyler, K., Hoppe-Seyler, F., Hasskarl, J. & Burwinkel, B. Targeting Id1 and Id3 by a specific peptide aptamer induces E-box promoter activity, cell cycle arrest, and apoptosis in breast cancer cells. *Breast Cancer Res. Treat.* **124**, 623–633 (2010).
155. Sharma, P., Patel, D. & Chaudhary, J. Id1 and Id3 expression is associated with increasing grade of prostate cancer: Id3 preferentially regulates CDKN1B. *Cancer Med.* **1**, 187–197 (2012).
156. Zhang, X. *et al.* Identification of a novel inhibitor of differentiation-1 (ID-1) binding partner, caveolin-1, and its role in epithelial-mesenchymal transition and resistance to apoptosis in prostate cancer cells. *J. Biol. Chem.* **282**, 33284–33294 (2007).
157. Lin, J. *et al.* Inhibitor of differentiation 1 contributes to head and neck squamous cell carcinoma survival via the NF-κB/survivin and phosphoinositide 3-kinase/Akt signaling pathways. *Clin. Cancer Res.* **16**, 77–87 (2010).
158. Monticelli, L. A. *et al.* Transcriptional regulator Id2 controls survival of hepatic NKT cells. *Proc. Natl Acad. Sci. USA* **106**, 19461–19466 (2009).
159. Swarbrick, A., Roy, E., Allen, T. & Bishop, J. M. Id1 cooperates with oncogenic Ras to induce metastatic mammary carcinoma by subversion of the cellular senescence response. *Proc. Natl Acad. Sci. USA* **105**, 5402–5407 (2008).
160. Nair, R. *et al.* Redefining the expression and function of the inhibitor of differentiation 1 in mammary gland development. *PLoS ONE* **5**, e11947 (2010).
161. Salomon, R., Young, L., Macleod, D., Yu, X. L. & Dong, Q. Probasin promoter-driven expression of ID1 is not sufficient for carcinogenesis in rodent prostate. *J. Histochem. Cytochem.* **57**, 599–604 (2009).
162. Kim, D., Peng, X. C. & Sun, X. H. Massive apoptosis of thymocytes in T-cell-deficient Id1 transgenic mice. *Mol. Cell Biol.* **19**, 8240–8253 (1999).
163. Morrow, M. A., Mayer, E. W., Perez, C. A., Adlam, M. & Siu, G. Overexpression of the helix-loop-helix protein Id2 blocks T cell development at multiple stages. *Mol. Immunol.* **36**, 491–503 (1999).
164. Henke, E. *et al.* Peptide-conjugated antisense oligonucleotides for targeted inhibition of a transcriptional regulator *in vivo*. *Nature Biotechnol.* **26**, 91–100 (2008). **This study describes how peptide-mediated delivery of ID1 antisense oligonucleotides to tumour blood vessels leads to the inhibition of growth of primary tumours and metastasis.**
165. de Candia, P. *et al.* Angiogenesis impairment in Id-deficient mice cooperates with an Hsp90 inhibitor to completely suppress HER2/neu-dependent breast tumors. *Proc. Natl Acad. Sci. USA* **100**, 12337–12342 (2003).
166. Valkov, E., Sharpe, T., Marsh, M., Greive, S. & Hyvonen, M. Targeting protein-protein interactions and fragment-based drug discovery. *Top. Curr. Chem.* **317**, 145–179 (2012).
167. Azzarito, V., Long, K., Murphy, N. S. & Wilson, A. J. Inhibition of α-helix-mediated protein-protein interactions using designed molecules. *Nature Chem.* **5**, 161–173 (2013).
168. Ciarapica, R., Rosati, J., Cesareni, G. & Nasi, S. Molecular recognition in helix-loop-helix and helix-loop-helix-leucine zipper domains. Design of repertoires and selection of high affinity ligands for natural proteins. *J. Biol. Chem.* **278**, 12182–12190 (2003).
169. Ciarapica, R. *et al.* Targeting Id protein interactions by an engineered HLH domain induces human neuroblastoma cell differentiation. *Oncogene* **28**, 1881–1891 (2009).
