CLINICAL STUDY

Unique expression pattern of the EMT markers Snail, Twist and E-cadherin in benign and malignant parathyroid neoplasia

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Abstract

Background: Epithelial and mesenchymal transitions (EMT) are essential for embryonic development and progression of non-invasive tumor cells into malignant, metastatic carcinomas. During embryogenesis, the parathyroid glands develop from pharyngeal pouches and migrate to their final destinations, densely enclosed by mesenchymal neural crest cells. In this study, we examined the expression of the EMT markers Snail, Twist and E-cadherin in normal parathyroid glands and benign and malignant parathyroid diseases.

Methods: Using immunohistochemistry, we compared expression of E-cadherin, Snail and Twist in 25 patients with parathyroid adenoma, 25 patients with parathyroid hyperplasia, and nine patients with parathyroid cancer with normal parathyroid glands.

Results: Normal parathyroid glands, parathyroid adenomas, and parathyroid hyperplasias showed a typical membranous E-cadherin staining pattern. Expression of Snail was found in 22/25 parathyroid adenomas and in all parathyroid hyperplasias. Twist was expressed in 22/25 of parathyroid adenomas and in 20/25 parathyroid hyperplasias. Snail and Twist positive cells were homogeneously distributed throughout the gland. However, in all nine parathyroid carcinomas, membranous E-cadherin staining was lost. In addition, the expression pattern of Snail and Twist was changed and mostly limited to the invasive front of cancer tissue samples.

Conclusion: Expression of Snail and Twist at the invasive front and consecutive loss of E-cadherin in parathyroid carcinomas suggests a key role of EMT in the tumorigenesis of this cancer. The unique expression pattern could help to distinguish between an adenoma and a non-metastatic carcinoma. Loss of E-cadherin and change of the expression pattern of Snail and Twist together should result in an *en bloc* resection or a close follow-up.

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Introduction

In epithelial–mesenchymal transition (EMT), epithelial cells acquire fibroblast-like properties and show reduced intercellular adhesion and increased motility (1). During progression to metastatic competence, carcinoma cells acquire mesenchymal gene-expression patterns and properties. This results in changed adhesive properties and the activation of proteolysis and motility, which allows tumor cells to invade into surrounding stroma and finally to metastasize and establish secondary tumors at distant sites (2). During EMT, the E-cadherin promoter is frequently repressed by specific transcriptional repressors. E-cadherin levels become limiting, which results in the loss of E-cadherin-dependent intercellular epithelial (3). Snail,

a highly unstable protein, is rapidly phosphorylated by glycogen synthase kinase-3 β (GSK-3 β) and subsequently degraded. Conversely, inhibition of GSK-3 β function results in upregulation of Snail by an NF- κ B-dependent pathway, loss of E-cadherin expression, and EMT. Additional protein modification further stabilizes Snail protein and promotes EMT and tumor invasion (4). Expression of Snail in epithelial tumors increases their aggressiveness, as seen in experimentally induced breast tumors, where high Snail expression correlates with an increased risk of tumor relapse and poor survival rates in human breast cancer (5). Twist is a highly conserved basic helix-loop-helix transcription factor that has important regulatory functions during embryogenesis. In *Drosophila*, Twist protein is crucial for proper gastrulation and mesoderm formation (6).

While Twist proteins are only expressed in a subset of mesodermally and ectodermally derived tissues, Twist is overexpressed in various human solid tumors including numerous types of carcinomas as well as sarcomas, gliomas, neuroblastomas, and melanomas. The role of Twist in tumor progression has been convincingly associated with the metastatic process (7). Exogenous overexpression of Twist increases the invasive and metastatic abilities of cancer cells by promoting the downregulation of E-cadherin and the induction of an EMT (7).

