Epithelial-mesenchymal Transition (EMT) Markers in Human Pituitary Adenomas Indicate a Clinical Course

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Abstract. *Background/Aim: Pituitary adenomas are brain tumors with invasive properties. Epithelial-mesenchymal-transition (EMT) is a cellular process linked to the transformation to an aggressive cancer phenotype. In the present study, we investigated the expression of a panel of EMT markers, namely E-cadherin, N-cadherin, SLUG, SNA1 and TWIST in a cohort of human pituitary adenomas. Materials and Methods: Fresh-frozen human pituitary tumors (n=95) were collected immediately after surgery for histology. Gene transcripts of the EMT markers were quantified using quantitative-polymerase chain reaction (PCR) analysis. Levels of expression were analyzed against clinical, pathological, invasion and endocrine functions. Results: Levels of E-cadherin and N-cadherin had a negative and positive correlation with the appearance of intratumoral cystic lesions of pituitary tumors. E-cadherin and TWIST were associated with tumor size and staging. There was a significant link between SLUG/TWIST and the destruction of the sella fosa bones (p<0.030). EMT markers also showed links with the endocrine functions of pituitary tumors. In pituitary tumors, SLUG and SNA1 had significant correlation with N-cadherin. Conclusion: EMT markers are significant indicators of the appearance of cystic lesions, tumor progression, bone destruction and endocrine functions. These markers are valuable biomarkers in assessing the clinical course of pituitary adenomas.*

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Pituitary adenoma is a common benign tumor of the central nervous system with a number of biological behaviours similar to malignant tumors, including invasion of the surrounding structures, such as the cavernous sinus, hypothalamus and sphenoid sinus, where it is named invasive adenoma (1). Most invasive pituitary adenomas are characterized by rapid growth, large size, poor treatment efficacy and a high recurrence rate. However, some small tumors show obvious invasive behaviour and apoplexy early on during the disease. Although there exists little consensus about what constitutes an invasive pituitary adenoma, the utility of biomarkers is rapidly evolving. Potential markers of pituitary adenoma invasiveness that have been studied include oncogenes, such as pituitary tumour transforming gene (*PTTG*); proliferation-related factors, such as Ki-67, proliferation cell nuclear antigen (PCNA) and P53 and angiogenic factors, such as vascular endothelial growth factor (VEGF) (2, 3).

The events that convert adherent epithelial cells into individual migratory cells, capable of invading the extracellular matrix, have been collectively referred to as the epithelial-mesenchymal transition (EMT). At a biomedical and molecular level, EMT is characterized by such changes as the cadherin switch, loss of cytokeratin, increased expression of vimentin and collagen, spindle-like morphology and gain of migration (4). EMT involves a sequence of changes in gene expression patterns during which epithelial cells dissipate their epithelial features and acquire characteristics typical of mesenchymal cells. EMT has been shown to play an important role during tumor progression

Table I. *Clinical and pathological information of patients.*

Group		n
Sex	Male	51
	Female	44
Age	<45years	45
	>45years	50
Tumour size	T<1cm	2
	T1-2cm	17
	T2-3cm	34
	T>3cm	42
Intratumoral haemorrhage	No	77
	Yes	18
Suprasella invasion	No	59
	Yes	36
Invasion	No	68
	Yes	27
Knosp grade	0-2	70
	3-4	25
Endocrine tumor	No	59
	PRL	5
	GH	8
	FSH	2
	ACTH	6
	TSH	4
	LH	2
	Mixture	9

ACTH: Adrenocorticotropic hormone; FSH: follicle stimulating hormone; GH: growth hormone; LH: luteinizing hormone; PRL: prolactin; TSH: thyroid stimulating hormone.

where the cells acquire mesenchymal cell properties in order to gain enhanced motile and adhesive capacities allowing them to spread and metastasize (5). Mesenchymal-like cancer cells are associated with an aggressive phenotype (6). The key changes during EMT include loss of E-cadherin expression and increases in N-cadherin, SNAI1, SLUG (SNAI2), TWIST, vimentin, fibronectin and, accordingly, many of these molecules have been shown to be deregulated in cancer (5).

Epithelial cells present a highly polarized morphology, intimately linked by cell-cell junctions. Loss of these intercellular connections provides a critical step during EMT allowing for physical detachment of cancer cells from the primary tumour. Thus, EMT is characterized by the combined loss of epithelial cell junction proteins, including E-cadherin, α-catenin, claudins, occludin and ZO-1, an increased expression of mesenchymal markers, such as N-cadherin, vimentin and fibronectin, as well as re-organization of the cytoskeleton, which collectively results in the loss of apical-basal cell polarity and the attainment of a spindle-shaped morphology (7, 8).

Loss of expression of the cell-to-cell adhesion molecule E-cadherin is a characteristic trait of EMT in the development and in progression of epithelial tumors to invasive, metastatic cancers. The loss of E-cadherin is generally seen to coincide with a gain of expression of the mesenchymal cadherin, N-cadherin in many cancer types; this "cadherin switch" is thought to be necessary for tumor cells to gain invasive properties and is also a characteristic of EMT (9).

