The Expression of E-Cadherin in Somatotroph Pituitary Adenomas Is Related to Tumor Size, Invasiveness, and Somatostatin Analog Response

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Context: Appropriate cell-to-cell adhesion is fundamental for the epithelial phenotype of pituitary cells. Loss of the adhesion protein E-cadherin has been associated with invasiveness, metastasis, and poor prognosis in cancers of epithelial origin. In somatotroph adenomas, a variable and reduced expression of E-cadherin has been demonstrated. In addition, nuclear translocation of E-cadherin was found to correlate with pituitary tumor invasion.

Objective: The objective was to examine the protein expression of E-cadherin in somatotroph pituitary adenomas in relation to adenoma size, invasiveness, and somatostatin analog (SMS) efficacy.

Patients and Methods: Eighty-three patients were included, and 29 were treated preoperatively with SMS. Adenoma E-cadherin protein expression was analyzed by Western blot (61 patients) and immunohistochemistry (IHC) (80 patients) with antibodies directed against both extracellular and intracellular domains (IHC). The acute (direct surgery group) and long-term (preoperatively treated group) SMS responses were evaluated. Baseline tumor volume and invasiveness were measured on magnetic resonance imaging scans.

Results: Membranous E-cadherin was lost in several adenomas. Nine of these were nuclear E-cadherin positive. The E-cadherin protein expression correlated negatively to tumor size and positively to acute SMS response. Low E-cadherin levels (preoperatively treated group only) and loss of membranous E-cadherin correlated to tumor invasiveness. The E-cadherin level correlated positively to tumor reduction after SMS treatment, and adenomas with nuclear E-cadherin staining had lower IGF-I reduction and tumor shrinkage. Preoperatively treated adenomas had reduced E-cadherin protein levels, but the IHC expression was unaltered.

Conclusion: Reduced E-cadherin expression may correlate to a dedifferentiated phenotype in the somatotroph pituitary adenomas. **(*J Clin Endocrinol Metab* 95: 2334–2342, 2010)**

The pituitary somatotroph adenomas produce and secrete GH in supraphysiological levels, causing the clinical syndrome of acromegaly. These adenomas usually grow slowly and metastasize extremely rarely, but they may be locally invasive, which is of importance for surgical cure. At the time of diagnosis, the majority is macroadenomas with a low surgical cure rate, and a large proportion of patients is in need of medical treatment (1–3).

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Abbreviations: EMT, Epithelial-to-mesenchymal transition; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MRI, magnetic resonance imaging; RKIP, Raf kinase inhibitory protein; SIPAP, suprasellar, infrasellar, parasellar, anterior, and posterior; SMS, somatostatin analogs; SSTR, somatostatin receptor.

Somatostatin analogs (SMS) are usually the drug of choice, but the clinical response to treatment is variable. Biochemical control is achieved in up to 50% of patients primarily treated with SMS (4, 5). Mean tumor shrinkage is reported to be 25–40% in unselected *de novo* patients, but individually the tumor volume reduction is highly variable (4–7), with no correlation between biochemical response and tumor shrinkage (4, 8, 9). We and others have shown that biochemical SMS response correlates to the protein expression of somatostatin receptor (SSTR) subtype 2 in the adenoma (10–12). We have also demonstrated that SMS responses are attenuated in adenomas with low expression of Raf kinase inhibitory protein (RKIP) (13).

Anterior pituitary cells have an epithelial phenotype, in which appropriate cell-to-cell adhesion and polarity are fundamental. Expression of the protein E-cadherin is typical for epithelial cells, and this transmembrane adhesion protein provides a physical link to both the adjacent cells and the intracellular cytoskeleton. The extracellular domain of E-cadherin of one cell binds to E-cadherin molecules of the adjacent cell. The intracellular domain of the protein is linked to the actin cytoskeleton via a protein complex with catenins (14, 15). Epithelial-to-mesenchymal transition (EMT) is a process in which epithelial cells lose their intercellular adhesion and acquire a more mesenchymal phenotype with increased cell motility. Functional loss of E-cadherin is a hallmark of EMT (14, 15). This has been demonstrated in several cancer types, being most pronounced in poorly differentiated and invasive tumors and with correlation to metastasis and poor prognosis (16, 17). A variable and reduced expression of E-cadherin has also been demonstrated in somatotroph adenomas, particularly sparsely granulated adenomas (18–23). Hypermethylation of the E-cadherin promoter was seen in approximately 40% of adenomas, and was highly correlated to loss of E-cadherin expression (22, 23) Down-regulation of E-cadherin expression correlated to tumor aggressiveness in one but not other studies (18, 19, 22, 23). However, in a recent publication, nuclear staining of E-cadherin was found when using an antibody against the intracellular domain of the protein. This correlated to loss of membranous E-cadherin staining. In addition, tumor invasiveness correlated positively to nuclear E-cadherin staining but not to loss of membranous staining (20).