The increased motility and invasiveness of cancer cells in the first phase of metastasis are reminiscent of EMT during embryonic development. Following neural tube closure, multipotent neural crest cells undergo EMT, delaminate from the dorsal aspect of the neural tube and migrate extensively throughout the embryo before giving rise to a diverse set of derivatives, such as for the development of the mesoderm in amniotes, or the neural crest in all vertebrates (8). It has been demonstrated that as development proceeds, the neural crest mesenchyme contributes connective tissue elements to organs developing in the pharyngeal region, including thymus and parathyroid (9). EMT also explains why epithelial cells from one region can dissociate and migrate to a new location. One classic example for such a cell movement during embryogenesis is the descent of the parathyroid glands. The inferior parathyroid glands that originate from the third pharyngeal pouch migrate caudally with the thymus, normally only as far as the inferior poles of the thyroid gland, but may descend with the thymus gland into the thorax. The position of the larger pair of superior parathyroid glands, which develop from the fourth pharyngeal pouch is more constant, with 99% located behind the upper poles of the thyroid lobes.

Three parathyroid disorders can lead to an enlargement of one or more glands. Primary hyperparathyroidism (pHPT) is classically thought of as the somatic manifestation of hypercalcemia in which patients suffer from a variety of complaints including abdominal pain, nephrolithiasis, osteopenia, and mental status changes. Ninety percent of cases of pHPT are caused by a single enlarged parathyroid adenoma. Multiglandular involvement is less common and may be associated with the multiple endocrine neoplasia syndromes MEN I and II (10).

Secondary hyperparathyroidism (sHPT) usually develops in patients with chronic renal failure, where decreased levels of calcitriol with consecutive hypocalcemia and a reduced phosphate clearance lead to an increase of parathyroid hormone (PTH) synthesis and secretion (11).

Parathyroid carcinoma is a rare cause of pHPT, affecting 0.2 to 1% of patients undergoing surgery (12). The major cause of death in patients with parathyroid cancer is severe hypercalcemia with its metabolic complications, such as malnutrition, acute pancreatitis,

and heart arrest. Cure is dependent on precise diagnosis with consecutive surgical *en bloc* resection (12, 13). The only definite criteria for diagnosing parathyroid carcinoma are local recurrence or metastases, making it sometimes difficult. Most patients with parathyroid cancer have clinical manifestations that are virtually indistinguishable from those in patients with a parathyroid adenoma, although severe hypercalcaemia may be regarded as a risk factor for malignancy (13).

The molecular mechanism for most of these parathyroid disorders is unknown and poorly understood. Some groups tried to characterize the global gene expression profiles in a series of sporadic parathyroid adenomas in an attempt to obtain an improved picture of the genetic etiology behind parathyroid tumor development (14, 15).

Recently, our group described that Snail is overexpressed in a large subset of neuroendocrine tumors of the ileum, presenting the first evidence of Snail expression in endocrine tumors (16). In the present study, we analyzed the expression pattern of E-cadherin, Snail and Twist in normal parathyroid glands and parathyroid disorders. For the first time, we show that Snail and Twist are expressed in parathyroid carcinomas. Furthermore, we demonstrate that E-cadherin, Snail and Twist are expressed simultaneously in normal parathyroid gland and benign parathyroid disorders, based on the embryonic background of epithelial and mesenchymal cells of the glands.

Patients and methods

Patients and tissue collection

Tissue from 25 patients with pHPT due to parathyroid adenoma, 25 patients with sHPT due to renal failure, nine patients with parathyroid cancer, and two patients with normal parathyroid glands were obtained from the tissue bank of the Department of Pathology at the University Hospital of Marburg, Germany. All patients underwent parathyroid surgery in the Department of Surgery from the University Hospital of Marburg, Germany. Patients with a hereditary background of the parathyroid disease, e.g., MEN I, were excluded from the study. Furthermore, patients with pHPT due to double adenomas or with persistent hypercalcaemia after previous surgery were excluded. Also patients with sHPT with an unidentified gland during operation were excluded. The study was approved by the local Ethics Committee.

Diagnosis

Histological diagnosis was confirmed by an experienced pathologist (A R). The sporadic parathyroid adenomas (pHPT) all showed a single enlarged hypercellular parathyroid gland with or without a rim of normal parathyroid tissue and a biopsy of at least one other

parathyroid gland with findings consistent with normal parathyroid tissue. The renal-induced hyperplasias (sHPT) all showed hypercellular parathyroid tissue involving three or more glands. A parathyroid tumor was defined as carcinoma only when it showed invasion of the tumor capsule or of surrounding structures. The presence of lymph node and/or distant metastasis was also considered diagnostic of malignancy.