It is evident from recent studies that EMT-inducing signals are, in part, initiated by growth factors, including hepatocyte growth factor (HGF), epidermal growth factor (EGF) and transforming growth factor-β (TGFβ). These induce downstream activation of a number of EMT-inducing transcription factors including SNAIL, SLUG, TWIST and zinc finger E-box binding homeobox 1 (ZEB1) (10).

The prsent study aimed to elucidate the expression levels of a set of known EMT markers in human pituitary tumors and to determine whether these levels are related to clinical outcome.

Materials and Methods

Clinical and pathological demographics of patients. Patients' clinical history, diagnostic images and endocrine tests were routinely recorded. This study included a total of 95 patients with pituitary adenomas who underwent trans-sphenoidal or craniotomy surgical resection between January 2012 and December 2012 at the Department of Neurosurgery of Beijing TianTan Hospital. Age, gender and hormonal functioning were also reviewed. The diagnosis was confirmed by postoperative pathology. Immunohistochemistry was used to determine the endocrine type. Preoperative magnetic resonance imaging (MRI) was performed to determine image

Table II. *Primers used in the study.*

	Forward (5'-3')	Reverse (5'-3')
E-cadherin (*CDH1*)	CAGAAAGTTTTCCACCAAAG	ACTGAACCTGACCGTACAAAATGTGAGCAATTCTGCTT
N-cadherin (*CDH2*)	ATTCTCAACCCCATCT	ACTGAACCTGACCGTACATTCTCCACTTGATTTCCATT
SLUG (*SNAI2*)	CTCCAAAAAGCCAAACTACA	ACTGAACCTGACCGTACAGAGGATCTCTGGTTGTGGTA
SNA1	TCTTTCCTCGTCAGGAAGC	ACTGAACCTGACCGTACACTGCTGGAAGGTAAACTCTG
TWIST	AAGCTGAGCAAGATTCAGAC	ACTGAACCTGACCGTACAGAGGACCTGGTAGAGGAAGT
Actin (*ACTB*)	GGACCTGACTGACTACCTCA	ACTGAACCTGACCGTACAAGCTTCTCCTTAATGTCACG
GAPDH	CTGAGTACGTCGTGGAGTC	ACTGAACCTGACCGTACACAGAGATGATGACCCTTTTG

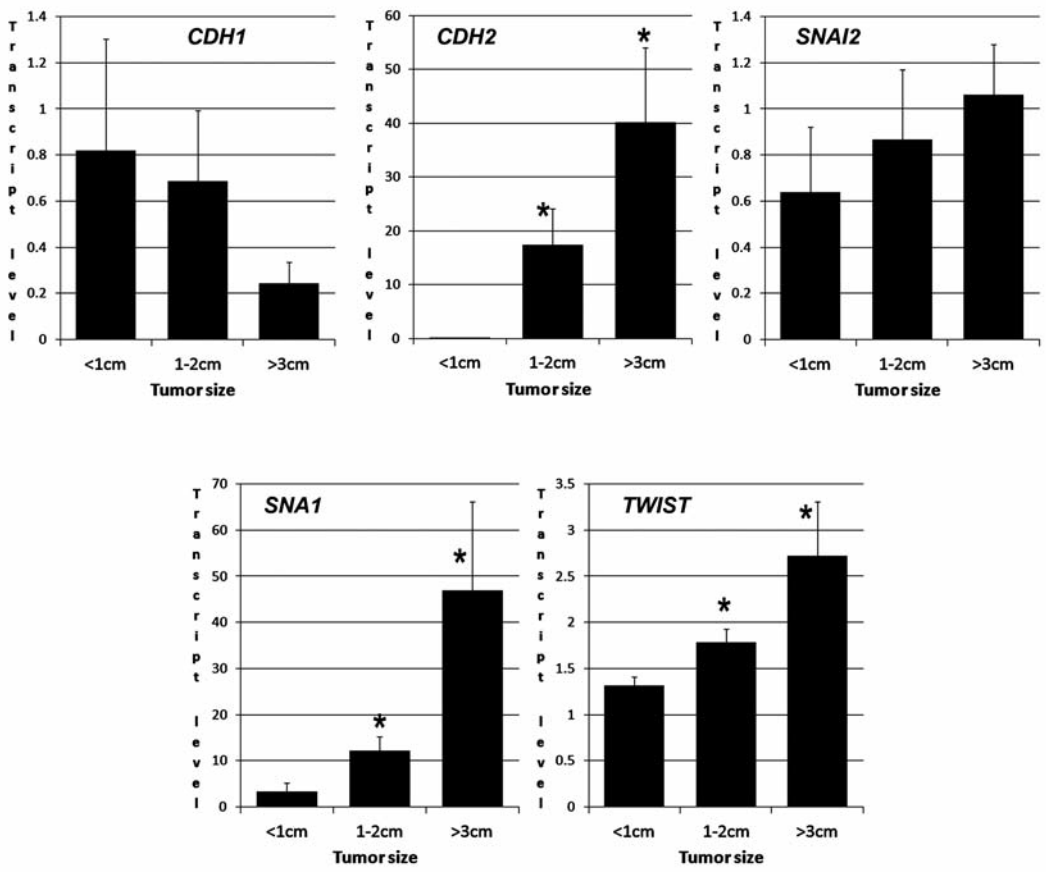

Figure 1. *EMT markers and the size of pituitary tumors.* *Indicates the respective levels of transcript significantly different from small (<1 cm) tumors.

