Overexpression of Slug is associated with malignant progression of esophageal adenocarcinoma

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Abstract

AIM: To characterise expression of known E-cadherin repressors; Snail, Slug and Twist in the development of esophageal adenocarcinoma.

METHODS: E-cadherin, Slug, Snail and Twist mRNA expression in Barrett's metaplasia and esophageal adenocarcinoma specimens was examined by real-time reverse transcription-polymerase chain reaction (RT-PCR). Semi-quantitative immunohistochemistry was used to examine cellular localization and protein levels. The effect of Slug on epithelial mesenchymal transition (EMT) markers was examined by transfection of Slug into an adenocarcinoma line OE33.

RESULTS: Cellular localization of Slug in Barrett's metaplasia was largely cytoplasmic whilst in adenocarcinoma it was nuclear. Semi-quantitative analysis indicated that Slug was more abundant in adenocarcinoma compared to matched Barrett's metaplastic specimens. Snail and Twist were expressed in adenocarcinoma but were cytoplasmic in location and not induced compared to Barrett's mucosa. These observations were supported by mRNA studies where only Slug mRNA was shown to be over-expressed in adenocarcinoma and inversely correlated to E-cadherin expression. Overexpression of Slug in OE33 mediated E-cadherin repression and induced the mesenchymal markers vimentin and fibronectin.

CONCLUSION: Progression to adenocarcinoma is associated with increased Slug expression and this may represent a mechanism of E-cadherin silencing.

Key words: Slug; Oesophagus; Cancer; Barrett's metaplasia; Epithelial-mesenchymal transition

INTRODUCTION

The incidence of esophageal adenocarcinoma is currently rising faster than any other cancer in the Western world. Alarmingly the cause of this increase is largely unknown[1]. The strongest known risk factor for esophageal adenocarcinoma is the condition Barrett's metaplasia which is characterized by a replacement of the native squamous lined esophagus with a columnar epithelium: a possible consequence of prolonged reflux of gastric contents into the lower esophagus[2].

To date, numerous proteins have been implicated in the malignant progression of Barrett's metaplasia to adenocarcinoma including E-cadherin[3-6]. E-cadherin is a calcium dependent cell adhesion molecule which is essential in the establishment of homotypic adhesion junctions[7]. In nearly all epithelial cancers E-cadherin has been reported to be repressed: an essential step for increased invasiveness and metastasis[8-10], thus this event commonly occurs during late tumourigenesis. In particular in the development of esophageal adenocarcinoma, E-cadherin repression has been reported to be a late event associated with high grade dysplastic Barrett's metaplasia...
and adenocarcinoma. However, it remains unclear what directs repression of E-cadherin expression in esophageal adenocarcinoma. Unlike in hereditary diffuse gastric cancer E-cadherin is not subject to mutation and neither is there evidence of E-cadherin promoter methylation as is common in colorectal cancers. A possible mechanism of E-cadherin silencing, which to date has not been addressed, is transcriptional repression by proteins involved in epithelial mesenchymal transition (EMT) including proteins in the Snail family: Snail and Slug and the transcription factor Twist.

Several studies have elegantly demonstrated in a variety of cell types that overexpression of Snail and Slug causes an EMT and this is associated with E-cadherin repression. Furthermore, it has been demonstrated that these effects are mediated through interaction with specific E-pal elements of the E-cadherin proximal promoter. In support of a role for these EMT regulators in E-cadherin repression and carcinogenesis, several studies have shown overexpression in several epithelial cancers including overexpression of Slug in gastric carcinomas, Snail in colorectal cancers and Twist in pancreatic cancers.

The primary aim of this study was to determine the expression profile of Snail, Slug and Twist in the development of esophageal adenocarcinoma and determine if overexpression of these proteins in an esophageal background is able to mediate a repression in E-cadherin.

MATERIALS AND METHODS

Ethics
This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved ethically by University Hospital Birmingham Trust (LREC 2002/166). All patients provided informed written consent.