Surgery

Standard surgical treatment of sporadic pHPT consisted of a bilateral exploration and identification of all four parathyroid glands with consecutive removal of the enlarged gland. Standard surgical treatment of sHPT consisted of a total parathyroidectomy with or without autotransplantation and with bilateral thymectomy. Standard surgical treatment of parathyroid carcinomas consisted of an *en bloc* resection. This procedure includes the resection of the ipsilateral thyroid lobe together with the isthmus, as well as a lymphadenectomy of the central compartment of the neck. All areas suspicious for local invasion must be resected, even if important structures (e.g., recurrent laryngeal nerve, esophagus or great vessels) are affected. Avoiding rupture of the tumor capsule is of utmost importance and a complete resection of all tumor bearing tissue is inevitable to avoid local recurrence.

Biochemical data

Preoperative levels of serum calcium and plasma PTH were obtained from clinical records. The values corresponding to the first hospital visit before any medical treatment, and of drugs with possible influence on calcium metabolism were registered. The normal range for intact PTH was 11–65 pg/ml. The normal range for calcium was 2.2–2.7 mmol/l.

Immunostaining

For immunolabeling, formalin-fixed and paraffin embedded archived tumor samples and corresponding normal tissues were stained as previously described (17). Concentrations and sources of primary antibodies were used as follows: α -E-cadherin 1:200 (Zymed, San Francisco, CA, USA), α -Twist and α -SNAIL 1:100 (Santa Cruz, Santa Cruz, CA, USA). Briefly, slides from archived normal parathyroid glands, parathyroid adenomas, parathyroid hyperplasias, and parathyroid carcinomas were heated to 60 °C for 1 h, deparaffinized using xylene, and hydrated by a graded series of ethanol washes. Antigen retrieval was accomplished by microwave heating in 10 mM sodium citrate buffer of pH 6.0 for 10 min. For immunohistochemistry, endogenous peroxidase activity was quenched by 10 min incubation in 3% H₂O₂. Non-specific binding was blocked with 10% serum. Sections were then probed with primary

Table 1 Clinical characteristics and results of E-cadherin, Snail and Twist immunohistochemistry in 25 patients with PHPT.

Patient number	Age (years)	Sex	Diagnosis	Ca ²⁺ (mmol/l)	PTH (pg/l)	Gland weight (g)	E-cadherin expression	Snail expression	Twist expression
1	41	M	pHPT	2.7	128	1.1	++	++	+
2	72	F	pHPT	2.8	151	1.8	++	+	+
3	67	M	pHPT	3	230	2.3	++	+	+
4	66	M	pHPT	2.8	93	0.2	++	++	++
5	66	F	pHPT	2.8	149	0.8	++	+	++
6	81	M	pHPT	2.5	99	0.3	++	++	+
7	67	M	pHPT	2.4	81	0.4	++	Negative	Negative
8	64	M	pHPT	3.3	243	1.4	++	+	+
9	53	F	pHPT	2.8	88	1.8	++	+	+
10	56	M	pHPT	3	134	2.2	++	+	+
11	48	M	pHPT	3.4	1418	16.0	+	++	++
12	69	M	pHPT	2.9	182	1.0	++	Negative	Negative
13	68	F	pHPT	2.9	147	1.1	++	++	++
14	54	F	pHPT	3.3	191	3.1	+	++	+
15	63	F	pHPT	2.6	84	0.3	++	++	++
16	26	F	pHPT	2.6	157	2.0	++	++	++
17	38	M	pHPT	2.7	359	0.7	++	+	+
18	60	M	pHPT	2.8	150	1.4	++	+	+
19	57	F	pHPT	2.8	110	2.1	++	+	++
20	62	F	pHPT	2.8	183	0.7	++	++	+
21	53	M	pHPT	2.8	140	4.1	+	+	+
22	43	M	pHPT	2.6	190	0.5	++	++	++
23	43	M	pHPT	2.6	106	1.3	++	+	+
24	46	F	pHPT	2.8	129	0.8	++	Negative	Negative
25	47	M	pHPT	2.6	108	0.4	++	+	+