characteristic: tumour size, cystic lesion, intratumoural haemorrhage and invasion type. The greatest diameter of tumour obtained on the gadolinium (Gd)-enhanced T1WI was measured as tumour size. Intratumoural haemorrhage and cystic lesion were defined by preoperative MRI and confirmed intraoperatively. The invasion type was determined based on the invasion site of the tumour and included cavernous sinus invasion, sphenoid sinus invasion and suprasellar invasion. The criterion for suprasellar invasion was tumour growth toward the suprasellar region with invasion in the third ventricle and/or lateral ventricle. The criterion for sphenoid sinus invasion was tumour growth downward to the sphenoid sinus cavity or tumour growth into the clivas. The cavernous sinus invasion was defined as tumour extending lateral to the lateral tangent of the intra- and supracavernous internal carotid artery (ICA) or beyond that (Grade 3 or 4). With reference to the classification by Knosp *et al*. and the method proposed by Vieira *et al*. (11-13), the full details of the patients' information are shown in Table I.

Collection of pituitary adenomas. Pituitary adenoma samples were freshly collected immediately after microsurgical resection at the Department of Neurosurgery of Beijing TianTan Hospital, supported by an ethics approval by the Local Research Ethics Committee with patient's consent. A total of 95 patients entered the current study. The tissues were immediately frozen and stored in liquid nitrogen until use.

Tissue processing and generation of genetic materials for genetic-based analyses. Frozen tumour tissues were frozen sectioned at 10 μm. Small number of sections were used for histological evaluation and the rest were combined and, then, homogenised in an RNA extraction solution. Total RNA was extracted based on manufacturer's instructions and quantified using a spectrophotometer. Equal amounts of total RNA were used to generate complementary DNA, cDNA, using a reverse transcription kit from Promega (Southampton, UK). The quality of cDNA samples were verified using *GAPDH* as a housekeeping control.

Quantitative gene transcript analyses. This was based on the Amplifluor™ Technology in quantitative gene transcript analysis. Briefly, pairs of specific primers were designed to amplify a region of E-cadherin (*CDH1*), N-cadherin (*CDH2*), SLUG (*SNAI2*), SNA1 (*SNA1*) and TWIST (*TWIST*). To one primer of the primer sets was added a Z sequence, which is complementary to the Amplifluor probe (FAM tagged, synthesised by Biosearch Technologies, Inc., Novato, CA, USA). Each reaction was comprised of a forward primer, a reverse Z primer at one tenth of the strength, the Amplifluor probe, cDNA from tumours and custom master mix (Life Technologies, Paisley, UK) (Table II). An internal standard was included as control. Quantitative polymerase chain reaction (PCR) analysis was conducted using the SteponePlus instrument (ABI, Paisley, Scotland, UK).

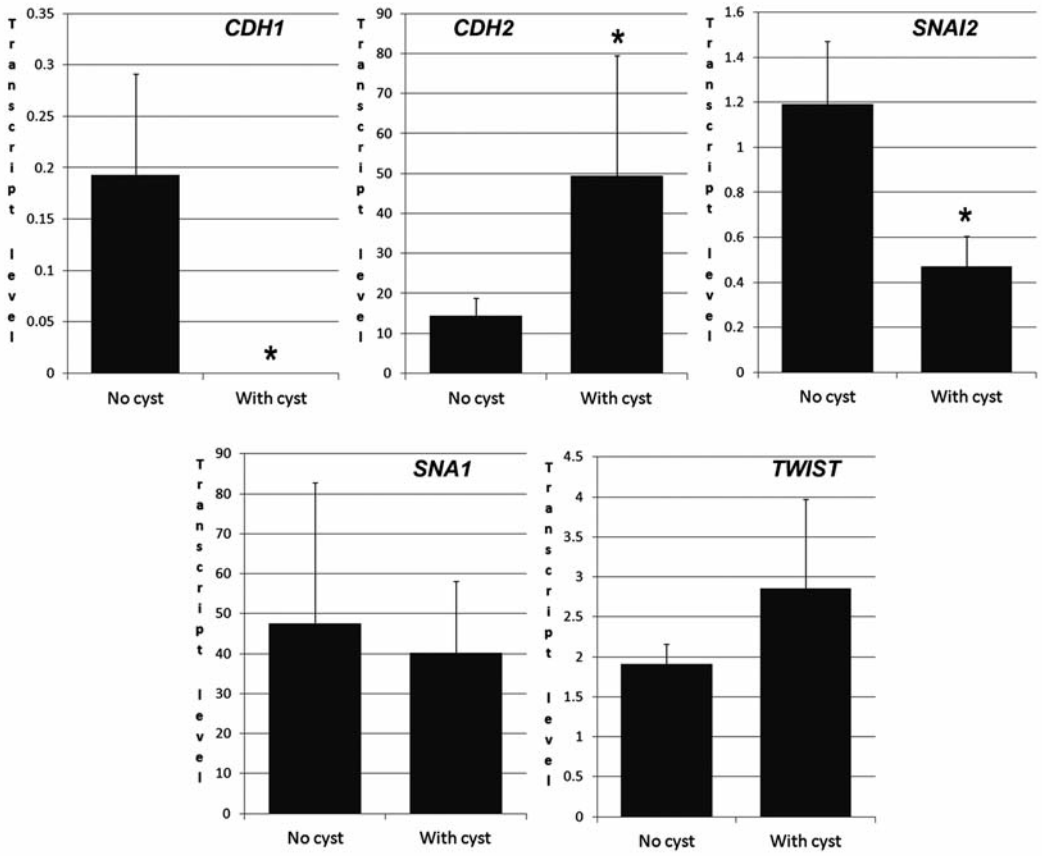

Figure 2. *Levels of cadherin transcripts are correlated with the appearance of intratumoral cysts in pituitary tumors.* *Levels of the respective transcripts significantly different from pituitary tumors without cystic lesions.

