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Commentary & View

The role of the transcriptional regulator snail in cell detachment, reattachment and migration

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In order to metastasize, cancer cells must first detach from the primary tumor, migrate, invade through tissues and attach to a second site. The transcription factor snail is an important mediator of epithelial-mesenchymal transitions and is involved in tumor progression. Recent data have provided evidence for a requirement for snail expression in metastatic dissemination. Although very little is known about the molecular mechanisms governing metastatic dissemination, we review the possible roles of snail expression in this process. We also review the regulation of snail expression.

Local tissue invasion represents the first step of the metastatic cascade of carcinomas. Invasion of carcinoma cells requires changes in cell-cell or cell-matrix adhesion, cell polarity and cell migratory properties of the tumor cells. These changes are collectively known as the epithelial-mesenchymal transition (EMT). Downregulation of E-cadherin is an essential event for EMT.[1] Snail is a prominent inducer of EMT and strongly represses E-cadherin expression. Snail also plays important roles in tumor growth and lymph node metastasis of human breast cancer MDA-MB cells.[2] In these cells snail knockdown induces a decrease in proinvasive markers such as matrix metalloproteinase-9 (MMP9) as well as lymph node metastasis.[2] In addition snail knockdown inhibited skin carcinoma cell dissemination into the spleen[3] and ovarian cancer dissemination.[4] However, the role of snail in the process of dissemination remains to be clarified.

The Role of Snail in Cell Detachment From Extracellular Matrices (ECM)

We have shown that exogeneous expression of snail in epithelial MDCK and A431 cells enhanced trypsin-induced cell detachment from the ECM.[5] This observation suggested that snail influences cell-ECM adhesion most likely through alteration of ECM components secreted by the cells or through modulation of the cellular ECM receptors such as integrins. Basement membranes (BM) are sheets of ECM generated by cells at epithelial mesenchymal interfaces. Changes in BM proteins and their cellular receptors are associated with the progression of human carcinomas.[6,7] The major classes of BM proteins are laminins that are trimers of α, β and γ chains and which bind to a variety of cell surface integrin receptors. To date, 16 different types of laminin trimers have been identified.[8] Modulation of specific forms of laminin or their cell surface receptors may play an important role in snail-mediated metastasis. Laminin-332 (laminin-5) has been reported to bind strongly to integrin $\alpha6\beta4$ and to form hemidesmosomes.[9] However snail-transfected oral squamous cell carcinoma (43A-SNA) cells showed no laminin-332 synthesis.[10] Furthermore, laminin-$\gamma2$ knockdown resulted in increased detachment of oral squamous cell carcinoma JHU-022-SCC cells.[11] We also confirmed that the expression of laminin-332 and integrin $\alpha6$, $\alpha3$ was reduced in snail-expressing MDCK and A431 cells. However, we did not observe enhancement of cell detachment by laminin-$\gamma2$ knockdown in A431 cells or by laminin-$\alpha3$ knockdown in MDCK cells.[5] Cell adhesion to laminin-332 occurs by binding through $\alpha3\beta1$ and $\alpha6\beta4$ integrins.[12] Thus, a reduction in integrin $\alpha3$ and $\alpha6$, as well as a decrease in laminin-332, could cause detachment of snail-expressing MDCK and A431 cells. In addition to laminin-332, laminin-$\alpha5$ is also downregulated in snail transfected SCC cells.[13] In contrast, in the same cells, neoexpression of laminin-$\alpha4$ mRNA and synthesis of laminin-411 (laminin-8) was observed. Laminin-511 is regarded as the most widely expressed laminin and is found in most epithelial BMs. Laminin-511 or -521 (lamin-10/11) interacts with $\alpha3\beta1$, $\alpha6\beta1$ and $\alpha6\beta4$ integrins.[14] Laminin-411 also binds $\alpha6\beta1$ integrins.[15,16] SCC cells potently adhered to laminin-511 whereas adhesion to laminin-411 was minimal.[13] Furthermore, the laminin-$\alpha4$ chain has been reported to play a role in the detachment of renal carcinoma cells from fibronectin.[17] Laminin-411 decreased adhesion of SCC cells to laminin-511 and to fibronectin by blocking binding sites in the fibronectin molecule.[13] In parental SCC cells integrin $\alpha6$ is paired with the $\beta4$ subunit. When paired with the $\beta4$ subunit, integrin $\alpha6\beta4$ mediates the formation of hemidesmosomes which link the intermediate filament cytoskeleton to BM laminin-332. Integrin