The aim of this study was to evaluate E-cadherin protein expression in somatotroph adenomas from a large and well-described cohort of patients with acromegaly in relation to: 1) tumor size and invasiveness, 2) acute and long-term responses to somatostatin analogs, and 3) the expression of SSTR2a in the adenomas.

Subjects and Methods

Subjects

Eighty-three patients with active acromegaly were included, and all underwent transsphenoidal pituitary surgery in the period 1996–2008. The diagnosis was based on clinical symptoms and was biochemically verified by elevated serum IGF-I and failure to suppress GH in an oral glucose tolerance test. All patients had a pituitary tumor visualized on a magnetic resonance imaging (MRI) scan.

Three patients had previously undergone transsphenoidal surgery, one of them twice. Twenty-nine patients received treatment with a somatostatin analog before surgery (median 6 months, range 1–32 months). One had used pasireotide as participant in a clinical study. Two patients were treated preoperatively with pegvisomant for 3–4 months (one not 6 months before surgery). Six patients had been treated with a dopamine agonist preoperatively, four of them also the last months before surgery. None had received radiation therapy. Table 1 provides an overview of the study population.

TABLE 1. The study population

Groups	Direct surgery	Preoperative SMS
Demographics		
n	54	29
Age (yr)	52 (42–57)	47 (40–57)
Women/men (n)	32/22	11/18
Tumor size (n = 74)		
Tumor volume (cm^3)	1.04 (0.50–2.04)	1.24 (0.68–2.28)
Biochemistry (n = 81)		
Serum GH (mU/liter)	20.3 (13.3–41.6)	35.4 (18.1–79.5)
Serum IGF-I (nmol/liter)	87.6 (72.6–113.8)	87.7 (71.3–102.0)
SMS response		
GH reduction (%) (n = 50)[a]	83 (73–91)	
IGF-I reduction (%) (n = 26)[b]		50 (27–60)
GH reduction (%) (n = 25)[b]		85 (55–91)
Tumor size reduction (%) (n = 23)[b]		26 (2–59)
Tumor protein levels[c]		
RKIP ratio (n = 63)[d]	1.22 (0.87–2.09)	1.66 (0.75–2.60)
SSTR2a ratio (n = 48)[e]	0.08 (0.05–0.10)	0.05 (0.03–0.07)
E-cadherin ratio (n = 61)	0.31 (0.10–0.45)	0.10 (0.04–0.21)

Unless stated, data are given as median (interquartile range).

[a] During acute octreotide test in patients not preoperatively treated with a somatostatin analog; [b] after 6 months (median) preoperative SMS treatment; [c] protein levels measured by Western blot, normalized to GAPDH; [d] previously published data in 51 of the patients (13); [e] previously published data, normalized β-actin (10).

Because the vast majority of the SMS-treated patients were treated with octreotide depot injections, they were expected to have therapeutic concentrations at the time of surgery. The patient treated with pasireotide, however, had not received treatment for the last 3 months before surgery.

The study was approved by the local ethical committee and conducted according to the Declaration of Helsinki II. Written informed consent was obtained from all patients.

Biochemical measurements

Blood samples were drawn after an overnight fast and serum isolated. Serum GH was measured by immunoassay (detection limit 0.3 mU/liter) and in most patients estimated as a mean GH level of several daytime samples. An acute somatostatin test was performed in 62 of the patients before medical treatment with a somatostatin analog (10). The percentage reduction of GH was calculated for each patient. Serum IGF-I was measured by RIA (Nichols Institute, Nijmegen, The Netherlands) in most patients. In 38 patients, IGF-I was measured in additionally stored serum in one run to minimize between-assay variability and compared with previous values ($r = 0.866$; $n = 38$; $P < 0.001$). When available, this value was used for statistical analysis. In the eight latest included patients, IGF-I was measured by Immulite 2000 (Siemens, Munich, Germany). The same method was used when IGF-I levels were compared before and after SMS treatment except for one patient, in which the IGF-I level after treatment was adjusted after cross-calibration of the methods.

MRI measurements

Reanalyses of the primary MRI scans were performed as previously described in 74 of the patients (10, 13). In 23 of these, scans both before and after SMS treatment were available for reevaluation. The formula width × height × length × 0.5 was used to estimate tumor volume (24), and for each tumor, the SIPAP (suprasellar, infrasellar, parasellar, anterior, and posterior) grading score was determined (25).