Ca²⁺, serum calcium level at diagnosis; PTH, serum parathyroid hormone level at diagnosis; pHPT, primary hyperparathyroidism due to parathyroid adenoma.

antibodies overnight at 4 °C. For immunohistochemistry, bound antibodies were detected using the avidin–biotin–complex (ABC) peroxidase method (ABC Elite Kit, Vector Labs, Burlingame, CA, USA). Final staining was developed with the Sigma FAST DAB peroxidase substrate kit (Sigma). To avoid misleading results, we used the exact same amount of time for all sections to be developed.

The immunohistochemistry results for E-cadherin, Snail and Twist were scored as described previously (16): negative=less than 5% cells positive; + = <30% cells positive; ++ = >30% cells positive.

Results

Patients

Altogether, 59 patients with parathyroid disorders were included in the study. For evaluating E-cadherin, Snail and Twist in pHPT, fifteen males and ten females with a median age of 56 years (range 26 to 81 years) at the time of surgery were included. Clinical and biochemical characteristics are listed in Table 1. To study expression of EMT-markers in sHPT, thirteen females and twelve males with a median age of 53 years (range 29 to 78 years) at the time of surgery were enclosed. Clinical and biochemical characteristics are listed in Table 2. Five males and four females presenting with parathyroid carcinoma with a median age of 46 years (range 21 to

65 years) at the time of surgery were also included. Clinical and biochemical characteristics are listed in Table 3.

E-cadherin, Snail and Twist are simultaneously expressed in normal parathyroid glands

First, we analyzed the expression of E-cadherin, Snail and Twist in normal parathyroid tissue from two patients with thyroid diseases and concurrently resected normal parathyroid glands. Immunohistochemical staining revealed expression of E-cadherin (Fig. 1A), Snail (Fig. 2A) and, Twist (Fig. 3A) in both glands. The pattern of E-cadherin expression showed a typical membranous staining. Cells with cytoplasmic Snail and Twist expression were distributed throughout larger areas of the glands.

E-cadherin, Snail and Twist are simultaneously expressed in benign parathyroid disorders

All parathyroid glands obtained from patients with pHPT or sHPT showed a strong membranous staining of E-cadherin (Fig. 1B and C, Tables 1 and 2).

Immunohistochemical staining revealed expression of Snail in 22/25 (88%) of parathyroid adenomas out of patients with pHPT (Fig. 2B, Table 1) and in all 25

Table 2 Clinical characteristics and results of E-cadherin, Snail and Twist immunohistochemistry in 25 patients with sHPT.

Patient number	Age (years)	Sex	Diagnosis	Ca ²⁺ (mmol/l)	PTH (pg/l)	Gland weight (g)	E-cadherin expression	Snail expression	Twist expression
1	31	F	sHPT	2.6	1090	1.3	++	++	+
2	58	F	sHPT	2.8	793	0.9	+	++	+
3	56	F	sHPT	2.4	1667	0.1	++	++	+
4	36	M	sHPT	2.7	1208	1.6	++	++	++
5	58	M	sHPT	2.4	1536	0.1	++	++	Negative
6	63	F	sHPT	2.6	1274	0.3	++	+	+
7	61	F	sHPT	2.6	2271	1.1	++	++	+
8	48	F	sHPT	2.8	870	0.9	++	++	++
9	52	M	sHPT	2.8	1198	1.5	++	+	Negative
10	61	F	sHPT	2.8	322	0.1	++	++	+
11	46	M	sHPT	2.4	1129	0.5	++	++	++
12	51	M	sHPT	2.7	518	0.5	++	+	+
13	45	F	sHPT	2.9	588	1.1	+	+	Negative
14	65	M	sHPT	2.7	3011	2.3	++	++	++
15	68	F	sHPT	2.7	1739	0.6	+	+	+
16	44	M	sHPT	2.6	794	0.9	++	++	+
17	46	F	sHPT	1.9	554	0.3	++	++	++
18	62	M	sHPT	2.7	557	1.4	++	+	Negative
19	63	F	sHPT	2.7	2123	1.4	++	+	+
20	35	M	sHPT	2.8	1090	1.0	+	+	Negative
21	29	F	sHPT	2.4	1116	0.3	++	++	++
22	59	F	sHPT	2.3	708	0.6	++	+	+
23	78	M	sHPT	2.7	1324	1.4	++	+	+
24	74	M	sHPT	2.9	1318	1.8	++	+	+
25	27	M	sHPT	2.6	2800	0.8	++	++	++