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$\alpha6\beta4$ also mediates cell adhesion to laminin-511.[13] However, in 43A-SNA cells integrin $\alpha6$ is paired with the $\beta1$ subunit.[13] Integrin $\alpha6\beta1$ is the main receptor for laminin-411. A reduction in integrin $\alpha6\beta4$ levels has been shown in snail transfected SCC cells.[10] We have detected a loss of integrin $\beta4$ expression in snail-expressing cells.[5] It has been proposed that a reduction in the level of the integrin $\beta4$ subunit allows snail transfected SCC cells to escape from hemidesmosomal contacts and to use the laminin-411 receptor, integrin $\alpha6\beta1$, to become motile.[13]

Collagen IV is another major component of BM. Since normal production and assembly of BM is disrupted during malignant cancer progression it has been suggested that collagen IV $\alpha5/\alpha6$ chains might protect against rapid cancer progression.[18] We have confirmed a reduction in collagen IV in snail-expressing MDCK cells. Detachment of these cells was significantly suppressed when the cells were plated in wells that had been precoated with collagen IV. Thus, a reduction in collagen IV might be at least partly responsible for the increased detachment of MDCK/snail cells.[5]

Other molecules besides ECM proteins might be involved in the detachment of snail-expressing cells. For example, plasminogen activator inhibior-1 (PAI-1) has been reported to induce detachment of cells from extracellular matrices by inactivating integrins. The binding of urokinase plasminogen activator (uPA) to its cell surface receptor (uPAR) promotes cell adhesion by increasing the affinity of the receptor for both vitronectin (VN) and integrins. PAI-1 can disrupt uPA-uPAR, uPAR-VN and integrin-VN interactions thereby leading to cell detachment.[19] Genetic profiling of snail-expressing MDCK cells revealed enhanced expressions of PAI-1.[20] We have also confirmed induction of PAI-1 in snail-expressing cells (unpublished data). Another candidate for the mediation of snail-induced cell detachment is the protein p63. p63, a member of the p53-family, has a pivotal role in epithelial development. Knockdown of p63 expression resulted in the down-regulation of cell adhesion-associated genes such as integrin $\beta4$, $\beta1$, $\alpha6$, fibronectin and laminin $\gamma2$ and caused cell detachment.[21] Transfection of snail induced downregulation of p63 protein in SCC cells by inhibiting p63 promoter activity.[22]

Degradation of the ECM is involved in the process of cell detachment from the substratum and cell migration. Thus upregulation of the matrix metalloproteinases MMP-9 and MMP-2 in snail expressing cells has been observed.[23-26] Furthermore, snail-expressing HepG2 cells enhanced the expression of MMP-1, MMP-2, MMP-7 and MT1-MMP.[26] Although we also observed MMP-3 induction in snail expressing cells, neither the MMP-3 inhibitor nor MMP inhibitors efficiently suppressed cell detachment. In malignant mesothelioma cells, snail protein expression showed a positive association with MT1-MMP and TIMP-2 mRNA expression, but was unrelated to MMP-2 and MMP-9 expression or activity.[27] Since the ECM is significantly changed in snail expressing cells, the sensitivity of these ECM proteins to proteases might be altered.

The Role of Snail in Apoptosis

There are several reports that implicate snail in cell survival. During embryonic development, expression of the snail gene in chicken and mouse is inversely correlated with cell death in different developing tissues.[28] Snail downregulation by antisense oligonucleotides has been shown to increase cell death in colon tumors in a mouse model.[29] Snail also confers resistance to cell death induced by the withdrawal of survival factors and by pro-apoptotic signals.[28] The MAPK and PI3K survival pathways are highly active in snail expressing cells.[28] Furthermore, snail expression also enhanced resistance to cell death elicited by DNA damage. A detailed molecular analysis of this phenomenon revealed that snail directly repressed the transcriptional of multiple factors that have well-documented roles in programmed cell death such as p53, BID and caspase-6.[30] Anoikis refers to apoptosis induced by a loss of cell-matrix interactions. For a tumor cell to metastasize to a distant site, it needs to overcome anoikis.[31] Metastatic dissemination generally occurs when cancer cells overcome anoikis after detachment from the primary tumor site. Although the transcription factor slug, that is another inducer of EMT, was reported to be essential for resistance to anoikis of human breast cells,[31] snail did not confer resistance to anoikis.[5] Loss of E-cadherin from cell-cell contacts is involved in the onset of anoikis.[32] Given that snail represses the expression of E-cadherin, snail-expressing cells would fail to be resistant to anoikis.