Patient tissue
Esophageal adenocarcinoma resection specimens: Samples of normal squamous esophagus (n = 40) and esophageal adenocarcinoma (n = 40) were collected during surgery. Half of the esophageal adenocarcinoma resection specimens (n = 20) collected also had associated intestinal Barrett’s metaplasia. In addition, samples (n = 20) of long segment (≥ 3 cm) Barrett’s metaplasia, defined as columnar mucosa with intestinal type goblet cells were also collected during endoscopy. All specimens were divided in two, half for RNA extraction and half for immunohistochemistry.

Immunohistochemistry: Immunohistochemistry for E-cadherin was performed using microwave antigen retrieval as previously described with an E-cadherin monoclonal antibody (clone 36, BD Biosciences, Oxford, UK) used at a concentration of 1:300. Immunohistochemistry for Snail, Slug and Twist was performed as follows: Slides were immersed in W-cap buffer (Bio-Optica, Milan, Italy) and cycled in a Pixel antigen retriever (CellPath, Newtown, UK) for 60 min, washed in running water and placed in methanol:hydrogen peroxide (10:1) for 5 min. Sections were then incubated in a primary antibody to either Snail, 1:10, (SNA1 clone E18, Autogen Bioclear, Wiltshire, UK), Slug, 1:20 (SNA12 clone G18 Autogen Bioclear, UK) or Twist, 1:50 (clone C17 Autogen Bioclear, UK) in TBS 7.5 × buffer (Bios Europe Ltd, Skelmersdale, UK) at 4°C overnight, washed with TBS and reacted with peroxidase-linked rabbit anti-sheep antibody (Dako, Ely, UK) at 1:100 dilution in TBS for 1 h. The immunoreactivity was then revealed as above using DAB. Slides were then dipped in hematoxylin, dehydrated and mounted. The slides were scored by a previously described method for (1) intensity of staining (0 = negative, 1 = weak, 2 = moderate, 3 = intense) and (2) percentage of epithelial cells staining (0 = 0%-5%; 1 = 6%-25%; 2 = 26%-50%; 3 = 51%-75%; 4 = 76%-100%); these two scores were multiplied to yield a final staining score. In addition, cellular localization (nuclear, cytoplasmic, cell surface) was assessed. All sections were scored independently by two observers (PJ and CT).

In the series of immunofluorescent experiments following primary antibody incubations, sections were washed extensively and then incubated with either FITC goat anti-mouse or goat anti-rabbit (Jackson Immunoresearch, USA, 1:500) for 1 h. Sections were then washed and incubated in 4’, 6-Diamidino-2-phenylindole dihydrochloride hydrate (DAPI) (1:10 000) for 1 min prior to visualisation. Omission of primary antibody was employed as a negative control. Images were visualized using an Olympus BX40 microscope and digital images taken using a Sensys Photometrics camera (Middlesex, UK). Desksoft SmartCapture 2 software was used for image acquisition (Desksoft, USA).

Real time RT-PCR
Real time RT-PCR reactions were performed as previously described using 18S ribosomal RNA as an internal standard (PE Biosystems, Roche, USA). Each reaction was performed in triplicate and contained one of the following sets of probes and primers: (1) E-cadherin (probe 5’FAM AAATTCCACTCTGCCCAGGACCGGTT3’), forward primer (5’-GGCGCCACCTCGAGAGA-3’) and reverse primer (5’-TGTGACCCGTGTCATCTT3’); (2) Slug (probe 5’FAM’CACATACGTTATTTC CC CCGATCTCTATGAGATTAMRA3’), forward primer (5’-GAGTGGGCTGGTCGTA-3’) and reverse primer (5’-TGTGACCCGTGTCATCTT3’); (3) Snail (probe 5’FAM’GGTGAGTTTACCTTCCAGCTAMRA3’), forward primer (5’-AAGGCAAATCTACGGAAGT-3’) and reverse primer (5’-GGTGAGTTTACCTTCCAGCTAMRA3’), forward primer (5’-GCTGGGCTGGTCGTA-3’) and reverse primer (5’-GAGTGGGCTGGTCGTA-3’), or (4) Twist (probe 5’FAMCAATGACACTTGG TCTC-3’), forward primer (5’-GCTGAGATCTCTA GACTTTCAATT-3’) and reverse primer (5’-GGGGCCTGTTCTAGTCT-3’).