Protein extraction and Western blot

Adenoma tissue was frozen at −70 C shortly after pituitary surgery in 61 patients. The tissue was homogenized in TRIzol reagent (Invitrogen Corp., Carlsbad, CA), and protein was extracted following the manufacturer's instructions. The proteins were precipitated, washed, protein concentration measured, and Western blot performed as described (10, 13) with 15 μg of total protein applied per lane. One previous blot and stripping were done before the membranes were cut at approximately 80 kDa. The parts containing the largest proteins were incubated with a mouse monoclonal anti-E-cadherin antibody (1:1000, ab1416; Abcam PLC, Cambridge, UK) and the secondary antibody antimouse IgG (1:10 000, Jackson ImmunoResearch Laboratories Europe, Suffolk, UK). The other part of the membranes were incubated with a mouse monoclonal antiglyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:10,000, G8795; Sigma-Aldrich Corp., St. Louis, MO) and the antimouse IgG (1:10,000). Multi Gauge software (Fuji film Corp., Tokyo, Japan) was used for data analysis of the protein levels. The band signal for each antibody was adjusted for background. The E-cadherin to GAPDH ratio for each adenoma was calculated (E-cadherin ratio) and used as a measure of E-cadherin protein level.

The SSTR2a protein level has previously been analyzed by Western blot in 43 of the patients and standardized to the β-actin signal (10) and the RKIP protein level in 51 patients, normalized to GAPDH (13). RKIP analyses as described in this study were performed in additional 12 patients.

Immunohistochemistry

Paraffin sections were available in 80 patients. Immunohistochemical analyses were performed on sections from tissue arrays consisting of two 1-mm cores of tumor tissue from each of 66 pituitary adenomas and on whole sections from 14 adenomas using the Dako EnVision Flex+ System (K8012; Dako, Glostrup, Denmark) and Dakoautostainer. Sections were deparaffinized and epitopes unmasked using PT-Link (Dako) and EnVision Flex target retrieval solution (high pH). Two E-cadherin antibodies were used. One was identical with the antibody used in the Western blot and directed against the extracellular domain (1:100; Abcam); the other was directed against the intracellular domain of the E-cadherin protein (1:1000; BD Biosciences, Franklin Lakes, NJ). The sections were incubated with mouse anti-E-cadherin, EnVision Flex+ Mouse (linker), and EnVision Flex/horseradish peroxidase each for 30 min and 3′3 diaminobenzidine tetrahydrochloride for 10 min. Normal skin was used as positive control. Negative controls were performed in the absence of the primary antibody. Both controls gave satisfactory results.

The adenomas were semiquantitatively scored in three classes by a neuropathologist blinded for clinical data: antibody directed against the extracellular domain of E-cadherin, class 0, E-cadherin negative (maximum 20% of the cells weakly stained); class 1, intermediate group; and class 2, preserved E-cadherin (minimum 50% of the cells strongly stained or minimum 80% of the cells moderately or strongly stained). For the antibody directed against the intracellular domain, the classes are: class 0, adenomas with nuclear staining; class 1, intermediate group (reduced membranous staining but no nuclear staining); and class 2, strong membranous staining in all cells.

Primary culture

Adenoma tissue from five patients was used for primary culture to examine the *in vitro* effects of octreotide treatment on E-cadherin expression. Approximately 1×10^5 cells were cultured in wells with and without octreotide for 6 h, in triplicate or quadruplicate. For procedure details, see the supplementary file, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

Real-time quantitative RT-PCR

Total RNA was extracted from the cultured adenoma cells using TRIzol (Invitrogen) and then purified by RNeasy microkit (Qiagen, Dusseldorf, Germany) and stored in nuclease-free water at −80 C. The RT-PCR was performed as described earlier (26). All samples were run in duplicate with 400 ng total RNA in each reaction. Sequence-specific oligonucleotide primers were designed using Primer Express software version 2.0 (Applied Biosystems, Foster City, CA): E-cadherin, forward, 5′-GCATTGCCACATACACTCTCTTCT-3′ and reverse, 5′-TCGGTTACCGTGATCAAAATCTC-3′ and β-actin, forward, 5′-AGGCACCAGGGCGTGAT-3′ and reverse, 5′-TCGTCCCAGTTGGTGACGAT-3′. β-Actin was included as endogenous normalization control to adjust for unequal amounts of RNA. Quantification of mRNA was performed using standard curve method of the ABI Prism 7500 (Applied Biosystems).