Table 1 **Adhesion-related genes differentially expressed in snail-expressing cells**

	Genes downregulated in snail-expressing cells	Genes upregulated in snail-expressing cells
Integrins	$\alpha3$[5], $\alpha6$[5], $\beta4$[5]	$\alpha5$[5]
Laminins	$\alpha3$[5], $\alpha5$[13], $\beta3$[5], $\gamma2$[5]	$\alpha4$[13]
Other extracellular matrix molecules	Aggrecan,[69] Type II collagen,[69] Type IV collagen,[5] Type X collagen[70]	Fibronectin[71]
Matrix metalloproteinases		MMP-1,[26] MMP-2,[24-26] MMP-3,[5] MMP-7,[26] MMP-9,[23] MT1-MMP[26]
Other detachment-related genes	p63,[22]	PAI-1,[20]

The Role of Snail in Cell Attachment to ECM

To complete metastasis, tumor cells must adhere to some extracellular matrix ligands for migration and for reattachment to the second site. Snail expressing cells have enhanced expression of integrin αv or $\alpha5$ and expression of $\alpha v\beta3$ integrin stimulates tumor cell adhesion to vitronectin.[33] Thus it is possible that snail-expressing cells might show enhanced reattachment to ECM that contains fibronectin or vitronectin. Indeed, we did observe enhanced attachment of snail-expressing MDCK and A431 cells to tissue culture wells coated with fibronectin or fetal calf serum which could be detected as early as 30 min after plating. Decreased cell adhesion to laminin-332,[34] within 72 hr after plating has been reported in slug-expressing epidermal keratinocytes. The production of ECM proteins and their corresponding

receptors in snail-expressing tumor cells is changed from the basement membrane type, e.g., laminin332, to the stromal type, e.g., fibronectin. Rapid cell attachment to specific ECM ligands would prevent anoikis, induce migration and enhance the re-growth of metastasized snail-expressing tumor cells. Therefore blocking of integrin-cell attachment might provide a therapeutic benefit for the treatment of snail-expressing tumors.

The Role of Snail in Cell Migration

The snail genes are implicated in biological processes that involve cell movement during embryonic development such as migration of the neural crest of Xenoopus[35] and of the axial mesendoderm of zebrafish.[36] Snail also triggers migration of hepatoma HepG2,[37] and oesophageal squamous cell carcinoma cells.[38] Snail silencing dramatically reduced the ability of breast carcinoma MDA-MB231 cells to migrate into collagen IV.[2] Snail accelerates the migration of human bone mesenchymal stem cells (BMSC) by a mechanism dependent on the PI3-kinase signaling pathway.[24] Since higher expression of integrin αv or α5 was detected in snail-expressing MDCK cells compared to control cells, we analyzed the migration of these cells to fibronectin and osteopontin that are ligands for the integrins αvβ3 and α5β1. We observed enhanced migration of snail-expressing cells to fibronectin and osteopontin compared to the parental MDCK cells. Furthermore, a blocking antibody against integrin αvβ3 completely inhibited snail-expressing cell migration to osteopontin,[5] indicating that the migration of snail-expressing cells depends on integrin αvβ3. Expression of integrin αvβ3 in breast cancer cells is reported to promote not only migration to vitronectin, osteopontin and bone-derived soluble factors but also spontaneous metastasis to bone in vivo.[28] Moreover, melanoma cells (M21) are also known to migrate towards bone sialoprotein (BSP) through an interaction with integrin αvβ3.[39] The combined data suggest that snail-expressing cells might migrate to bone and form metastasis at this site.