Western blotting
Western blotting was performed as previously described with antibodies to (1) E-cadherin (1:2000, clone 36, Autogen Bioclear UK); (2) Fibronectin (1:1000, Clone...
PIF11 Autogen Bioclear, UK); (3) Vimentin (1:1000, clone C-20, Autogen Bioclear, UK); (4) Cytokeratin 19 (CK-19) (1:2000; Oncogene Research Products USA) and (5) GFP (1:2000, Clone AB290 Abcam, UK). Immunoreactive bands were then subject to densitometry using NIH Image 1.62 software.

**Cell culture**

Cell lines derived from esophageal adenocarcinoma (OE33[22], SEG-1[23] and oesophageal squamous carcinoma TE-7[24]) were all routinely cultured in Dulbecco’s modified Eagles medium (Gibco, USA) with 10% fetal calf serum supplemented with 1 × 10^5 Units/L penicillin and 1 g/L streptomycin. Upon reaching 70% confluence cells were lysed into Trizol reagent (Gibco, UK) for mRNA extraction and evaluation of Slug mRNA expression by Real-Time PCR.

**Transfection studies**

Cell line OE33 was transiently transfected with either control empty vector, pMSCV-GFP or full-length human Slug-GFP tagged construct, pMSCV-Slug-GFP[25] or beta-galactosidase plasmid (beta-GAL) using Lipofectamine Plus according to manufacturer’s instruction. 48 h post transfection cells were either assayed for transfection efficiency using beta-galactosidase assay to determine transfection efficiency, lysed into RIPA buffer for Western blotting or lysed in Trizol for real time RT-PCR studies. Data analysis was only performed in experiments where a minimum of 40% transfection efficiency was achieved as determined by beta-galactosidase assay.

To determine the effect of Slug on the human E-cadherin promoter, an E-cadherin promoter assay was performed as previously described[26]. Briefly, OE33 cells were transiently transfected with full length human slug (pCDNA3-Slug[27]), Renilla luciferase plasmid pRL-TK and the wild-type human E-cadherin promoter cloned into the pGL3 basic luciferase reporter plasmid (EproWT) a kind gift from Professor Frans van Roy). Controls included empty pCDNA3 and empty pGL3 basic vectors. 48 h post transfection cells were assayed for firefly and Renilla luciferase activities using the dual-reporter assay kit Stop ‘N’ Glow (Promega) according to manufacturer’s instruction. Firefly luciferase activity was normalized to Renilla luciferase activity as a transfection control. Promoter activity was expressed as a fold change in relative luciferase units (RLU) compared to that obtained in pGL3 basic control transfected cells. Results shown represent the means ± SE of three independent experiments.

**Statistical analysis**

All data are presented as means ± SE. Statistical significance was calculated using unpaired Student’s t test. To assess the association between Slug and E-cadherin, linear regression analysis was performed on log-transformed mRNA fold change values using log Slug expression as the independent variable and log E-cadherin expression as the dependent variable. The R^2 value was determined to demonstrate the extent to which expression of Slug was correlated to E-cadherin. A Bonferroni adjustment was applied to P values attained from multiple comparisons during the analysis of prognostic factors. In univariate analyses significance was accepted at P ≤ 0.05, following Bonferroni adjustment, P ≤ 0.0125 was considered significant. All analyses were performed using SPSS version 10.0 (SPSS Inc, USA).

**RESULTS**

**Immunolocalization of Slug, Slug and Twist in esophageal adenocarcinoma**

In normal stratified squamous esophagus, there was no detectable expression of Slug and only weak diffuse cytoplasmic immunoreactivity for Twist, while there was strong nuclear immunoreactivity for Slug in basal and supra-basal layers (Figure 1). In Barrett’s metaplasia, Slug and Slugs were generally localised to the cytoplasm with staining being patchy and diffuse in nature. In contrast, Twist was largely nuclear in localization (Figure 1). In esophageal adenocarcinoma, all three proteins were abundantly expressed with Snail and Twist being mostly localised in the cytoplasm whilst Slug was almost exclusively nuclear in all adenocarcinoma specimens (n = 20) (Figure 1).