Table III. *Correlation coefficients between the EMT markers.*

Gene transcript name		N-cadherin (CDH2)	SLUG (SNAI2)	*SNA1*	*TWIST*
E-cadherin	r[a]	0.974	-0.0300	0.133	0.0214
(*CDH1*)	*p*-Value	0.347	0.772	0.198	0.838
N-cadherin	r		0.379	0.211	0.0913
(*CDH2*)	*p*-Value		0.000164	0.0402	0.381
SLUG	r			0.342	0.0451
(*SNAI2*)	*p*-Value			0.000748	0.665
SNA1	r				–0.0734
	p-Value				0.481

[a]Correlation coefficients by the Spearman's correlation test using Sigmaplot (v11).

GAPDH (coding the Glyceraldehyde 3-phosphate dehydrogenase protein) transcript, which was also simultaneously quantified, was used as a housekeeping control. The primers used are shown in Table II.

Statistical analysis. The Statistical package used was SigmaPlot (Version 11, Systat Software Inc., London, UK). The Student's *t* test and ANOVA were used for normally distributed data and Mann-Whitney U test and Kruskall-Wallis test for non-normally distributed data.

Results

EMT markers and tumor staging. High levels of TWIST were associated with the size of tumor, *i.e.* tumors greater than 2 cm had significantly higher levels of TWIST than smaller tumors (Figure 1). This trend was also observed with *CDH2* and *SNA1* transcripts. Although lower levels of *CDH1* transcript were seen in large tumors, this finding was not statistically significant. *CDH1* transcript was expressed at low levels and *TWIST* transcript were expressed at lower levels in late-stage tumors, namely KNOSP3/4 tumors (p=0.011 and p=0.05 *vs.* KNOSP1/2 tumors, respectively). No significant difference was seen with KNOSP staging and *CDH2, SNAI2* or *SNA1* transcript.

EMT markers and intratumoral cystic lesions and haemorrhage. One of the most interesting findings was that

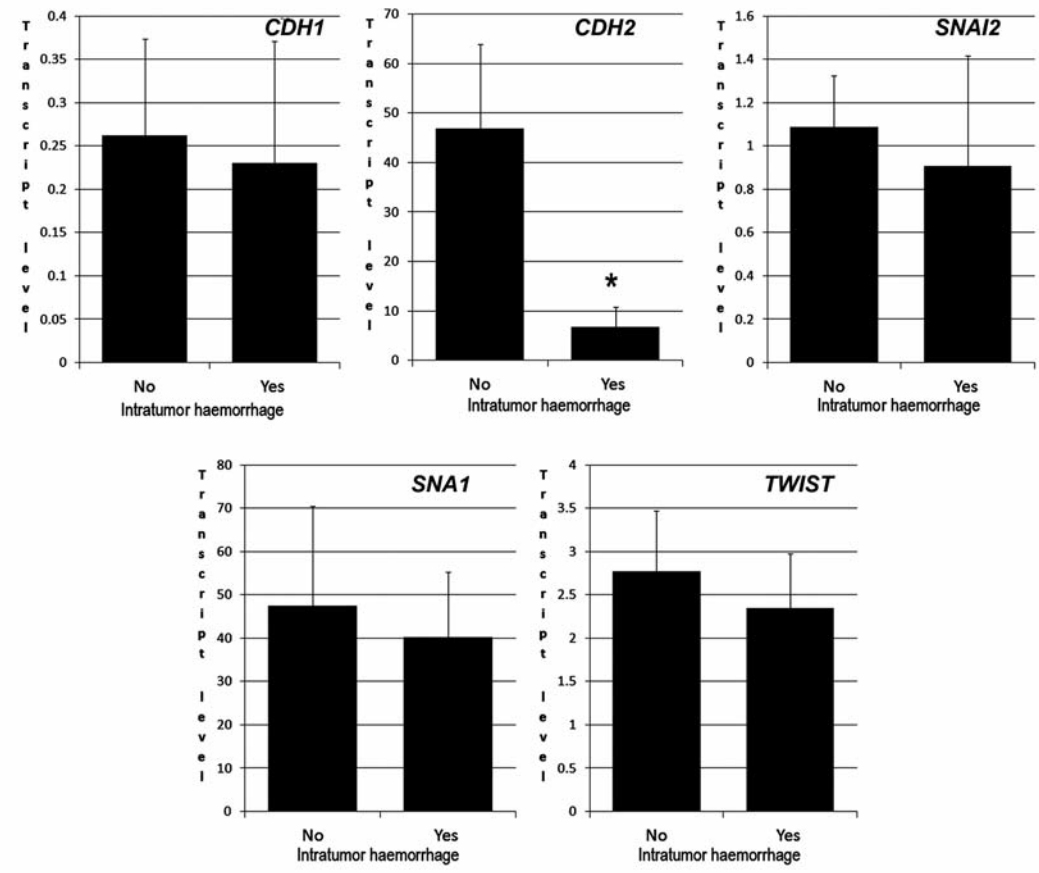

Figure 3. *EMT markers and intratumoural haemorrhage.* [*]*transcript levels significantly different from that of tumours without intratumoural haemorrhage.*