Regulation of Snail Expression

Many signaling pathways have been reported to regulate snail expression. Receptor tyrosine kinase signaling, activated by fibroblast growth factor (FGF) or epidermal growth factor (EGF), induces snail expression.[40,41] VEGF and the VEGF receptor Neuropilin-1 increase snail expression by suppression of Glycogen Synthase Kinase-3beta (GSK-3β), an established inhibitor of snail transcription and protein stability.[42] Transforming growth factor beta (TGFβ) and bone morphogenic proteins (BMPs) are also involved in the induction of snail.[43,44] It has been demonstrated that knockdown of either Myc or the TGFβ effectors SMAD3/4 in epithelial cells eliminates snail induction by TGFβ.[45] Snail induction by TGFβ is dependent on cooperation with active Ras signals.[46,47] TGFβ-mediated induction of the snail promoter is blocked by a dominant negative form of H-Ras (N17Ras). H-Ras mediated induction of snail depends on both MAPK and phosphatidylinositol 3-kinase (PI3K) activities.[47] These results are consistent with the data that snail expression is triggered by constitutively active Akt, a kinase downstream of PI3K.[48] The p70 S6 kinase (p70(S6K)) is another downstream effector of PI3K and is frequently activated in human ovarian cancer. Activation of p70(S6K) also stimulates the expression of snail.[49] In regulating snail gene expression, the TGFbeta/Smad pathway cooperates with high mobility group A2 (HMGA2) that directly binds to the snail promoter and acts as a transcriptional regulator of snail expression.[50] Gli mediate constitutive Hedgehog signaling in the common skin cancer, basal cell carcinoma. Snail is rapidly induced by Gli1.[51] Notch directly upregulates snail expression in two ways: first by binding of the Notch intracellular domain to the snail-1 promoter and second by Notch potentiation of hypoxia-inducible factor 1alpha (HIF-1α) recruitment to the lysyl oxidase (LOX) promoter and elevation of the hypoxia-induced upregulation of LOX, which stabilizes the snail-1 protein.[52] An human snail promoter that contains the site of initiation of transcription has been characterized.[53] This promoter was activated in response to addition of the phorbol ester PMA or to overexpression of integrin-linked kinase (ILK) or oncogenes such as Ha-ras or v-Akt. Although other regions of the promoter were required for complete stimulation by Akt or ILK, a minimal fragment (-78/+59) was sufficient to maintain mesenchymal specificity. Activity of this minimal promoter and snail RNA levels were dependent on the ERK signaling pathway. NFkappaB/p65 also stimulates snail transcription through a region located immediately upstream of the minimal promoter, between -194 and -78.[53] The endothelin A receptor (ET(A)R)/endothelin-1 (ET-1) autocrine pathway increases the level of snail. Activation of ET(A)R by ET-1 triggers an ILK-mediated signaling pathway leading to GSK-3β inhibition and snail stabilization.[54] Overexpression of ILK stimulates snail expression and inhibition of ILK resulted in the inhibition of snail gene transcription.[55] Ultraviolet radiation (UVR), which activates MAPK cascades, also stimulates snail expression in epidermal keratinocytes. This induction was mediated, at least in part, through the ERK and p38 MAPK cascades.[56] Reactive oxygen species (ROS) stimulate the expression of snail.[57] Exposure of mouse mammary epithelial cells to MMP-3 induces the expression of Rac1, which causes an increase in ROS and expression of snail.[57] Snail mRNA expression was increased under hypoxic conditions in ovarian cancer cell lines.[58] Hypoxia is known to induce hypoxia-inducible factor-alpha (HIF-1α), which binds to hypoxia-responsive elements of target genes and activates the transcription of these genes. HIF-1α has been proposed to activate snail via HIF-1α engagement of the hypoxia-responsive element found in the snail promoter at position -86 to -82.[59] The product of the von Hippel-Lindau gene (VHL) ubiquitylates HIF-1α leading to oxygen-dependent HIF-1α destruction. Therefore, reintroduction of wild-type VHL into CC-RCC [VHL(-/-)] cells markedly reduced the expression of snail.[59] Signaling of the estrogen receptor negatively regulates snail expression.[60] The product of human MTA3 (metastasis-associated gene) is an estrogen-dependent component of the Mi-2/NuRD transcriptional co-repressor and constitutes a key component of an estrogen-dependent pathway. The absence of estrogen receptor or of MTA3 leads to aberrant expression of snail.[60,61] Recent studies have shown that snail binds to its own promoter and represses its activity. These results indicate the existence of a feed-back mechanism of regulation of snail