In addition, all slides were scored and data are shown in Table 1. To determine if Slug, Slugs and Twist were over-expressed in adenocarcinoma specimens relative to non-matched Barrett’s metaplastic specimens (BM-ve), statistical analysis was performed and results showed that both Slug and Snail were significantly over-expressed (P < 0.05) in esophageal adenocarcinoma. Twist, however, was not significantly induced. To validate this data, we further performed immunohistochemistry on a further 20 sections of adenocarcinoma with matched Barrett’s metaplastic tissue (BM+ve). When comparing the staining scores of matched Barrett’s metaplastic specimens with adenocarcinoma, only Slug was significantly over-expressed (P < 0.05).

Since there is a growing body of evidence implicating Slug as a repressor of E-cadherin, we further sought to address if Slug overexpression was associated with depressed E-cadherin expression in sections of both Barrett’s metaplasia and adenocarcinoma using dual immunofluorescence with anti-Slug (FITC) and E-cadherin antibodies (Texas Red) (Figure 2). Sections of Barrett’s
metaplasia mostly demonstrated preserved cell border E-cadherin immunoreactivity consistent with its role in cell-cell adhesion whilst there was little detectable expression of Slug. Conversely in sections of adenocarcinoma in areas of abundant Slug immunoreactivity, there was almost a complete loss of E-cadherin immunoreactivity with the expression mostly localised to the cytoplasm.

**mRNA expression of E-cadherin, Slug, Snail and Twist in Barrett’s metaplasia and esophageal adenocarcinoma specimens**

To determine if Slug, Snail, Twist and E-cadherin were modulated at the transcriptional level in esophageal adenocarcinoma, 10 specimens of esophageal adenocarcinoma each with matched intestinal Barrett’s
metaplasia were analysed by real-time PCR (Figure 3).

*E-cadherin* mRNA was repressed in 70% of adenocarcinoma specimens whilst *Slug, Snail* and *Twist* was over-expressed in 70%, 40% and 50%, respectively. The mean fold decrease for *E-cadherin* in the ten specimens examined was 0.73 whilst *Slug, Snail* and *Twist* had mean fold increases of 2.07, 1.47 and 1.50 respectively. We then sought to determine if the mean fold changes for *Slug*, *Snail* and *Twist* across the ten specimens were significantly different from *E-cadherin* values. This revealed that only *Slug* was significantly elevated compared to *E-cadherin* expression (*P* < 0.005). Additionally we assessed the association between *E-cadherin* and these EMT regulators using linear regression analysis. Results of this showed that again only *Slug* was negatively correlated with *E-cadherin* expression (*R*² -0.677, *P* < 0.03) (Figure 4).

**Figure 2** Co-immunofluorescence of Slug and E-cadherin in Barrett’s metaplasia and esophageal adenocarcinoma. Sections of Barrett’s metaplasia (BM) mostly demonstrate preserved cell border E-cadherin (Texas Red) immunoreactivity consistent with its role in cell-cell adhesion. Slug (FITC) immunoreactivity in BM however was minimal and appeared non specific. Conversely in sections of adenocarcinoma (ADC) in areas of abundant Slug immunoreactivity, there was negligible E-cadherin immunoreactivity. DAPI staining was used to highlight nuclei in sections (× 40).

**Figure 3** Exogenous overexpression of Slug in the cell line OE33 induces an EMT phenotype

*Slug* mRNA expression was examined in a panel of three esophageal cell lines OE33, SEG1 and TE-7 by real-time PCR and results showed that the cell line OE33 had the lowest expression of *Slug* mRNA whilst the highest *Slug* expressing cell line was TE-7. In this regard, the cell line OE33 was chosen for *Slug* overexpression studies.

The cell line OE33 was transiently transfected with either full length human *Slug*-GFP vector or the control empty GFP vector. Forty-eight h after transfection, cells were either lysed and processed for mRNA and protein analysis or fixed for immunofluorescence.

mRNA analysis revealed an approximate 6.5 × induction in *Slug* mRNA in cells transfected with full length human *Slug*-GFP compared to cells transfected with empty-GFP vector (*P* < 0.05) (Figure 5A). Exogenous *Slug* expression was further verified indirectly (due to a lack of a commercially available *Slug* antibody suitable for Western blotting) by Western blotting for GFP (Figure 5B).