the appearance of intratumoural cysts was inversely correlated with *CDH1* gene transcript expression but was positively correlated with *CDH2* transcript expression. In other words, low levels of *CDH1* gene transcript and high levels of *CDH2* transcript are associated with the development of cysts (Figure 2). Intratumoral haemorrhage was significantly more frequently seen in tumors with low levels of *CDH2* transcript (p=0.023). There was, otherwise, no significant correlation between the haemorrhage and *CDH1, SNAI2, TWIST* or *SNA1* transcript levels (Figure 3).

EMT markers and tumor invasion and sella destruction. Sella destruction was significantly more common in tumors with lower levels of *SNAI2* transcrip (p=0.015) and higher levels of *TWIST* (p=0.030) (Figure 4). There was, otherwise, no significant correlation between the destruction and *CDH1, CDH2* nor *SNA1*. None of the markers used was found to have a significant relationship with tumor invasiveness except for pituitary tumors, that invaded the surrounding bones, and had low levels of *CDH1* transcript (p=0.11).

Endocrine functions of pituitary tumors and EMT markers. Due to the variance of the expression levels of *CDH1* transcript, we did not find any correlation between levels of *CDH1* transcript and the endocrine functions (Figure 5). Significantly lower levels of *CDH2* transcript were seen in prolactin (PR), growth hormone (GH) and mixed-hormone-secreting tumors (p=0.0087, 0.0086 and 0.044, *vs.* non-active tumours, respectively). Likewise, significantly low levels of *CDH2* transcript were seen in prolactin (PR), growth hormone (GH) and mixed-hormone-secreting tumors (seen in thyroid-stimulating hormone (TSH)- and luteinizing hormone (LH)-secreting tumors (p=0.05 and 0.031 *vs.* non-active tumors) and low levels of SNA1 in follicle-stimulating hormone (FSH)- and adrenocorticotropic hormone (ACTH)-secreting tumors (p=0.05 and p=0.047, respectively). No significant correlation was seen between *TWIST* and the endocrine functions of pituitary tumors.

Correlation of EMT markers. We further analyzed the correlation among the EMT markers in order to establish if there was a possible relationship between these markers. As

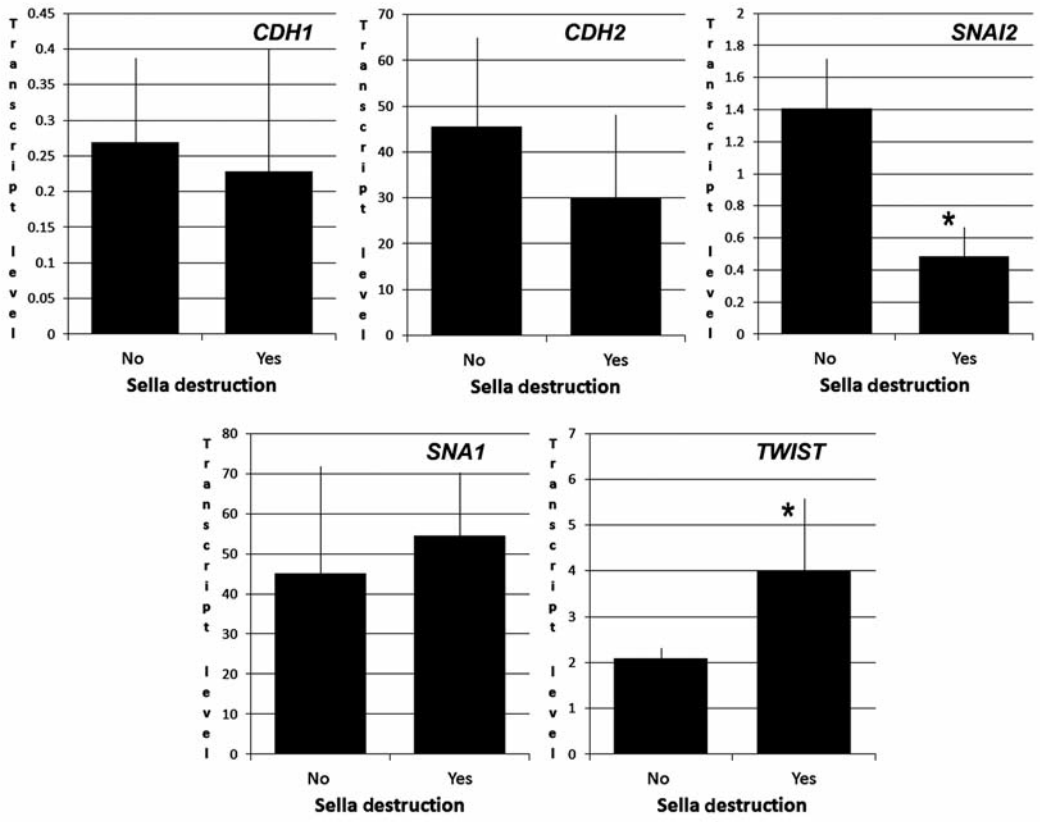

Figure 4. *EMT markers and sella destruction.* *Transcript levels significantly different from that of tumours without signs of sella destruction.

shown in Table III, *SNAI2* and *SNA1* were significantly correlated (r=0.342, p<0.001). It is interesting to note that both *SNAI2* and *SNA1* transcripts were significantly correlated with the CDH2 transcript (r=0.379, p=0.00016) and (r=0.211, p=0.04), respectively).