Ca²⁺, serum calcium level at diagnosis; PTH, serum parathyroid hormone level at diagnosis; sHPT, secondary hyperparathyroidism due to renal failure.

Table 3 Clinical characteristics and results of E-cadherin, Snail and Twist immunohistochemistry in 9 patients with parathyroid carcinoma.

Patient number	Age (years)	Sex	Diagnosis	Ca ²⁺ (mmol/l)	PTH (pg/l)	Gland weight (g)	E-cadherin expression	Snail expression	Twist expression
1	59	M	Pc	4.0	489	4.0	Negative	++	++
2	34	F	Pc	3.3	1365	6.0	Negative	+	++
3	21	F	Pc	5.8	880	1.2	Negative	++	++
4	64	M	Pc	4.9	1120	11.5	Negative	++	Negative
5	44	F	Pc	4.0	714	1.4	Negative	+	+
6	65	M	Pc	4.2	1480	12.0	Negative	++	++
7	58	F	Pc	3.4	358	7.3	Negative	++	+
8	31	M	Pc	3.8	2319	10.5	Negative	+	+
9	42	M	Pc	3.8	480	1.0	Negative	++	+

Ca²⁺, serum calcium level at diagnosis; PTH, serum parathyroid hormone level at diagnosis; Pc, parathyroid carcinoma.

parathyroid tissues from patients with sHPT (Fig. 2C, Table 2). Furthermore, immunohistochemical staining revealed expression of Twist in 22/25 (88%) patients with pHPT (Fig. 3B, Table 1) and in 20/25 (80%) patients with sHPT (Fig. 3C, Table 2).

Snail and Twist positive cells were homogeneously distributed throughout the whole gland, comparable with the pattern seen in normal parathyroid glands.

Expression of E-cadherin is lost in parathyroid carcinoma

In all nine parathyroid carcinomas analyzed, membranous E-cadherin staining was lost (Fig. 1D, Table 3). Immunohistochemical staining now revealed a cytoplasmic expression of E-cadherin protein, which is a hallmark of EMT.

Figure 1 IHC staining for E-cadherin in normal parathyroid gland and parathyroid disorders. (A) Representative example of E-cadherin expression in a normal parathyroid gland, showing a typical membranous staining pattern for the cell-adhesion marker. (B) Strong membranous staining of E-cadherin in pHPT and (C) sHPT. (D) By contrast, in parathyroid carcinoma, the membranous pattern of E-cadherin expression was lost, showing now a cytoplasmic expression of E-cadherin protein, which is a hallmark of EMT. Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-08-0662>.

The expression patterns of Snail and Twist are changed in parathyroid cancer

Consistent with loss of E-cadherin expression in parathyroid carcinomas, the expression pattern of Snail (Fig. 2D, Table 3) and Twist (Fig. 3D, Table 3) changed in malignant tumors. Snail was no longer expressed homogeneously throughout the whole tumor, but was mostly limited to the invasive front (Fig. 2D, arrows). In addition, Twist was now stronger and expressed along the front of the tumor.

Discussion

EMT occurs during embryonic morphogenesis in multicellular organisms, in which embryonic mesenchymal cells are formed and become motile following the loss of epithelial cell polarity. In recent years, EMT has also been recognized as a potential mechanism for cancer progression (18). A central event in EMT is

Figure 2 IHC staining for Snail in normal parathyroid gland and parathyroid disorders. (A–C) Snail expression was found in normal parathyroid gland (A) and in benign parathyroid disorders (B, C). Snail positive cells were distributed throughout larger areas of the glands. (D) By contrast, in parathyroid cancer, Snail was no longer expressed homogeneously throughout the whole tumor, but was mostly limited to the invasive front (arrows). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-08-0662>.