transcription.[62] Although, the expression of snail can be induced by different pathways that act at the transcriptional level, a non-transcriptional mechanism that regulates snail activity has been described.[63] Snail is highly unstable, with a short half-life of about 25 min. GSK-3β binds to and phosphorylates snail at consensus motifs and regulates ubiquitylation of snail by β-Trcp. A variant of snail (snail-6SA), which cannot be phosphorylated at these sites, is much more stable.[63] In agreement with these findings, Wnt signaling inhibits snail phosphorylation and consequently increases snail protein levels.[64] The lysine residues at position 98 and 137 of snail are essential for snail stability, its functional cooperation with LOXL2/3 and for snail induction of EMT.[65] LOXL2 appears to attenuate GSK3β-dependent snail degradation.[65] Oxidation of snail K98 and/or K137 by LOXL2 generates an intramolecular linkage in snail thereby inducing a conformational change which would mask GSK-3β-dependent regulatory motifs. Blockage of the GSK-3β phosphorylation sites leads to a more stable and a nuclear-localized snail protein.[66] Snail function is controlled by its intracellular location. The cytosolic distribution of snail depends on its nuclear export by a CRM1-dependent mechanism, and a nuclear export sequence (NES) has been located in the regulatory domain of snail. Export of snail is controlled by phosphorylation of a Ser-rich sequence adjacent to this NES.[67] In contrast, phosphorylation of snail on Ser(246) by p21-activating kinase 1 (PAK1) promotes snail's accumulation in the nucleus as well as its repressor functions.[68] On the other hand, GSK-3β phosphorylates the NES of snail and induces its export to the cytoplasm.[63] Importantly, the phosphorylation and subcellular distribution of snail are also controlled by cell attachment to the extracellular matrix. Suspended cells show higher levels of phosphorylated snail and an augmented snail extranuclear localization compared to cells attached to culture plates. These findings show the existence of an effective and finely tuned nontranscriptional mechanism of regulation of snail activity that is dependent on the extracellular environment.[67]