Discussion

In the present study, we report that EMT markers have a significant link to the development of pituitary tumors including tumor size and staging, destruction of neighbouring bone tissues, cystic lesions and endocrine functions.

EMT is a cellular process that permits polarized epithelial (-like) cells to acquire phenotypic changes, which results in cells with increased migration and invasive characteristics relative to that of a cell with mesenchymal/fibroblast-like morphology (14). Although the prominent change of these cells is mostly morphological in nature, a number of cellular functions are also modified during the EMT process. This includes a loss of cell-cell adhesion and tight junction function, as well as an increase in cell migration and invasion (15). Moreover, associated with these cellular function changes are certain key biochemical and molecular alterations, that include the loss of epithelial markers, such as E-cadherin, claudins and occludin, *etc*. and the attainment of mesenchymal markers, including N-cadherin, vimentin and fibronectin (16). Other key EMT markers include the transcription factors that regulate the expression of EMT proteins including SLUG, SNA1 and TWIST (17, 18). EMT and EMT markers have been shown to be closely linked to disease progression in a number of human solid cancers.

In human breast cancer, SLUG, SNAI and TWIST have been demonstrated to have inappropriate expression, which is thought to contribute to the progression of the disease. SLUG and TWIST were found up-regulated in human breast cancer, whereas SNAIL was found down-regulated (18). In bladder cancer, SLUG and TWIST are up-regulated, whereas SNAIL is down-regulated. Such disparate expression levels may contribute to the progression of bladder tumors (19). It has been also shown that over-expression of TWIST-induced morphological changes, such as occurrence of EMT. TWIST over-expression also increased cell migration and invasion ability, accompanied by an alteration in E-cadherin, N-cadherin, c-fos and matrix metallopeptidase 9 (MMP-9) expression in hypopharyngeal carcinoma (20). Furthermore, immunohistochemical assays showed that TWIST over-