170. Soucek, L. *et al.* Modelling Myc inhibition as a cancer therapy. *Nature* **455**, 679–683 (2008).
171. Chen, C. H., Kuo, S. C., Huang, L. J., Hsu, M. H. & Lung, F. D. Affinity of synthetic peptide fragments of MyoD for Id1 protein and their biological effects in several cancer cells. *J. Pept. Sci.* **16**, 231–241 (2010).

172. Geng, H. *et al.* ID1 enhances docetaxel cytotoxicity in prostate cancer cells through inhibition of p21. *Cancer Res.* **70**, 3239–3248 (2010).
173. Hernandez-Vargas, H. *et al.* Transcriptional profiling of MCF7 breast cancer cells in response to 5-Fluorouracil: relationship with cell cycle changes and apoptosis, and identification of novel targets of p53. *Int. J. Cancer* **119**, 1164–1175 (2006).
174. Soroceanu, L. *et al.* Id-1 is a key transcriptional regulator of glioblastoma aggressiveness and a novel therapeutic target. *Cancer Res.* **73**, 1559–1569 (2013).
175. Yap, W. N. *et al.* γ-tocotrienol suppresses prostate cancer cell proliferation and invasion through multiple-signalling pathways. *Br. J. Cancer* **99**, 1832–1841 (2008).
176. Bedford, L. *et al.* Id4 is required for the correct timing of neural differentiation. *Dev. Biol.* **280**, 386–395 (2005).
177. Liu, H. *et al.* p53 regulates neural stem cell proliferation and differentiation via BMP-Smad1 signaling and Id1. *Stem Cells Dev.* **22**, 913–927 (2013).
178. Yun, K., Mantani, A., Garel, S., Rubenstein, J. & Israel, M. A. Id4 regulates neural progenitor proliferation and differentiation *in vivo*. *Development* **131**, 5441–5448 (2004).
179. Arai, F. *et al.* Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* **118**, 149–161 (2004).
180. Cotsarelis, G., Sun, T. T. & Lavker, R. M. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* **61**, 1329–1337 (1990).
181. Lim, D. A. *et al.* Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* **28**, 713–726 (2000).
182. Zhang, J. *et al.* Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* **425**, 836–841 (2003).
183. Jones, D. L. & Wagers, A. J. No place like home: anatomy and function of the stem cell niche. *Nature Rev. Mol. Cell Biol.* **9**, 11–21 (2008).
184. Morrison, S. J. & Spradling, A. C. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* **132**, 598–611 (2008).
185. O'Brien, L. E. & Bilder, D. Beyond the niche: tissue-level coordination of stem cell dynamics. *Annu. Rev. Cell Dev. Biol.* **29**, 107–136 (2013).
186. Voog, J. & Jones, D. L. Stem cells and the niche: a dynamic duo. *Cell Stem Cell* **6**, 103–115 (2010).
187. Hsu, Y. C., Pasolli, H. A. & Fuchs, E. Dynamics between stem cells, niche, and progeny in the hair follicle. *Cell* **144**, 92–105 (2011).
188. Ihrie, R. A. & Alvarez-Buylla, A. Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. *Neuron* **70**, 674–686 (2011).
189. Joshi, P. A., Di Grappa, M. A. & Khokha, R. Active allies: hormones, stem cells and the niche in adult mammopoiesis. *Trends Endocrinol. Metab.* **23**, 299–309 (2012).
190. Kordes, C. & Haussinger, D. Hepatic stem cell niches. *J. Clin. Invest.* **123**, 1874–1880 (2013).
191. Moore, K. A. & Lemischka, I. R. Stem cells and their niches. *Science* **311**, 1880–1885 (2006).
192. Mounier, R., Chretien, F. & Chazaud, B. Blood vessels and the satellite cell niche. *Curr. Top. Dev. Biol.* **96**, 121–138 (2011).
193. Discher, D. E., Mooney, D. J. & Zandstra, P. W. Growth factors, matrices, and forces combine and control stem cells. *Science* **324**, 1673–1677 (2009).
194. Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677–689 (2006).
195. Guilak, F. *et al.* Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell* **5**, 17–26 (2009).
196. Kshitiz *et al.* Matrix rigidity controls endothelial differentiation and morphogenesis of cardiac precursors. *Sci. Signal.* **5**, ra41 (2012).