To ascertain whether overexpression of *Slug* in OE33 cells mediated an EMT phenotype, Western blotting was performed to examine protein expression of three EMT markers *E-cadherin*, vimentin and fibronectin (Figure 6). Densitometric analysis of Western blots (*n* = 6) revealed *Slug* overexpression was associated with a repression in *E-cadherin* (mean fold decrease of 53% ± 15%, *P* < 0.05) and increased vimentin (41% ± 11%, *P* < 0.05) and fibronectin expression (32% ± 12%, *P* < 0.05). In addition, the cell adhesion and signalling molecule beta-catenin was also examined and shown to be significantly repressed (57% ± 19%, *P* < 0.05).

To address whether *Slug* mediated *E-cadherin* protein repression was through a direct effect at the level of the *E-cadherin* promoter and consequently a transcriptional effect, a luciferase *E-cadherin* promoter assay and real-time PCR was employed respectively as previously described[21,25]. Overexpression of *Slug* in OE33 resulted in a significant reduction (35% ± 10%, *P* < 0.05) in *E-cadherin* promoter activity (Figure 7A) which was mirrored by decreased *E-cadherin* mRNA expression (20% ± 5%, *P* < 0.05) (Figure 7B).

**DISCUSSION**

Recent reports have highlighted the importance of
We have demonstrated for the first time a modulation in the expression and localization of these proteins in the malignant progression of normal squamous esophagus to adenocarcinoma.

Twist was only weakly expressed in squamous esophagus consistent with the results of Yuen et al. Their studies reported over 95% of non-neoplastic squamous esophageal samples were either negative or weak for Twist immunoreactivity as assessed by immunohistochemistry. However, in our studies we did observe nuclear Twist staining in Barrett’s metaplasia which in adenocarcinoma had become cytoplasmic in localization. This pattern of cytoplasmic expression for Twist in cancer has also been observed in squamous cell cancers of the esophagus.

We were unable to show a difference in Twist expression between matched Barrett’s and adenocarcinoma specimens.
at both the mRNA and protein level, and neither was its expression associated with prognostic end points such as stage and nodal involvement.

In the case of Snail, this was comparably either very weak or nondetectable in stratified squamous oesophagus whilst in both Barrett’s metaplasia and adenocarcinoma Snail was detectable in the cell cytoplasm. Despite moderate expression of Snail in adenocarcinoma specimens, there was no difference in expression between matched adenocarcinoma and Barrett’s specimens at both the protein and mRNA level. A recent study has also reported Snail immunoreactivity in adenocarcinomas of the upper gastrointestinal tract including the esophagus. In their study Snail expression was reported in 11% of esophageal adenocarcinomas examined and no correlation was found between the expression of Snail and tumor grade and stage.

Why both Snail and Twist are localised to the cytoplasm in esophageal adenocarcinoma is unclear though a recent study has suggested that Snail’s localization could in part be determined by post-translational phosphorylation with proteins such as Pak1. Interestingly, when Pak1 mediated phosphorylation of Snail was inhibited, this not only caused a nuclear to cytoplasmic accumulation but also attenuated its repressor activity.

Immunolocalization studies for the transcription factor Slug showed that it was strongly expressed in nuclei of basal and suprabasal esophageal keratinocytes of the stratified squamous epithelium consistent with previous reports in murine studies and other human stratified epithelia including epidermis. Whilst Slug nuclear expression was lost in Barrett’s metaplasia, it was retained in adenocarcinoma and furthermore there appeared to be an overexpression of this protein in adenocarcinoma compared to matched Barrett’s metaplasia at both the mRNA and protein level. Interestingly Uchikado et al have reported that Slug is also over-expressed in esophageal squamous cell carcinomas (SCC). Their study showed that the presence of Slug was associated with depth of tumor invasion, lymph node metastasis, stage and lymphatic invasion. Consistent with our study, Slug expression in SCC was significantly correlated with reduced E-cadherin expression.

Thus our data would suggest that of the three EMT regulators examined, Slug was the only transcription factor to show a significant increase at both the mRNA and protein level in the progression from Barrett’s metaplasia to adenocarcinoma. This coupled with nuclear expression in adenocarcinoma and an association with E-cadherin repression suggests functionality in oesophageal tumourigenesis.
The consequence of overexpression of Slug in adenocarcinoma is likely to be complex, as exemplified by the ever-growing list of direct and indirect downstream target genes attributed to be modulated as a consequence of its expression\[25,32,33\]. As anticipated, many of these targets appear to be involved in cellular survival, proliferation and mesenchymal transition. The latter was exemplified by E-cadherin, a protein commonly silenced in epithelial cancers including esophageal adenocarcinoma\[64,8\]17\].