Figure 3 IHC staining for Twist in normal parathyroid gland and parathyroid disorders. Expression of Twist was found in normal parathyroid gland (A) and in patients with pHPT (B) and sHPT (C). The expression pattern was comparable with Snail (Fig. 2), with a distribution throughout the gland. (D) In parathyroid carcinoma, the staining pattern changed again. Twist was more strongly expressed along the front of the tumor (arrows). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-08-0662>.

downregulation of membranous E-cadherin expression (19), which leads to the loss of cell–cell contact and the consecutive progression of the cells towards a malignant phenotype. The transcription factor Snail is one major suppressor of E-cadherin and a strong inducer of EMT. Snail downregulates E-cadherin in different types of tumors e.g., hepatocellular carcinomas (20), carcinomas from the esophagus, cardia, stomach (21), and colorectal carcinomas (22). Recently, our group was the first to describe activation of Snail in endocrine tumors (16).

The presented study is now the first to show that Snail, Twist and E-cadherin are expressed simultaneously in normal parathyroid glands and benign parathyroid disorders; at first glance a surprising result, having these opposed characters of EMT expressed at the same time. But, most likely, the explanation could be found in organogenesis of the parathyroid glands. The pharyngeal glandular organs in mammals have complex developmental origins. The parathyroid, thymus, and ultimobranchial primordial develop from the pharyngeal pouches and migrate to their final destinations. During their descent to the neck, these pharyngeal organs are surrounded by mesenchyme derived from the cranial neural crest (9). The cranial neural crest arising from the embryonic midbrain and hindbrain plays a critical role in the development of the pharyngeal arches and pouches, initially by providing the mesenchymal cells which populate this region. As development proceeds, the neural crest mesenchyme contributes directly to the formation of some structures in the pharyngeal region, including thymus and parathyroid, and forms the calcitonin producing cells of thyroid gland (9, 23, 24). The molecular basis for control of these events is largely unknown. Recently, it has been shown

that neural crest cells undergo EMT, delaminate from the neural epithelium, and migrate throughout the embryo, differentiating at their destination sites into a wide array of cell types. Subsequent to the specification of neural crest progenitors at the neural plate border, a group of genes that primarily encode transcription factors (25), including the Snail family genes Snail and Slug, are induced in neural crest progenitor cells (26). EMT can be triggered by different signaling molecules, such as bone morphogenetic proteins (BMPs) (8). Thériault *et al.* recently demonstrated an upregulation of Snail mRNA and protein in response to exogenous BMP4 in ovarian cancer cells (27). Interestingly, BMP4 also plays a critical role in thymus and parathyroid organogenesis (28) and induces Snail during neural crest development (29). Very recently, Franci *et al.* showed that at day E9.5 in the mouse, Snail activity can be detected in the pharyngeal arches (30).

In the present study, in normal parathyroid glands and tissue obtained out of patients with benign parathyroid disorders, Snail positive cells were distributed throughout large areas of the glands. Furthermore, we found the same expression pattern for the transcription factor Twist. It has been shown that Twist is required for the maintenance of cell viability and proliferation in pharyngeal arch tissues (31). In mouse embryos, Twist-positive cells were found at E9.5 along the migratory paths of the hindbrain neural crest and in branchial arches (32). Twist is also required for the activation of Snail, which is crucial for proper gastrulation and for maintenance of Twist expression (33). We also found a typical membranous staining of E-cadherin in normal parathyroids and benign parathyroid disorders, which has been reported before (34). By contrast, in parathyroid carcinoma, the membranous pattern of E-cadherin expression is lost, showing now a cytoplasmic expression of E-cadherin protein. Cytoplasmic expression of E-cadherin protein and/or transcriptional repression of its mRNA are hallmarks of EMT, both in embryonic development and in cancer progression (4). Our results are in line with the results reported by Haven *et al.* They undertook an expression profiling of 53 hereditary and sporadic parathyroid tumors and found an upregulation of E-cadherin mRNA in parathyroid carcinomas, with aberrant staining noted, indicating loss of function in cell adhesion (35).