197. Higgins, S. *et al.* Fibroblast growth factor 2 reactivates G1 checkpoint in SK-N-MC cells via regulation of p21, inhibitor of differentiation genes (*Id1-3*), and epithelium-mesenchyme transition-like events. *Endocrinology* **150**, 4044–4055 (2009).
198. Tournay, O. & Benezra, R. Transcription of the dominant-negative helix-loop-helix protein Id1 is regulated by a protein complex containing the immediate-early response gene *Egr-1*. *Mol. Cell Biol.* **16**, 2418–2430 (1996).
199. Nishimori, H. *et al.* The *Id2* gene is a novel target of transcriptional activation by EWS-ETS fusion proteins in Ewing family tumors. *Oncogene* **21**, 8302–8309 (2002).
200. Langenfeld, E. M., Kong, Y. & Langenfeld, J. Bone morphogenetic protein 2 stimulation of tumor growth involves the activation of Smad-1/5. *Oncogene* **25**, 685–692 (2006).
201. Yan, W. *et al.* High incidence of T-cell tumors in E2A-null mice and E2A/Id1 double-knockout mice. *Mol. Cell Biol.* **17**, 7317–7327 (1997).
202. Hacker, C. *et al.* Transcriptional profiling identifies Id2 function in dendritic cell development. *Nature Immunol.* **4**, 380–386 (2003).
203. Mori, S., Nishikawa, S. I. & Yokota, Y. Lactation defect in mice lacking the helix-loop-helix inhibitor Id2. *EMBO J.* **19**, 5772–5781 (2000).
204. Russell, R. G., Lasorella, A., Dettin, L. E. & Iavarone, A. Id2 drives differentiation and suppresses tumor formation in the intestinal epithelium. *Cancer Res.* **64**, 7220–7225 (2004).
205. Yokota, Y. *et al.* Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. *Nature* **397**, 702–706 (1999).
206. Pan, L., Sato, S., Frederick, J. P., Sun, X. H. & Zhuang, Y. Impaired immune responses and B-cell proliferation in mice lacking the *Id3* gene. *Mol. Cell Biol.* **19**, 5969–5980 (1999).
207. Rivera, R. R., Johns, C. P., Quan, J., Johnson, R. S. & Murre, C. Thymocyte selection is regulated by the helix-loop-helix inhibitor protein, Id3. *Immunity* **12**, 17–26 (2000).
208. Fraidenraich, D. *et al.* Rescue of cardiac defects in id knockout embryos by injection of embryonic stem cells. *Science* **306**, 247–252 (2004).
209. Ding, Y. *et al.* Significance of Id-1 up-regulation and its association with EGFR in bladder cancer cell invasion. *Int. J. Oncol.* **28**, 847–854 (2006).
210. Stighall, M., Manetopoulos, C., Axelson, H. & Landberg, G. High ID2 protein expression correlates with a favourable prognosis in patients with primary breast cancer and reduces cellular invasiveness of breast cancer cells. *Int. J. Cancer* **115**, 403–411 (2005).
211. Umetani, N. *et al.* Aberrant hypermethylation of *ID4* gene promoter region increases risk of lymph node metastasis in T1 breast cancer. *Oncogene* **24**, 4721–4727 (2005).
212. Zeng, W., Rushing, E. J., Hartmann, D. P. & Azumi, N. Increased inhibitor of differentiation 4 (id4) expression in glioblastoma: a tissue microarray study. *J. Cancer* **1**, 1–5 (2010).
213. Wilson, J. W. *et al.* Expression of Id helix-loop-helix proteins in colorectal adenocarcinoma correlates with p53 expression and mitotic index. *Cancer Res.* **61**, 8803–8810 (2001).
214. Umetani, N. *et al.* Epigenetic inactivation of ID4 in colorectal carcinomas correlates with poor differentiation and unfavorable prognosis. *Clin. Cancer Res.* **10**, 7475–7483 (2004).
215. Luo, K. J. *et al.* Prognostic relevance of Id-1 expression in patients with resectable esophageal squamous cell carcinoma. *Ann. Thorac Surg.* **93**, 1682–1688 (2012).