References

1. Thiery JP. Epithelial-mesenchymal transitions in tumor progression. Nat Rev Cancer 2002; 2:23-6.
2. Olmeda D, Moreno-Bueno G, Flores JM, Fabra A, Portillo F, Cano A. Snai1 is required for tumor growth and lymph node metastasis of human breast carcinoma MDA-MB-231 cells. Cancer Res 2007; 67:11721-31.
3. Olmeda D, Montes A, Moreno-Bueno G, Flores JM, Portillo F, Cano A. Snai1 and Snai2 collaborate on tumor growth and metastasis properties of mouse skin carcinoma cell lines. Oncogene 2008; 27:4690-701.
4. Yagi H, Yotsumoto F, Miyamoto S. Heparin-binding epidermal growth factor-like growth factor promotes transcoelomic metastasis in ovarian cancer through epithelial-mesenchymal transition. Mol Cancer Ther 2008; 7:3441-51.
5. Haraguchi M, Okubo T, Miyashita Y, Miyamoto Y, Hayashi M, Crotti TN, et al. Snail regulates cell-matrix adhesion by regulation of the expression of integrins and basement membrane proteins. J Biol Chem 2008; 283:23514-23.
6. Guo W, Giancotti FG. Integrin signaling during tumor progression. Nat Rev Mol Cell Biol 2004; 5:816-26.
7. Patarroyo M, Tryggvason K, Virtanen I. Laminin isoforms in tumor invasion, angiogenesis and metastasis. Semin Cancer Biol 2002; 12:197-207.
8. Aumailley M, Bruckner-Tuderman L, Carter WG, Deutzmann R, Edgar D, Ekblom P, et al. A simplified laminin nomenclature. Matrix Biol 2005; 24:326-32.
9. Baker SE, Hopkinson SB, Fitchmun M, Andreason GL, Frasier F, Plopper G, et al. Laminin-5 and hemidesmosomes: role of the alpha3 chain subunit in hemidesmosome stability and assembly. J Cell Sci 1996; 109:2509-20.
10. Takkunen M, Grenman R, Hukkanen M, Korhonen M, García de Herreros A, Virtanen I. Snail-dependent and -independent epithelial-mesenchymal transition in oral squamous carcinoma cells. J Histochem Cytochem 2006; 54:1263-75.
11. Yuen HW, Ziober AF, Gopal P, Nasrallah I, Falls EM, Meneguzzi G, et al. Suppression of laminin-5 expression leads to increased motility, tumorigenicity and invasion. Exp Cell Res 2005; 309:198-210.
12. Niessen CM, Hogervorst F, Jaspars LH, de Melker AA, Delwel GO, Hulsman EH, et al. The alpha6beta4 integrin is a receptor for both laminin and kalinin. Exp Cell Res 1994; 211:360-7.
13. Takkunen M, Ainola M, Vainionpää N, Grenman R, Patarroyo M, García de Herreros A, et al. Epithelial-mesenchymal transition downregulates laminin alpha5 chain and upregulates laminin alpha4 chain in oral squamous carcinoma cells. Histochem Cell Biol 2008; 130:509-25.
14. Kikkawa Y, Sanzen N, Fujiwara H, Sonnenberg A, Sekiguchi K. Integrin binding specificity of laminin-10/11: laminin-10/11 are recognized by alpha3beta1, alpha6beta1 and alpha6beta4 integrins. J Cell Sci 2000; 113:869-76.
15. Geberhiwot T, Ingerpuu S, Pedraza C, Neira M, Lehto U, Virtanen I, et al. Blood platelets contain and secrete laminin-8 (alpha4beta1gamma1) and adhere to laminin-8 via alpha6beta1 integrin. Exp Cell Res 1999; 25:723-32.
16. Fujiwara H, Kikkawa Y, Sanzen N, Sekiguchi K. Purification and characterization of human laminin-8. Laminin-8 stimulates cell adhesion and migration through alpha-3beta1 and alpha6beta1 integrins. J Biol Chem 2001; 276:17550-8.
17. Vainionpää N, Lehto VP, Tryggvason K, Virtanen I. Alpha4 chain laminins are widely expressed in renal cell carcinomas and have a de-adhesive function. Lab Invest 2007; 87:780-91.