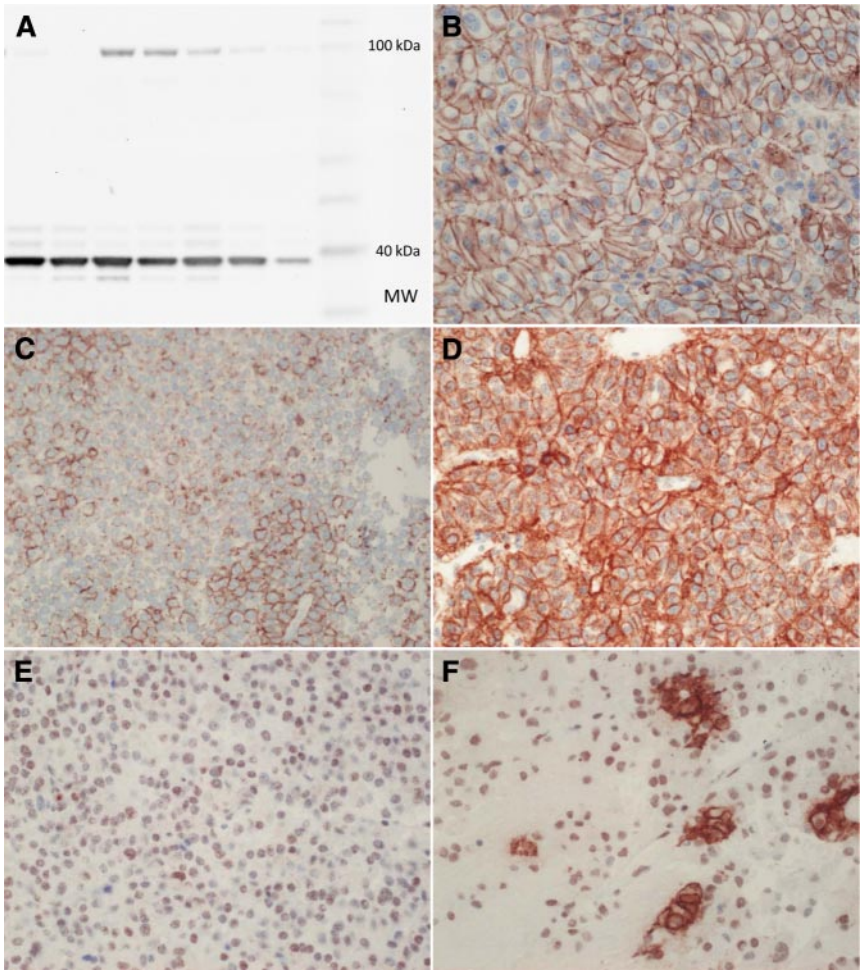

FIG. 1. A, A representative Western blot of E-cadherin (*upper panel*, the band at ~100 kDa) and GAPDH (*lower panel*, the band at ~36 kDa). Each lane represents one adenoma. MW, Molecular weight. B–F, Immunohistochemical staining of E-cadherin (magnification ×400). The extracellular domain antibody (B and C), an adenoma with strong membranous staining of all cells (B) and an adenoma with focal expression (C), is shown. The intracellular domain antibody (D–F) is shown: an adenoma class 2 with strong membranous staining of all cells (D), a class 0 adenoma with no membranous staining but instead a weak nuclear positivity (E), and an adenoma with moderate nuclear staining and membranous staining in s few small groups of cells.

Statistics

Differences between groups were analyzed using Mann-Whitney test and Kruskal-Wallis test when more than two groups were compared. Relationships between variables were tested by Spearman correlation (R) or Pearson correlation analysis (r) when normal distribution of the data were obtained by log transformation. $P < 0.05$ was considered significant. The statistical analyses were performed using SPSS software (version 16.0; SPSS, Chicago, IL).

Results

E-cadherin protein expression

Staining with the E-cadherin antibody gave a single band at 100–120 kDa in the Western blot analysis (Fig.1A). The E-cadherin to GAPDH ratio (E-cadherin ratio) was used as a measure of the E-cadherin protein level.

Immunohistochemical studies with two E-cadherin antibodies recognizing either the extracellular or intracellular domain demonstrated a highly variable degree of E-cadherin immunoreactivity in the adenomas (Fig.1, B–D). Using the extracellular domain antibody, only membranous staining was observed. Forty-one adenomas had preserved high E-cadherin expression, 21 adenomas were E-cadherin negative, and 18 were in the intermediate group.

With the intracellular domain antibody, nuclear expression of E-cadherin was demonstrated in nine adenomas. The nuclear staining was weak in all class 0 adenomas except one with moderate immunoreactivity, and they also displayed none or very low membranous staining. Seven adenomas had reduced E-cadherin membranous expression and no nuclear staining. The remaining 64 adenomas had strong membranous and cytoplasmic staining in all cells. A particularly heterogeneous pattern with strong membranous staining in 50% of the cells and nuclear positivity in the remaining 50% of cells was observed in one of the adenomas. It was scored as class 0 due to the nuclear positivity.

There was a significant correlation between the immunohistochemical results of the two antibodies ($P < 0.0001$) and between the protein level assessed by Western blot and the immunohistochemical grading ($P < 0.001$ for both antibodies, Table 2).

E-cadherin and baseline characteristics

Whereas the immunohistochemical expression of E-cadherin did not correlate to preoperative medical treatment, the E-cadherin protein level was significantly lower in patients who received somatostatin analog treatment before surgery (median 0.306 *vs.* 0.105 in the direct surgery group, $P = 0.01$). Patients preoperatively treated with dopamine agonist or pegvisomant did not differ in E-cadherin protein expression.