To support our hypothesis that Slug might be responsible for the observed repression of E-cadherin in adenocarcinoma, Slug was exogenously over-expressed in an oesophageal cell line OE33. Our results demonstrate that Slug could repress E-cadherin transcription at the level of the E-cadherin promoter. Slug mediated E-cadherin promoter repression has been previously demonstrated in other lineages and this is likely to be mediated through one or more of the E-box elements present within the promoter\[37\].

To verify the extent of EMT, we further examined the expression of the mesenchymal proteins vimentin and fibronectin\[13\]. Indeed both proteins were induced as a consequence of Slug expression and consistent with other studies Slug also mediated a repression in beta-catenin, a protein involved in both cell-cell adhesion and Wnt signalling\[39\].

Abrogating Slug induction may thus represent a potential therapeutic strategy since it appears central to the EMT phenotype observed in esophageal adenocarcinoma. A potential approach might be to silence or block known inducers of Slug. In this regard, the extracellular signals FGF, TGF-beta, and Wnt, which have been reported to be over-expressed and implicated in the pathogenesis of esophageal adenocarcinoma, have been found to induce Slug expression\[13,14,36-38\]. Thus abrogating the expression of these extracellular signals might represent a mechanism of repressing Slug and potentially mediating a mesenchymal to epithelial transition which is likely to impact on development of disease and ultimately patient survival.

In summary of the EMT regulators examined, Slug appears to be the only transcription factor over-expressed in esophageal carcinogenesis. A downstream consequence of Slug overexpression is likely to be silencing of E-cadherin and ultimately in concert with other signalling molecules induction of invasion and metastasis.

**COMMENTS**

**Background**

The incidence of esophageal adenocarcinoma is currently rising faster than any other cancer in the Western world though the cause of this increase is largely unknown. Progression of this disease is associated with a repression in the cell adhesion molecule E-cadherin; a crucial event in invasion and metastasis.

**Research frontiers**

E-cadherin silencing is a common event in nearly all epithelial malignancies and in several cancers this is either due to mutation, gene deletion or promoter methylation. However, how E-cadherin is silenced in esophageal adenocarcinoma has not been unequivocally addressed. In this study, the authors demonstrate and the overexpression of Slug could be a potential mechanism for mediating E-cadherin repression.

**Innovations and breakthroughs**

Recent reports have highlighted the importance of epithelial mesenchymal regulators including Snail, Slug and Twist in gastrointestinal carcinogenesis. In particular in esophageal squamous cell cancers, Slug is over-expressed. This is the first study to report that Slug is also over-expressed in esophageal adenocarcinomas. Furthermore, our in vitro studies would suggest that this protein may be the cause of the repression in E-cadherin observed in this cancer.

**Applications**

By understanding how Slug is induced and by blocking its expression, this study may represent a future strategy for therapeutic intervention in the treatment of patients with esophageal adenocarcinoma.

**Terminology**

Slug, Snail and Twist are all proteins involved in the process called epithelial mesenchymal transition. This is when epithelial cells lose cell-cell adhesion and behave more like fibroblasts. Such a mechanism is thought to be crucial in invasion and metastasis of cancer. Non-surprisingly, the cell adhesion molecule E-cadherin is repressed during this process.

**Peer review**

The authors examined the expression of E-cadherin and its repressors; Snail, Slug and Twist in squamous esophagus, Barrett’s metaplasia and esophageal adenocarcinoma. It revealed that Slug was increased in adenocarcinoma and in OE33 cells, the increase of Slug expression was inversely correlated to E-cadherin expression (mRNA and promoter activity) and induced EMT. The results are interesting and may represent a molecular mechanism of esophageal carcinogenesis.