18. Ikeda K, Iyama K, Ishikawa N, Egami H, Nakao M, Sado Y, et al. Loss of expression of type IV collagen alpha5 and alpha6 chains in colorectal cancer associated with the hypermethylation of their promoter region. Am J Pathol 2006; 168:856-65.
19. Czekay RP, Aertgeerts K, Curriden SA, Loskutoff DJ. Plasminogen activator inhibitor-1 detaches cells from extracellular matrices by inactivating integrins. J Cell Biol 2003; 160:781-91.
20. Moreno-Bueno G, Cubillo E, Sarrió D, Peinado H, Rodríguez-Pinilla SM, Villa S, et al. Genetic profiling of epithelial cells expressing E-cadherin repressors reveals a distinct role for Snail, Slug and E47 factors in epithelial-mesenchymal transition. Cancer Res 2006; 66:9543-56.
21. Carroll DK, Carroll JS, Leong CO, Cheng F, Brown M, Mills AA, et al. p63 regulates an adhesion programme and cell survival in epithelial cells. Nat Cell Biol 2006; 8:551-61.
22. Higashikawa K, Yoneda S, Tobiume K, Taki M, Shigeishi H, Kamata N. Snail-induced downregulation of DeltaNp63alpha acquires invasive phenotype of human squamous cell carcinoma. Cancer Res 2007; 67:9207-13.
23. Jordà M, Olmeda D, Vinyals A, Valero E, Cubillo E, Llorens A, et al. Upregulation of MMP-9 in MDCK epithelial cell line in response to expression of the Snail transcription factor. J Cell Sci 2005; 118:3371-85.
24. Zha YH, He JF, Mei YW, Yin T, Mao L. Zinc-finger transcription factor snail accelerates survival, migration and expression of matrix metalloproteinase-2 in human bone mesenchymal stem cells. Cell Biol Int 2007; 31:1089-96.
25. Yokoyama K, Kamata N, Fujimoto R, Tsutsumi S, Tomonari M, Taki M, et al. Increased invasion and matrix metalloproteinase-2 expression by Snail-induced mesenchymal transition in squamous cell carcinomas. Int J Oncol 2003; 22:891-8.
26. Miyoshi A, Kitajima Y, Sumi K, Sato K, Hagiwara A, Koga Y, et al. Snail and SIP1 increase cancer invasion by upregulating MMP family in hepatocellular carcinoma cells. Br J Cancer 2004; 90:1265-73.
27. Sivertsen S, Hadar R, Elloul S, Vintman L, Bedrossian C. Reich, et al. Expression of Snail, Slug and Sip1 in malignant mesothelioma effusions is associated with matrix metalloproteinase, but not with cadherin expression. Lung Cancer 2006; 54:309-17.
28. Vega S, Morales AV, Ocaña OH, Valdés F, Fabregat I, Nieto MA. Snail blocks the cell cycle and confers resistance to cell death. Genes Dev 2004; 18:1131-43.
29. Roy HK, Iversen P, Hart J, Liu Y, Koetsier JL, Kim Y, et al. Downregulation of SNAIL suppresses MIN mouse tumorigenesis: modulation of apoptosis, proliferation and fractal dimension. Mol Cancer Ther 2004; 3:1159-65.
30. Kajita M, McClinic KN, Wade PA. Aberrant expression of the transcription factors snail and slug alters the response to genotoxic stress. Mol Cell Biol 2004; 24:7559-66.
31. Leong KG, Niessen K, Kulic I, Raouf A, Eaves C, Pollet I, et al. Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin. J Exp Med 2007; 204:2935-48.
32. Fouquet S, Lugo-Martínez VH, Faussat AM, Renaud F, Cardot P, Chambaz J, et al. Early loss of E-cadherin from cell-cell contacts is involved in the onset of Anoikis in enterocytes. J Biol Chem 2004; 279:43061-9.
33. Sloan EK, Pouliot N, Stanley KL, Chia J, Moseley JM, Hards DK, et al. Tumor-specific expression of alphavbeta3 integrin promotes spontaneous metastasis of breast cancer to bone. Breast Cancer Res 2006; 8:20.
34. Turner FE, Broad S, Khanim FL, Jeanes A, Talma S, Hughes S, et al. Slug regulates integrin expression and cell proliferation in human epidermal keratinocytes. J Biol Chem 2006; 281:21321-31.