216. Yang, H. Y. *et al.* Expression and prognostic values of Id-1 and Id-3 in gastric adenocarcinoma. *J. Surg. Res.* **167**, 258–266 (2011).
217. Li, X. *et al.* Prognostic significance of Id-1 and its association with EGFR in renal cell cancer. *Histopathology* **50**, 484–490 (2007).
218. Sun, W. *et al.* Id-1 and the p65 subunit of NF-κB promote migration of nasopharyngeal carcinoma cells and are correlated with poor prognosis. *Carcinogenesis* **33**, 810–817 (2012).
219. Tobin, N. P., Sims, A. H., Lundgren, K. L., Lehn, S. & Landberg, G. Cyclin D1, Id1 and EMT in breast cancer. *BMC Cancer* **11**, 417 (2011).
220. Wang, H., Wang, X. Q., Xu, X. P. & Lin, G. W. ID4 methylation predicts high risk of leukemic transformation in patients with myelodysplastic syndrome. *Leuk. Res.* **34**, 598–604 (2010).
221. Renne, C. *et al.* Aberrant expression of ID2, a suppressor of B-cell-specific gene expression, in Hodgkin's lymphoma. *Am. J. Pathol.* **169**, 655–664 (2006).
222. Lee, K. T. *et al.* Overexpression of Id-1 is significantly associated with tumour angiogenesis in human pancreas cancers. *Br. J. Cancer* **90**, 1198–1203 (2004).
223. Maruyama, H. *et al.* Id-1 and Id-2 are overexpressed in pancreatic cancer and in dysplastic lesions in chronic pancreatitis. *Am. J. Pathol.* **155**, 815–822 (1999).
224. Kleeff, J. *et al.* The helix-loop-helix protein Id2 is overexpressed in human pancreatic cancer. *Cancer Res.* **58**, 3769–3772 (1998).
225. Lee, S. H. *et al.* The Id3/E47 axis mediates cell-cycle control in human pancreatic ducts and adenocarcinoma. *Mol. Cancer Res.* **9**, 782–790 (2011).
226. Coppe, J. P., Itahana, Y., Moore, D. H., Bennington, J. L. & Desprez, P. Y. Id-1 and Id-2 proteins as molecular markers for human prostate cancer progression. *Clin. Cancer Res.* **10**, 2044–2051 (2004).
227. Yuen, H. F. *et al.* Id proteins expression in prostate cancer: high-level expression of Id-4 in primary prostate cancer is associated with development of metastases. *Mod. Pathol.* **19**, 931–941 (2006).
228. Ciarrocchi, A., Piana, S., Valcavi, R., Gardini, G. & Casali, B. Inhibitor of DNA binding-1 induces mesenchymal features and promotes invasiveness in thyroid tumour cells. *Eur. J. Cancer* **47**, 934–945 (2011).
229. Schindl, M. *et al.* Level of Id-1 protein expression correlates with poor differentiation, enhanced malignant potential, and more aggressive clinical behavior of epithelial ovarian tumors. *Clin. Cancer Res.* **9**, 779–785 (2003).
230. Ding, R. *et al.* Overexpressed Id-1 is associated with patient prognosis and HBx expression in hepatitis B virus-related hepatocellular carcinoma. *Cancer Biol. Ther.* **10**, 299–307 (2010).
231. Matsuda, Y. *et al.* Overexpressed Id-1 is associated with a high risk of hepatocellular carcinoma development in patients with cirrhosis without transcriptional repression of p16. *Cancer* **104**, 1037–1044 (2005).
232. Lee, J. Y. *et al.* Id-1 activates Akt-mediated Wnt signaling and p27(Kip1) phosphorylation through PTEN inhibition. *Oncogene* **28**, 824–831 (2009).
233. Nieborowska-Skorska, M. *et al.* Id1 transcription inhibitor-matrix metalloproteinase 9 axis enhances invasiveness of the breakpoint cluster region/abelson tyrosine kinase-transformed leukemia cells. *Cancer Res.* **66**, 4108–4116 (2006).
234. Bai, G. *et al.* Id sustains Hes1 expression to inhibit precocious neurogenesis by releasing negative autoregulation of Hes1. *Dev. Cell* **13**, 283–297 (2007).

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Competing interests statement
The authors declare no competing interests.