Another striking result of our study was the change of staining pattern of Snail and Twist in parathyroid cancer tissue. Snail and Twist were no longer expressed homogeneously throughout the whole tumor, but were mostly limited to the invasive front, a hallmark of EMT (2, 4). The invasive front of a tumor is formed by cells that migrate into and invade the surrounding tissue either as single cells (Figs 6 and 7) or in collective clusters (Figs 4 and 5) (4). In order to acquire motility and invasiveness, malignant cells must lose some of their epithelial characters and undergo EMT. While these steps are crucial for embryonic development, they

Figure 4 IHC staining for Snail (4AB) and Twist (4CD) in parathyroid carcinomas. B and D show selected areas from A and C marked by the box. Snail and Twist expression is found at the invasive front of the tumor where tumor cells invade the surrounding tissue either as single cells (Figs 6 and 7) or in collective clusters (Figs 4 and 5). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-08-0662>.

become fatal in pathological situations in the adult. The strong similarity between the process of tumor invasion and cell migration observed during organ development suggest that carcinoma cells can change their own morphology, motility, and ability to invade surrounding structures. In parathyroid tissue, in which Snail, Twist and E-cadherin are expressed simultaneously in normal and benign states, undefined oncogenic factors must

Figure 6 IHC staining for Snail (6AB) and Twist (6CD) in multiple samples of parathyroid carcinomas. B and D show selected areas from A and C marked by the box. Snail and Twist expression is found at the invasive front of the tumor where tumor cells invade the surrounding tissue either as single cells (Figs 6 and 7) or in collective clusters (Figs 4 and 5). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-08-0662>.

switch-on EMT, leading to the loss of E-cadherin and the typical staining pattern of Snail and Twist at the edges of the parathyroid carcinoma (Figs 6 and 7).

Parathyroid adenoma shares some histological features with parathyroid cancer and at the time of initial surgery differentiation from parathyroid cancer can be difficult. Hence, some lesions have been reported as parathyroid cancer, but their clinical behavior has

Figure 5 IHC staining for Snail (5AB) and Twist (5CD) in multiple samples of parathyroid carcinomas. B and D show selected areas from A and C marked by the box. Snail and Twist expression is found at the invasive front of the tumor where tumor cells invade the surrounding tissue either as single cells (Figs 6 and 7) or in collective clusters (Figs 4 and 5). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-08-0662>.

Figure 7 IHC staining for Snail (7AB) and Twist (7CD) in multiple samples of parathyroid carcinomas. B and D show selected areas from A and C marked by the box. Snail and Twist expression is found at the invasive front of the tumor where tumor cells invade the surrounding tissue either as single cells (Figs 6 and 7) or in collective clusters (Figs 4 and 5). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-08-0662>.

not always been consistent with this diagnosis. Indeed, the presence of local recurrence or metastatic disease is the only reliable feature that differentiates benign from malignant parathyroid disease. At the initial operation, features of presumed malignancy, such as firm texture, grey color and gross adherence to adjacent tissue, are not proven differentiators of APA from parathyroid cancer. Thus, the risk for the surgeon is overtreatment of an APA or undertreatment of a true parathyroid cancer (13). The presented findings could help to distinguish between an adenoma and a non-metastatic carcinoma. Loss of E-cadherin and change of the expression pattern of Snail and Twist together with a clinical suspicious lesion should result in an *en bloc* resection as described above. If this is not possible, these patients should undergo a very close follow up, because they might be at a higher risk of developing distant metastases.

In conclusion, we show for the first time, that EMT plays a role in the tumorigenesis of parathyroid carcinomas. Loss of membranous E-cadherin and aberrant Snail and Twist expression patterns along the invasive front in parathyroid cancer tissue samples as compared with benign tumors and histologically normal parathyroid tissues is in line with changes usually observed in EMT.

Declaration of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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