SSTR2a protein levels assessed by Western blot and immunohistochemistry previously (10) correlated to the E-cadherin results in this study. There was a correlation between E-cadherin and SSTR2a protein levels ($R = 0.39$, $P = 0.011$), and adenomas with a high proportion of

TABLE 2. E-cadherin protein expression

		E-cadherin (extracellular domain)			
	Score by IHC	0	1	2	E-cadherin ratio
E-cadherin (intracellular domain)	0	9	0	0	0.012 (0.005–0.020)
	1	6	1	0	0.069 (0.009–0.253)
	2	6	17	41	0.293 (0.119–0.435)
E-cadherin ratio		0.019 (0.006–0.253)	0.102 (0.057–0.194)	0.358 (0.200–0.516)	

Numbers of adenomas categorized in each class assessed by immunohistochemistry using the two antibodies. E-cadherin ratio assessed by Western blot for each group is presented as median (interquartile range) ($P < 0.001$ for both). IHC, Immunohistochemistry.

SSTR2a-positive cells had significantly higher E-cadherin protein levels ($P = 0.006$) and a higher proportion of immunohistochemically E-cadherin-positive cells ($P = 0.018$ E-cadherin extracellular domain, $P = 0.006$ intracellular domain, data not shown).

The RKIP levels obtained in this and partly in a previous study (13) correlated significantly to the E-cadherin protein level ($R = 0.32$, $P = 0.013$). RKIP levels were significantly higher in class 2 compared with grade 0 adenomas defined by the E-cadherin intracellular domain antibody ($P = 0.006$, data not shown).

Sex, age, baseline IGF-I and GH levels did not correlate to the adenoma E-cadherin expression assessed by both Western blot and immunohistochemistry.

Tumor size and invasiveness

The MRI scans were performed before the preoperative SMS treatment, and the E-cadherin levels were lower in the pretreated compared with the patients not pretreated. Therefore, the correlations between E-cadherin protein level and tumor characteristics were analyzed separately for the two groups. There were significant negative correlations between tumor size and E-cadherin in both the pretreated (Pearson $r = -0.70$, $P = 0.001$) and not pretreated group ($r = -0.33$, $P = 0.043$), using log-transformed variables (Fig. 2, A and B). Tumor invasiveness, defined as the sum of SIPAP score, correlated negatively to E-cadherin levels only in the group pretreated with SMS ($R = -0.53$, $P = 0.018$) but not the direct surgery group ($R = -0.21$, $P = 0.22$, Supplemental Fig. 1, A and B).

For the immunohistochemical E-cadherin expression, no differences were found between the pretreated and not pretreated groups, which were analyzed together. The immunohistochemical classification of the adenomas correlated negatively to tumor size ($P = 0.007$) and partly to invasiveness ($P = 0.011$) using the extracellular domain antibody. The intracellular domain antibody gave $P = 0.033$ and $P = 0.070$, respectively (Kruskal Wallis test). Class 0 adenomas were significantly larger compared with the class 2 adenomas defined by both extracellular ($P = 0.002$) and intracellular domain antibody ($P = 0.009$, Fig. 2, C and D). Extracellular domain class 0 adenomas were significantly more invasive compared with class 2 adenomas ($P = 0.005$, Supplemental Fig. 1C).

Biochemical response to SMS

A significant positive correlation between E-cadherin level in the tumors and the percentage serum GH reduction during acute octreotide test was observed in patients not preoperatively treated with SMS ($R = 0.48$, $P = 0.004$, Fig. 3A). The groups defined by extra- or intracellular E-cadherin domain immunoreactivity also correlated positively to the acute GH response to octreotide ($P = 0.010$ and $P = 0.006$, respectively). Patients with class 0 adenomas had significantly less GH reduction compared with patients with class 2 adenomas, assessed by both extracellular domain ($P = 0.003$, data not shown) and intracellular domain antibodies ($P = 0.006$, Fig. 3B).

The percentage IGF-I reduction after a median of 6 months preoperative SMS treatment did not correlate statistically significantly to E-cadherin levels ($R = 0.426$, $P = 0.054$, Fig. 3C). The immunohistochemical results from class 1 and 2 adenomas were combined for the statistical analyses due to the low number of patients in each group. Nuclear staining with the antibody against the intracellular domain of E-cadherin (class 0) associated with significantly poorer IGF-I response compared with the adenomas without nuclear staining ($P = 0.033$, Fig. 3D). However, there was no difference in IGF-I reduction for adenomas scored by E-cadherin extracellular domain antibody reactivity.

Tumor response to SMS

Among patients treated with SMS before surgery, percentage tumor shrinkage correlated significantly to the E-cadherin level of the adenoma ($R = 0.57$, $P = 0.013$, $n = 18$, Fig. 3E). Tumor volume reduction was significantly less for adenomas with immunohistochemical nuclear E-cadherin staining compared with adenomas with no nuclear staining ($P = 0.045$, Fig. 3F). There was no difference in tumor shrinkage based on extracellular domain antibody classification.