**REFERENCES**

1. Lagergren J. Adenocarcinoma of oesophagus: what exactly is the size of the problem and who is at risk? Gut 2005; 54 Suppl 1: i1-i5
Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 1991; 251: 1451-1455

Takeichi M. Cadherins in cancer: implications for invasion and metastasis. Curr Opin Cell Biol 1993; 5: 806-813

Birchmeier W. Behrens J. Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta 1994; 1198: 11-26

Behrens J. Cadherins and catenins: role in signal transduction and tumour progression. Cancer Metastasis Rev 1999; 18: 15-30

Wijnhoven BP, de Both NJ, van Dekken H, Tilanus NL, van der Wilt BJ, van der Zwaag AM. Fas/APO-1 (CD95) down-regulation of E-cadherin. Int J Cancer 2007; 120: 131-142

Braunschweig R, Pasquier N, Bosman FT, Signoretti S, Mennuni F, Blasi F, van de Wetering M. Slug is overexpressed in gastric cancer. J Pathol 2005; 206: 507-515

Eads CA. Lord RV, Kurumoo SK, Wickramasinghe K, Skinner ML, Long TL, Peters JH, DeMeester TR, Danenberg KD, Danenberg PV, Laird PW, Skinner KA. Fields of aberrant CpG island hypermethylation in Barrett's esophagus and associated adenocarcinoma. Cancer Res 2000; 60: 5021-5026

De Craene B, van Roy F, Bers G. Unravelling signalling cascades for the Snail family of transcription factors. Cell Signal 2005; 17: 535-547

Barral-Cimeno A, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development 2005; 132: 3151-3161

Kang Y. Massague J. Epithelial-mesenchymal transitions: twist in development and metastasis. Cell 2004; 118: 277-279

Batlle E, Sancho E, Franci C, Dominguez M, Monfar M, Baulida J, Garcia De Herreros A. The transcription factor Slug represses E-cadherin and catenin expression and tumour progression. Cancer Res 2000; 60: 5113-5120

De Craene B, van Roy F, Bers G. Unravelling signalling cascades for the Snail family of transcription factors. Cell Signal 2005; 17: 535-547

Barral-Cimeno A, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development 2005; 132: 3151-3161

Kang Y. Massague J. Epithelial-mesenchymal transitions: twist in development and metastasis. Cell 2004; 118: 277-279

Batlle E, Sancho E, Franci C, Dominguez M, Monfar M, Baulida J, Garcia De Herreros A. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat Cell Biol 2000; 2: 84-89

Bolos V, Peinado H, Perez-Moreno MA, Fraga MF, Esteller M, Cano A. The transcription factor Slug represses E-cadherin expression and induces epithelial-to-mesenchymal transitions: a comparison with Snail and E47repressors. J Cell Sci 2003; 116: 499-511

Castro Alves C, Rosivatz E, Schott C, Hollweck R, Becker L, Sarbia M, Carneiro F, Becker KF. Slug is overexpressed in gastric carcinomas and may act synergistically with SIP1 and Snail in the down-regulation of E-cadherin. J Pathol 2007; 209: 507-515

Shioiri M, Shida T, Koda K, Oda K, Seike K, Nishimura M, Takano S, Miyazaki M. Slug expression is an independent prognostic parameter for poor survival in colorectal carcinoma patients. Br J Cancer 2006; 94: 1816-1822

Ohuchida K, Mizumoto K, Ohhashi S, Yamaguchi H, Konomi H, Nagai E, Yamaguchi K, Tsuneysuo M, Tanaka M. Twist, a novel oncogene, is upregulated in pancreatic cancer: clinical implication of Twist expression in pancreatic juice. Int J Cancer 2007; 120: 1634-1640

Brookes MJ, Hughes S, Turner FE, Reynolds G, Sharma N, Imsa T, Berx G, McKie AT, Hotchin N, Anderson GJ, Iqbal T, Teslpe C. Modulation of iron transport proteins in human colorectal carcinogenesis. Gut 2006; 55: 1449-1460

Roeckel JC, Larkin K, Darnton SJ, Morris AG, Matthews HR. Five newly established esophageal carcinoma cell lines: phenotypic and immunological characterization. Br J Cancer 1997; 75: 258-263

Hughes SJ, Nambu Y, Solides OS, Hamstra D, Rehmentulla A, Lanzelotti MD, Orringer MB, Beer DG. Fas/APO-1 (CD95) is not translocated to the cell membrane in esophageal adenocarcinoma. Cancer Res 1997; 57: 5571-5578