35. Aybar MJ, Nieto MA, Mayor R. Snail precedes slug in the genetic cascade required for the specification and migration of the Xenopus neural crest. Development 2003; 130:483-94.
36. Blanco MJ, Barrallo-Gimeno A, Acloque H, Reyes AE, Tada M, Allende ML, et al. Snail1a and Snail1b cooperate in the anterior migration of the axial mesendoderm in the zebrafish embryo. Development 2007; 134:4073-81.
37. Hu CT, Wu JR, Chang TY, Cheng CC, Wu WS. The transcriptional factor Snail simultaneously triggers cell cycle arrest and migration of human hepatoma HepG2. J Biomed Sci 2008; 15:343-55.
38. Usami Y, Satake S, Nakayama F, Matsumoto M, Ohnuma K, Komori T, et al. Snail-associated epithelial-mesenchymal transition promotes oesophageal squamous cell carcinoma motility and progression. J Pathol 2008; 215:330-9.
39. Byzova TV, Kim W, Midura RJ, Plow EF. Activation of integrin alpha(V)beta(3) regulates cell adhesion and migration to bone sialoprotein. Exp Cell Res 2000; 254:299-308.
40. Ciruna B, Rossant J. FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. Dev Cell 2001; 1:37-49.
41. Lu Z, Ghosh S, Wang Z, Hunter T. Downregulation of caveolin-1 function by EGF leads to the loss of E-cadherin, increased transcriptional activity of beta-catenin, and enhanced tumor cell invasion. Cancer Cell 2003; 4:499-515.
42. Wanami LS, Chen HY, Peiró S, García de HA, Bachelder RE. Vascular endothelial growth factor-A stimulates Snail expression in breast tumor cells: implications for tumor progression. Exp Cell Res 2008; 314:2448-53.
43. Valdés F, Alvarez AM, Locascio A, Vega S, Herrera B, Fernández M, et al. The epithelial mesenchymal transition confers resistance to the apoptotic effects of transforming growth factor Beta in fetal rat hepatocytes. Mol Cancer Res 2002; 1:68-78.
44. Piedra ME, Ros MA. BMP signaling positively regulates Nodal expression during left right specification in the chick embryo. Development 2002; 129:3431-40.
45. Smith AP, Verrecchia A, Fagà G, Doni M, Perna D, Martinato F, et al. A positive role for Myc in TGFbeta-induced Snail transcription and epithelial-to-mesenchymal transition. Oncogene 2009; 28:422-30.
46. Gotzmann J, Huber H, Thallinger C, Wolschek M, Jansen B, Schulte-Hermann R, et al. Hepatocytes convert to a fibroblastoid phenotype through the cooperation of TGFbeta1 and Ha-Ras: steps towards invasiveness. J Cell Sci 2002; 115:1189-202.
47. Peinado H, Quintanilla M, Cano A. transforming growth factor beta-1 induces snail transcription factor in epithelial cell lines: mechanisms for epithelial mesenchymal transitions. J Biol Chem 2003; 278:21113-23.
48. Grille SJ, Bellacosa A, Upson J, Klein-Szanto AJ, van Roy F, Lee-Kwon W, et al. The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res 2003; 63:2172-8.
49. Pon YL, Zhou HY, Cheung AN, Ngan HY, Wong AS. p70 S6 Kinase Promotes Epithelial to Mesenchymal Transition through Snail Induction in Ovarian Cancer Cells. Cancer Res 2008; 68:6524-32.
50. Thuault S, Tan EJ, Peinado H, Cano A, Heldin CH, Moustakas A. HMGA2 and Smads co-regulate SNAIL1 expression during induction of epithelial-to-mesenchymal transition. J Biol Chem 2008; 283:33437-46.
51. Li X, Deng W, Nail CD, Bailey SK, Kraus MH, Ruppert JM, et al. Snail induction is an early response to Gli1 that determines the efficiency of epithelial transformation. Oncogene 2006; 25:609-21.
52. Sahlgren C, Gustafsson MV, Jin S, Poellinger L, Lendahl U. Notch signaling mediates hypoxia-induced tumor cell migration and invasion. Proc Natl Acad Sci USA 2008; 105:6392-7.
53. Barberà MJ, Puig I, Domínguez D, Julien-Grille S, Guaita-Esteruelas S, Peiró S, et al. Regulation of Snail transcription during epithelial to mesenchymal transition of tumor cells. Oncogene 2004; 23:7345-54.
54. Rosanò L, Spinella F, Di Castro V, Nicotra MR, Dedhar S, de Herreros AG, et al. Endothelin-1 promotes epithelial-to-mesenchymal transition in human ovarian cancer cells. Cancer Res 2005; 65:11649-57.
55. Tan C, Costello P, Sanghera J, Dominguez D, Baulida J, de Herreros AG, et al. Inhibition of integrin linked kinase (ILK) suppresses beta-catenin-Lef/Tcf-dependent transcription and expression of the E-cadherin repressor, snail, in $APC^{-/-}$ human colon carcinoma cells. Oncogene 2001; 20:133-40.
56. Hudson LG, Choi C, Newkirk KM, Parkhani J, Cooper KL, Lu P, et al. Ultraviolet radiation stimulates expression of Snail family transcription factors in keratinocytes. Mol Carcinog 2007; 46:257-68.
57. Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, et al. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 2005; 436:123-7.
58. Imai T, Horiuchi A, Wang C, Oka K, Ohira S, Nikaido T, et al. Hypoxia attenuates the expression of E-cadherin via upregulation of SNAIL in ovarian carcinoma cells. Am J Pathol 2003; 163:1437-47.
59. Evans AJ, Russell RC, Roche O, Burry TN, Fish JE, Chow VW. VHL promotes E2 box-dependent E-cadherin transcription by HIF-mediated regulation of SIP1 and snail. Mol Cell Biol 2007; 27:157-69.
60. Fujita N, Jaye DL, Kajita M, Geigerman C, Moreno CS, Wade PA. MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. Cell 2003; 113:207-19.
61. Toh Y, Nicolson GL. The role of the MTA family and their encoded proteins in human cancers: molecular functions and clinical implications. Clin Exp Metastasis 2009; 26:215-27.
62. Peiró S, Escrivà M, Puig I, Barberà MJ, Dave N, Herranz N, et al. Snail1 transcriptional repressor binds to its own promoter and controls its expression. Nucleic Acids Res 2006; 34:2077-84.
63. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, et al. Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol 2004; 6:931-40.
64. Yook JI, Li XY, Ota I, Fearon ER, Weiss SJ. Wnt-dependent regulation of the E-cadherin repressor snail. J Biol Chem 2005; 280:11740-8.
65. Peinado H, Del Carmen Iglesias-de la Cruz M, Olmeda D, Csiszar K. A molecular role for lysyl oxidase-like 2 enzyme in snail regulation and tumor progression. EMBO J 2005; 24:3446-58.
66. Peinado H, Portillo F, Cano A. Switching on-off Snail: LOXL2 versus GSK3beta. Cell Cycle 2005; 4:1749-52.
67. Domínguez D, Montserrat-Sentís B, Virgós-Soler A, Guaita S, Grueso J, Porta M, et al. Phosphorylation regulates the subcellular location and activity of the snail transcriptional repressor. Mol Cell Biol 2003; 23:5078-89.
68. Yang Z, Rayala S, Nguyen D, Vadlamudi RK, Chen S, Kumar R. Pak1 phosphorylation of snail, a master regulator of epithelial-to-mesenchyme transition, modulates snail's subcellular localization and functions. Cancer Res 2005; 65:3179-84.
69. Seki K, Fujimori T, Savagner P, Hata A, Aikawa T, Ogata N, et al. Mouse Snail family transcription repressors regulate chondrocyte, extracellular matrix, type II collagen and aggrecan. J Biol Chem 2003; 278:41862-70.
70. de Frutos CA, Vega S, Manzanares M, Flores JM, Huertas H, Martínez-Frías ML, et al. Snail1 is a transcriptional effector of FGFR3 signaling during chondrogenesis and achondroplasias. Dev Cell 2007; 13:872-83.
71. Guaita S, Puig I, Franci C, Garrido M, Dominguez D, Batlle E, et al. Snail induction of epithelial to mesenchymal transition in tumor cells is accompanied by MUC1 repression and ZEB1 expression. J Biol Chem 2002; 277:39209-16.