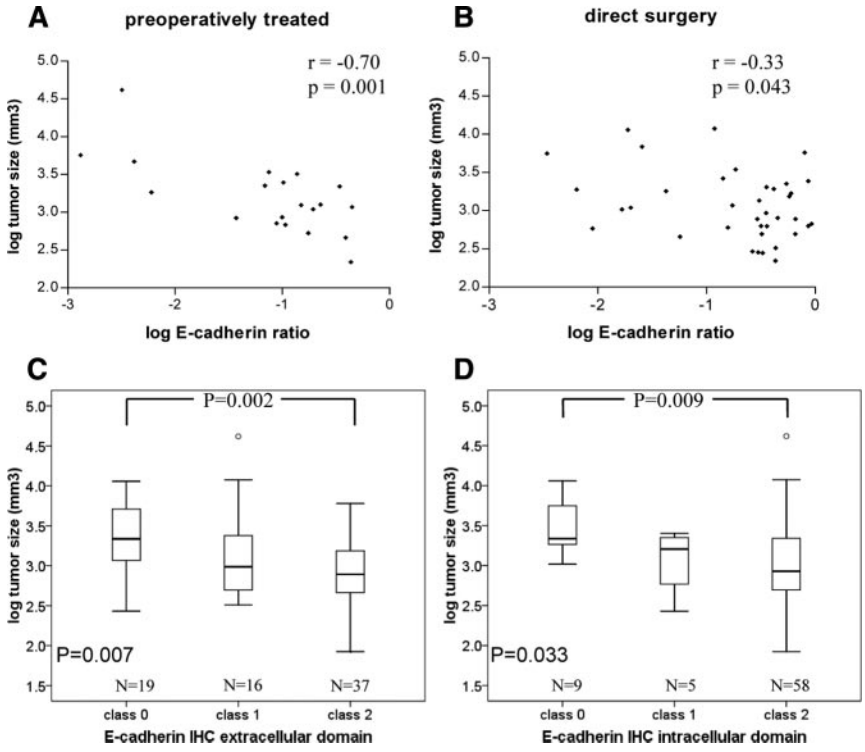

FIG. 2. The E-cadherin expression and tumor size. The *upper panels* show the correlation between E-cadherin protein levels assessed by Western blot and tumor size, both in logarithmic scale, in the preoperatively treated group (A) and the direct surgery group (B). The *lower panels* illustrate the association between tumor size (log transformed) and the immunohistochemical expression of E-cadherin with the extracellular domain antibody (C) (Kruskal Wallis test, $P = 0.007$) and the intracellular domain antibody (D) (Kruskal Wallis test, $P = 0.033$). Class 0 adenomas were significantly larger than class 2 adenomas. Box plot, The median value (*line*), the interquartile range (*box*), and the range of the data. Outlier (°), Cases with values between 1.5 and 3 box lengths from the *upper* or *lower edge of the box*.

In vitro study

In accordance with a previous study, the phenotype of primary cell cultures from five adenomas showed two different patterns (27). Cell cultures from four adenomas consisted of individual or small groups of rounded cells free floating or only weakly attached to the well. Two of these were from octreotide-treated patients (no. 331 and 334), but one received the last dose 3 months before surgery. No difference in E-cadherin mRNA was found in these cells in the absence or presence of octreotide *in vitro*. The other two patients had not been treated with SMS preoperatively. In one tumor (no. 324), the E-cadherin mRNA level was significantly lower in the *in vitro* octreotide-treated cells ($P = 0.043$). In the other tumor (no. 319), the reduction in E-cadherin mRNA after octreotide treatment did not reach statistical significance (Fig. 4).

E-cadherin mRNA was not detected in the primary culture of a large, rapidly growing, recurrent adenoma (no. 320) preoperatively treated with pasireotide. This adenoma had a very low protein level of E-cadherin measured by Western blot. The *in vitro* cultured cells were mainly spindle shaped and attached to the well.

For GH analyses of the supernatant, see Supplemental Fig. 2.

Discussion

In the present study, we have shown that E-cadherin expression in somatotroph pituitary adenomas is variable. A low E-cadherin level combined with a redistribution from the cell membrane to the nucleus correlated to large tumors and partly to tumor invasiveness. In addition, the E-cadherin protein expression seemed to be associated with SMS response, in particular to tumor shrinkage. Furthermore, E-cadherin correlated to the expression of SSTR2a in the adenomas.

Loss of E-cadherin is a hallmark of EMT and malignant progression in several cancers of epithelial origin. A causal role of E-cadherin in tumor progression has been demonstrated *in vivo* in a mouse model of pancreatic β-cell carcinogenesis (28). It has been suggested that loss of E-cadherin not only means loss of epithelial adhesion but also mediates direct activation of signaling pathways to cause invasive phenotypes (29). This study confirms the variable expression of E-cadherin previously shown in somatotroph adenomas (18–23), and in this large cohort, we found a significant negative correlation between E-cadherin protein expression and tumor size and also partly tumor invasiveness. Large and invasive adenomas seemed to have lost their membranous E-cadherin expression. The partial association with tumor invasiveness may be due to inaccurate measures of invasiveness or a true but weak association.