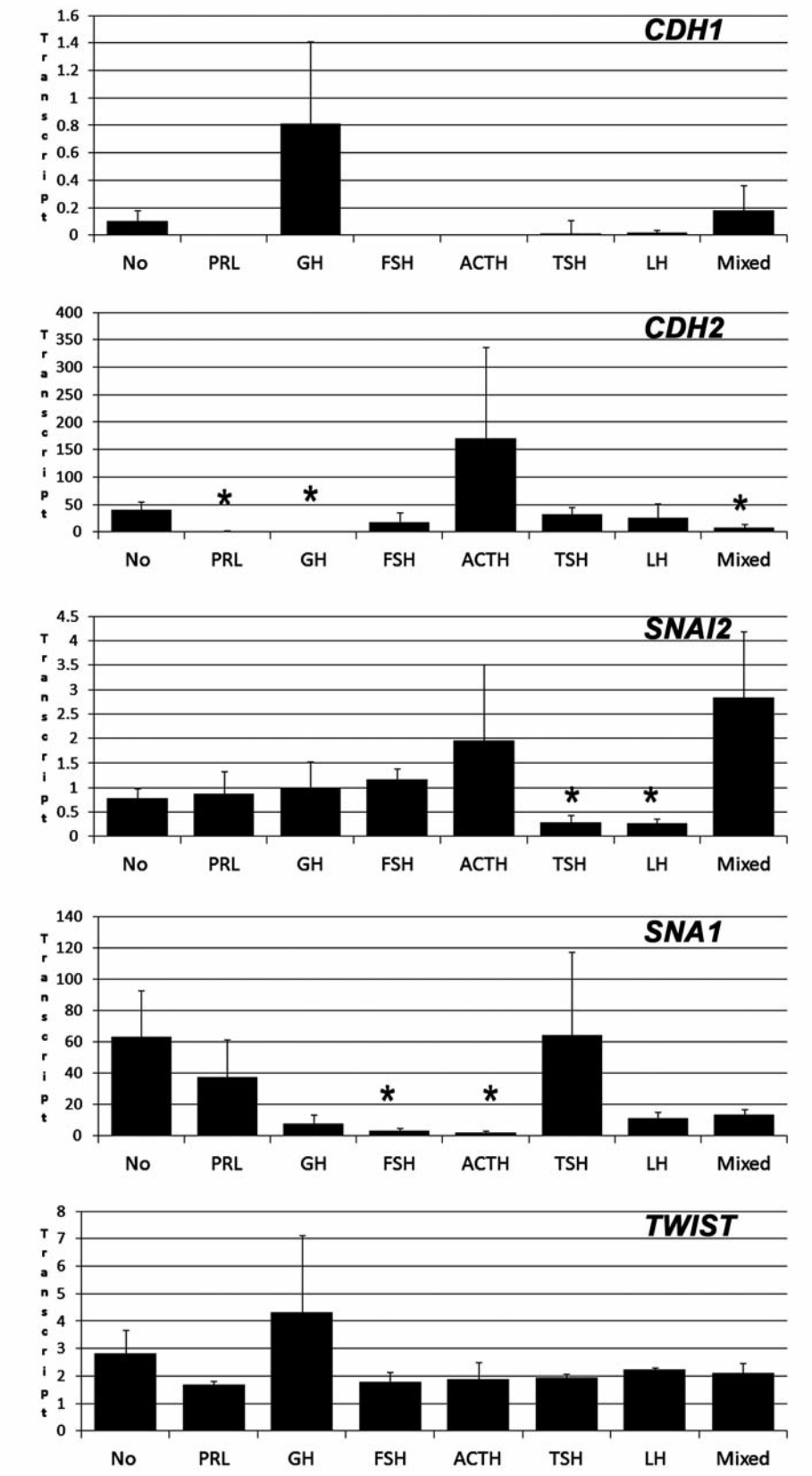

Figure 5. *EMT markers and the endocrine function of pituitary tumors.* **Transcript levels significantly different from endocrine inactive tumors.*

expression was related to tumor differentiation, tumor size and lymph node metastasis. Loss of the epithelial adhesion molecule E-cadherin is thought to enable metastasis by disrupting intercellular contacts - an early step in metastatic dissemination (21). Whereas the disruption of cell-cell contacts alone does not enable metastasis, the loss of E-cadherin protein does through induction of an epithelial-to-mesenchymal transition, invasiveness and anoikis resistance (21). Moreover, it has been shown that TWIST, is necessary for E-cadherin loss-induced metastasis and E-cadherin loss in tumors contributes to metastatic dissemination by inducing wide-ranging transcriptional and functional changes (21).

In the present study, we showed that low levels of E-cadherin and high levels of N-cadherin are associated with the appearance of intratumoural cystic lesions. The reason and source of cysts in pituitary tumors are thought to be due to two main reasons, the degenerative and residual space after absorbance of intratumoural haemorrhage. The link between E-/N-cadherin and cystic lesion is very interesting. E-cadherin acts as a strong cell adhesion mechanism for epithelial cells in the adherens junctions. This cell-cell adhesion structure not only confers cell-cell adhesion, it also controls the paracellular permeability. Damage to this mechanism, due to the loss/reduction of protein, may result in cells with increased mobility due -in part- to an increase in cell leakage throughout the monolayer, thus allowing formation of cystic lesions. Two typical clinical conditions associated with epithelial cadherin and cystic lesions are Pemphigus vulgaris and Pemphigus foliaceus, due to mutations in the desmosomal cadherins of the skin cells (22). It is possible that loss of E-cadherin in pituitary tumors also results in accumulation of fluids in the epithelial lumen. However, other possibilities also exist, *i.e.* due to intratumoural haemorrhage. After re-absorption of bleeding, the leaky epithelium keeps the space open by the leaked fluid from the interstitial tissues, *via* the paracellular space, to the cystic lesions. Clearly, more work is necessary to confirm this link.

Previously, it has been shown that SLUG protein is aberrant in non-functional pituitary tumors and is correlated with oestrogen receptor-alpha (ER alpha) in pituitary tumours (23). In addition, there has been an indication that the pituitary tumor transforming gene (*PTTG*) is able to induce EMT in tumor cells, such as ovarian cancer cells (24). The current findings that SNA1 and SLUG have significant correlation with N-Cadherin are very interesting. Both are known transcription factors for EMT markers, including E-cadherin. It is suggestive that the link with N-cadherin is novel and that the two transcription factors are central to the regulation of the mesenchymal phenotype by assisting cells to switch the E-cadherin (epithelial)–N-cadherin (mesenchymal) balance.

The other key finding of the study is the link between TWIST and SLUG and sella bone destruction. We found that these two EMT transcription factors are linked to this destruction. Presently, it is not clear why and how a benign pituitary tumor, which sits in a cosy sella fossa, begins to invade and destruct the fossa. It is possible that the rapid growth and suppression of blood vessels could perhaps cause the damage to the surrounding bones. However, the results in this study show that other mechanisms also operate. We have shown that SLUG is significantly correlated with N-cadherin in pituitary tumors. Taken together the high N-cadherin and low E-cadherin, the aberrant SLUG/TWIST expression and the function of these proteins, it can be argued that these biochemical changes could lead to the mesenchymal change of pituitary tumor cells, which could result in aggressive expansion into the surrounding tissues, including the fossa bony tissues. One of the key features of pituitary tumors is their ability to secret hormones, namely endocrine-active. This feature is reflected in the present study in that more than half of the pituitary tumors are endocrine-active.

In conclusion, we reported on the aberrant expression of several EMT markers in human pituitary adenomas and demonstrated a link between E-/N-cadherin balance and tumor cystic lesions, between EMT transcription factors and tumor invasiveness and bone destruction, as well as links with endocrine functions. These findings stress out the importance of EMT in the development and progression of pituitary adenoma.

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References

1 Blevins LS, Jr., Verity DK and Allen G: Aggressive pituitary tumors, Oncology (Williston Park) *12*: 1307-1312, 1315; discussion 1315-1308, 1998.