Boonstra JJ, van der Velden AW, Beerens EC, van Marion R, Morita-Fujimura Y, Matsui Y, Nishihara T, Teslpe C, Hainaut P, Lowe AW, Beerloov BH, van Dekken H, Tilanus HW, Djinns WN. Missedation of identity of widely used esophageal adenocarcinoma cell line TE-7. Cancer Res 2007; 67: 7996-8001

Turner FE, Brogden S, Kwon Y, Talma S, Hughes S, Teslpe C, Hotchin NA. Slug regulates integrin expression and cell proliferation in human epithelial keratinoctyes. J Biol Chem 2006; 281: 21321-21331

Rosivatz E, Becker KF, Kremmer E, Schott C, Blechschmidt K, Hofler H, Sarbia M. Expression and nuclear localization of Snail, an E-cadherin repressor, in adenocarcinomas of the upper gastrointestinal tract. Virchows Arch 2006; 448: 277-287

Yuen HF, Chan YP, Wong ML, Kwok WK, Chan KK, Lee PY, Srivastava G, Law SY, Wong YC, Wang X, Chan KW. Upregulation of Twist in esophageal squamous cell carcinoma is associated with neoplastic transformation and distant metastasis. Clin Cancer Res 2005; 11: 1174-1180

Uchikado Y, Natsukawa S, Okumura H, Setoyama T, Matsumoto M, Ishigami S, Aikou T. Slug Expression in the E-cadherin preserved tumors is related to prognosis in patients with esophageal squamous cell carcinoma. Clin Cancer Res 2005; 11: 1174-1180

Bolos V, Peinado H, Perez-Moreno MA, Fraga MF, Esteller M, Cano A. The transcription factor Slug represses E-cadherin expression and induces epithelial-to-mesenchymal transitions: a comparison with Snail and E47repressors. J Cell Sci 2003; 116: 499-511

Benni M, beta-Catenin: a pivot between cell adhesion and Wnt signalling. Cur Biol 2005; 15: R64-R67

Soslow RA, Petersen CG, Remotti H, Altorki N. Acicid fibroblast growth factor is expressed sequentially in the progression from Barrett's esophagus to esophageal adenocarcinoma. Dis Esophagus 2001; 14: 23-27

Hunt BH, Steven HJ, Feith M, Puhringer F, Theisen J, Siewert JR, Sarbia M. Overexpression of TGF-beta1 in Barrett's (esophageal) adenocarcinoma is associated with advanced stage of disease and poor prognosis. Mol Carcinog 2005; 45: 3179-3184

Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006; 7: 131-142

Biermejo-Rodriguez C, Perez-Beato M, Perez-Mancera PA, Sanchez-Beato M, Piris MA, Sanchez-Garcia I. Mouse CDNA microarray analysis uncovers Snail targets in mouse embryonic fibroblasts. Genomics 2006; 87: 113-118

Cajita M, Mcclenic KN, Wade PA. Aberrant expression of the transcription factors snail and slug alters the response to genotoxic stress. Mol Cell Biol 2004; 24: 7559-7566

Parent AE, Choi C, Caudy K, Gridley T, Kusewitt DF. The developmental transcription factor slug is widely expressed in tissues of adult mice. J Histochet Cytobast 2004; 52: 959-965

Bermajo-Rodriguez C, Perez-Beato M, Perez-Mancera PA, Sanchez-Beato M, Piris MA, Sanchez-Garcia I. Mouse CDNA microarray analysis uncovers Snail targets in mouse embryonic fibroblasts. Genomics 2006; 87: 113-118

Clement G, Braunischweig R, Pasquier N, Bosman FT, Benhattar J. Alterations of the Wnt signalling pathway during the neoplastic progression of Barrett's esophagus. Oncogene 2006; 25: 3084-3092

De Martino E, Wild CP, Rotimi O, Darnton JS, Ollivier RJ, Hardie LJ. IGFBP-3 and IGFBP-10 (CYR61) up-regulation during the development of Barrett's oesophagus and associated oesophageal adenocarcinoma: potential biomarkers of disease risk. Biomarkers 2006; 11: 547-561