The E-cadherin expression correlated positively to the expression of SSTR2a, an important receptor with respect to the clinical efficacy of somatostatin analog treatment (10). Ligand binding to SSTR2 has been shown to activate the tyrosine phosphatase Src homology domain phosphatase 1, which dephosphorylates E-cadherin and restores E-cadherin function (30). In a human neuroendocrine cell line, blocking SSTR signaling decreased the membranous E-cadherin levels and resulted in morphological changes with a more spindle-shaped fibroblastic appearance termed neuroendocrine-mesenchymal transition (31). However, we found a reduced E-cadherin protein level and no alterations in E-cadherin membranous

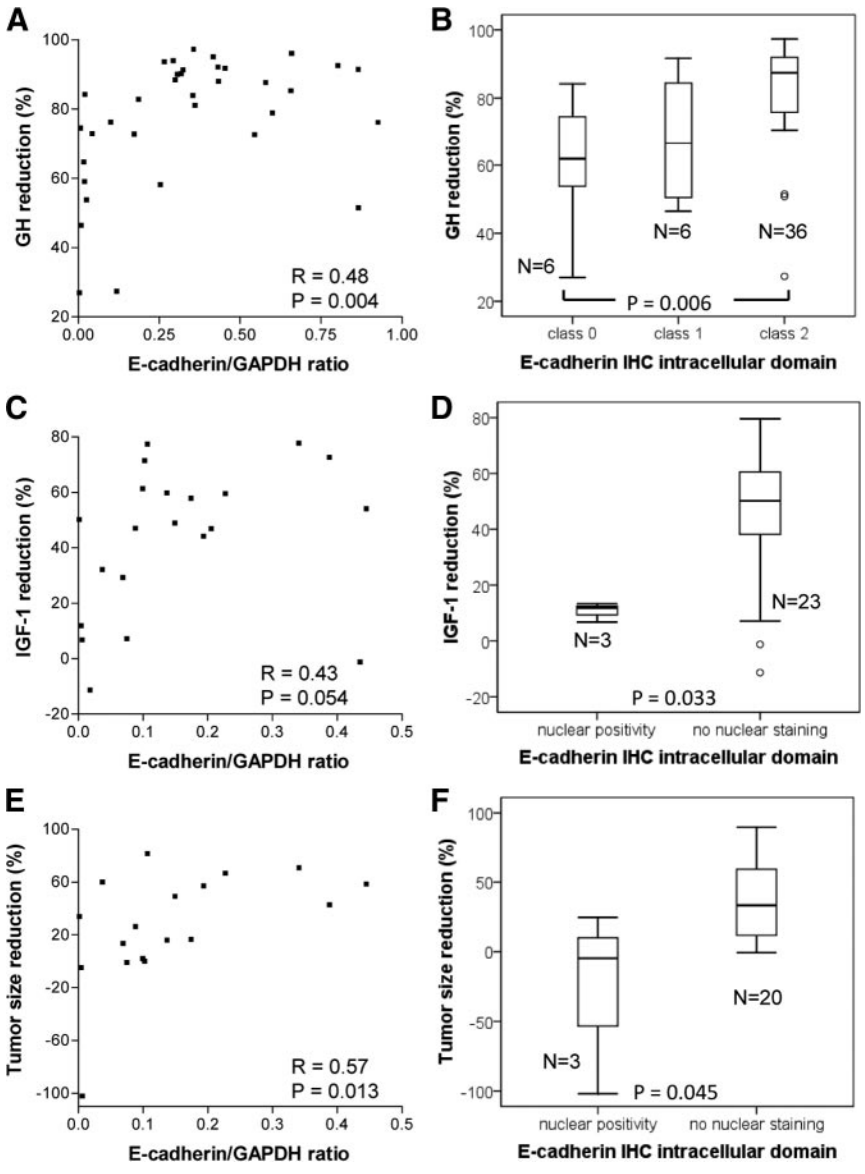

FIG. 3. The E-cadherin expression and SMS response. The *left panels* show the correlation between E-cadherin protein level assessed by Western blot and the response to SMS in an acute test (A) and percentage IGF-I reduction (C) and percentage tumor shrinkage (E) after long-term SMS preoperative treatment (median 6 months). The *right panels* show the immunohistochemical E-cadherin expression using the intracellular domain antibody related to percentage GH reduction in an acute SMS test (B) (Kruskal Wallis test, $P = 0.006$), the percentage IGF-I reduction (D), and percentage tumor shrinkage (F) after long-term SMS preoperative treatment (median 6 months). For box plot explanation, see legend Fig. 2.

staining in patients preoperatively treated with SMS. Indeed, in this cohort we also documented a decreased SSTR2a expression in the preoperatively treated patients (10). There seems to be a parallel regulation of SSTR2a and E-cadherin in the adenomas. This could be an indicator of lost epithelial differentiation. The similar phenomenon may also explain the observed correlation between E-cadherin and RKIP. We have previously shown that the adenoma RKIP protein level correlated to SMS biochemical response (13). The E-cadherin transcriptional repressor SNAIL has also been shown to inhibit RKIP transcription (32), which could explain the observed association between RKIP and E-cadherin. Overexpression of RKIP in a prostate cancer cell line led to reduced SNAIL and increased E-cadherin (both at protein level) and induced an epithelial-like phenotype (33).