2 Lloyd RV, Scheithauer BW, Kuroki T, Vidal S, Kovacs K and Stefaneanu L: Vascular Endothelial Growth Factor (VEGF) Expression in Human Pituitary Adenomas and Carcinomas. Endocr Pathol *10*: 229-235, 1999.

3 McCabe CJ, Khaira JS, Boelaert K, Heaney AP, Tannahill LA, Hussain S, Mitchell R, Olliff J, Sheppard MC, Franklyn JA and Gittoes NJ: Expression of pituitary tumour transforming gene (PTTG) and fibroblast growth factor-2 (FGF-2) in human pituitary adenomas: relationships to clinical tumour behaviour. Clin Endocrinol (Oxf) *58*: 141-150, 2003.

4 Larue L and Bellacosa A: Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. Oncogene *24*: 7443-7454, 2005.

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

6 Sarrio D, Rodriguez-Pinilla SM, Hardisson D, Cano A, Moreno-Bueno G and Palacios J: Epithelial-mesenchymal transition in breast cancer relates to the basal-like phenotype. Cancer Res *68*: 989-997, 2008.
7 Huber MA, Kraut N and Beug H: Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol *17*: 548-558, 2005.
8 Trimboli AJ, Fukino K, de Bruin A, Wei G, Shen L, Tanner SM, Creasap N, Rosol TJ, Robinson ML, Eng C, Ostrowski MC and Leone G: Direct evidence for epithelial-mesenchymal transitions in breast cancer. Cancer Res *68*: 937-945, 2008.
9 Cavallaro U and Christofori G: Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. Nat Rev Cancer *4*: 118-132, 2004.
10 Martin TA, Ye L, Sanders AJ, Lane J and Jiang WG: Cancer Invasion and Metastasis: Molecular and Cellular Perspective, in: R. Jandia (Ed.), Metastatic Cancer: Clinical and Biological Perspectives, Landes Bioscience, 2013.
11 Knosp E, Steiner E, Kitz K and Matula C: Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery *33*: 610-617; discussion 617-618, 1993.
12 Vieira JO, Jr., Cukiert A and Liberman B: Magnetic resonance imaging of cavernous sinus invasion by pituitary adenoma diagnostic criteria and surgical findings. Arq Neuropsiquiatr *62*: 437-443, 2004.
13 Vieira JO, Jr., Cukiert A and Liberman B: Evaluation of magnetic resonance imaging criteria for cavernous sinus invasion in patients with pituitary adenomas: logistic regression analysis and correlation with surgical findings. Surg Neurol *65*: 130-135; discussion 135, 2006.
14 Ombrato L and Malanchi I: The EMT universe: space between cancer cell dissemination and metastasis initiation. Crit Rev Oncog *19*: 349-361, 2014.
15 van Zijl F, Krupitza G and Mikulits W: Initial steps of metastasis: cell invasion and endothelial transmigration. Mutat Res *728*: 23-34, 2011.
16 May CD, Sphyris N, Evans KW, Werden SJ, Guo W and Mani SA: Epithelial-mesenchymal transition and cancer stem cells: a dangerously dynamic duo in breast cancer progression, Breast Cancer Res. *13*: 202, 2011.
17 Guaita S, Puig I, Franci C, Garrido M, Dominguez D, Batlle E, Sancho E, Dedhar S, De Herreros AG and Baulida J: Snail induction of epithelial to mesenchymal transition in tumor cells is accompanied by MUC1 repression and ZEB1 expression. J Biol Chem *277*: 39209-39216, 2002.
18 Martin TA, Goyal A, Watkins G and Jiang WG: Expression of the transcription factors snail, slug, and twist and their clinical significance in human breast cancer. Ann Surg Oncol *12*: 488-496, 2005.
19 Yu Q, Zhang K, Wang X, Liu X and Zhang Z: Expression of transcription factors snail, slug, and twist in human bladder carcinoma. J Exp Clin Cancer Res *29*: 119, 2010.
20 Yu L, Lu S, Tian J, Ma J, Li J, Wang H and Xu W: TWIST expression in hypopharyngeal cancer and the mechanism of TWIST-induced promotion of metastasis. Oncol Rep *27*: 416-422, 2012.
21 Onder TT, Gupta PB, Mani SA, Yang J, Lander ES and Weinberg RA: Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res *68*: 3645-3654, 2008.
22 Porro AM, Caetano Lde V, Maehara Lde S and Enokihara MM: Non-classical forms of pemphigus: pemphigus herpetiformis, IgA pemphigus, paraneoplastic pemphigus and IgG/IgA pemphigus. An Bras Dermatol *89*: 96-106, 2014.
23 Zhou W, Song Y, Xu H, Zhou K, Zhang W, Chen J, Qin M, Yi H, Gustafsson JA, Yang H and Fan X: In nonfunctional pituitary adenomas, estrogen receptors and slug contribute to development of invasiveness. J Clin Endocrinol Metab *96*: E1237-1245, 2011.
24 Shah PP and Kakar SS: Pituitary tumor transforming gene induces epithelial to mesenchymal transition by regulation of Twist, Snail, Slug, and E-cadherin. Cancer Lett *311*: 66-76, 2011.

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