In this study, we used two different antibodies directed against either the extracellular or intracellular domain of E-cadherin for the immunohistochemical analyses. There was high degree of concordance between the results. However, several adenomas showed membranous positivity in all cells using the intracellular domain antibody but had lost membranous E-cadherin using the extracellular domain antibody. This discordance could reflect a cleavage and loss of the extracellular domain before internalization of the protein. The cytoplasmic staining observed exclusively with the intracellular domain antibody could also be due to internalization of only the intracellular domain of the protein. Nuclear accumulation of the intracellular domain of E-cadherin has been demonstrated in several cancers, including endocrine pancreatic tumors (34–36). The catenin p120 is a possible mediator of this nuclear translocation (37). We found nuclear accumulation of E-cadherin combined with loss of membranous E-cadherin in a few adenomas (nine of 80) and in a lower proportion than in a recent study (20). These adenomas had very low E-cadherin protein levels assessed by Western blot, were larger compared with the adenomas with membranous positivity in all cells, and displayed a poor acute and long-term SMS response.

Preoperatively treated patients had significantly lower E-cadherin levels, suggesting down-regulation of E-cadherin during SMS treatment. There is a possibility that low E-cadherin levels were secondary to a selection bias for preoperative SMS treatment. However, a similar result was found when the analysis was restricted to the subgroup of patients who were randomized to preoperative SMS treatment or not in a clinical study (3). The observation was supported by our *in vitro* demonstration of reduced E-cadherin mRNA levels in octreotide-treated

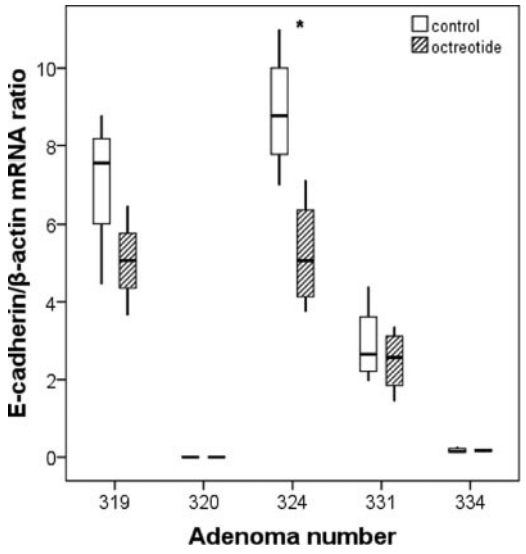

FIG. 4. E-cadherin expression in *in vitro* cultured adenoma cells analyzed with real-time RT-PCR. The E-cadherin mRNA levels were normalized to the level of the reference gene β-actin. The cells were cultured with or without octreotide at 10^{-8} M for 6 h, in triplicate (adenoma 319 and 320) or quadruplicate (adenoma 324, 331, and 334). For adenoma 319 and 320, the E-cadherin levels can be directly compared because the reverse transcription from these adenomas was performed in one run. Otherwise, comparisons can be made only between the controls and octreotide-treated cells for each adenoma. Adenoma 320 did not have detectable levels of E-cadherin mRNA, and this was clinically a large, recurrent, and rapidly growing adenoma with poor SMS response (pasireotide treatment before surgery, not the last 3 months). In the SMS analog naïve adenomas 319 and 324, octreotide-treated cells had significantly lower E-cadherin to β-actin ratio compared with the untreated cells in adenoma 324 (*, $P = 0.043$) but was not statistically significant in 319. Adenoma 331 and 334 had received preoperative octreotide treatment (for 334 not the last 3 months), and no difference in E-cadherin to β-actin ratio was detected in octreotide-treated compared with untreated cells.

cells from SMS-naïve patients, whereas we found no additional effect of octreotide on E-cadherin in tissue from SMS-pretreated patients. The immunohistochemical cellular distribution and expression of E-cadherin were not different in the two groups, and the pathophysiological importance and clinical relevance of this finding need to be explored more in detail.

To conclude, this study demonstrated that E-cadherin is down-regulated and redistributed in a substantial proportion of somatotroph adenomas. This was associated with large tumors and to some extent also to tumor invasiveness. A correlation between E-cadherin expression and clinical SMS efficacy, both hormonal and tumor response, was also shown. Translocation of E-cadherin to the nucleus with concomitant loss of membranous expression was demonstrated in a subset of adenomas and associated with large, SMS-resistant tumors. The E-cadherin expression, which may reflect a process involving epithelial to mesenchymal transition, seems to be of importance for the clinical behavior of somatotroph pituitary adenomas.

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