Downregulation of E-Cadherin and Its Undercoat Proteins in Pituitary Growth Hormone Cell Adenomas with Prominent Fibrous Bodies

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Abstract

The cadherin-catenin complex regulates cellular adhesion and motility, and genetic alterations in these molecules play a critical role in multistage tumorigenesis. In this study, the expression of three major type I classic cadherins—E-, N-, and P-cadherin—and their undercoat proteins—α-, β-, and γ-catenin, and pp120—was investigated in 127 pituitary adenomas and 10 normal adenohypophyseal glands using an immunohistochemical technique with highly specific monoclonal antibodies. In normal pituitary glands, E-cadherin, catenins, and pp120 were strongly expressed on almost all hormone-producing cell-cell boundaries, N-cadherin was weakly immunoreactive on a few cell-cell boundaries, and P-cadherin was negative. In pituitary adenomas, a correlation was not identified among expression of E-cadherin, catenins, or pp120 with patient age, sex, hormone level, tumor size, and/or invasiveness, respectively. Expression of E-cadherin, catenins, and pp120 was significantly reduced in 24 growth hormone (GH) cell adenomas with prominent fibrous bodies compared with the other subtypes of pituitary adenomas and normal pituitary glands (p < 0.0001, respectively). Methylation-specific polymerase chain reaction analysis revealed that the E-cadherin gene promoter region was methylated in 6 of 16 (37.5%) GH cell adenomas with prominent fibrous bodies examined, 2 of which displayed total methylation, but not in 10 GH cell adenomas without fibrous bodies. No mutation of exon 3 of the β-catenin gene was found in 16 GH cell adenomas with prominent fibrous bodies or in 10 other subtypes of pituitary adenomas that showed unremarkable intracellular presence of β-catenin protein. In conclusion, the decreased expression of the E-cadherin–catenin complex and methylation of the E-cadherin gene promoter region only in GH cell adenomas with prominent fibrous bodies may be an event associated with the formation of fibrous bodies.

Key Words: Cadherin; catenin; pituitary; adenoma.

Introduction

Cadherins, a multigene family of calcium-dependent cell-cell adhesion glycoproteins, control solid tissue morphogenesis including segregation of cell types, cell growth, differentiation, and support of particular tissue architecture [1,2]. The classic cadherins comprise a subgroup that includes three major type I classic cadherins: E-, N-, and P-cadherin. E-cadherin is expressed in virtually all of epithelial tissue [3]. N-cadherin is predominantly expressed in neural tissues but is also present in fibroblasts and skeletal muscle cells [4,5]. P-cadherin, originally described in mouse placenta, is also found in
epithelium of the lung and prostate and basal cells of the skin [3,6–8]. Cadherins, to exhibit their functional adhesion activity, must form complexes with cytoplasmic plaque proteins, β- or γ-catenin, which, in turn, bind α-catenin, which is attached to the actin microfilament-based cytoskeleton [9]. pp120, which is a tyrosine kinase substrate implicated in receptor ligand–induced signaling by growth factors and in transformation by src, colocalizes with E-, N-, or P-cadherin and binds to the juxtamembrane domain of the E-cadherin cytoplasmic tail [10].

Abnormalities in cadherins and catenins have been reported in a number of tumors, both malignant and benign [11,12], and are believed to be associated with tumor progression. The E-cadherin-mediated cell adhesion system in tumor cells is inactivated by multiple mechanisms, such as genetic alterations in E-cadherin and α- and β-catenin, aberrant tyrosine phosphorylation of members of the cadherin-catenin complex, and transcriptional inactivation of E-cadherin expression regulated by CpG methylation around the E-cadherin promoter region [13].

Furthermore, β-catenin, an important element in the Wingless-Wnt transduction signaling pathway, plays an important role in oncogenesis [14]. The adenomatous polyposis coli protein forms a macromolecular complex with glycogen synthase kinase-3β and β-catenin, thereby sequestering β-catenin and targeting it for degradation [15,16]. The binding of β-catenin by adenomatous polyposis coli requires phosphorylation of β-catenin by glycogen synthase kinase-3β on specific serine and threonine residues, all of which are encoded in exon 3 of the β-catenin gene [17]. Unphosphorylated β-catenin accumulates in the cytoplasm, where it can interact with the T-cell factor/lymphoid enhancer factor family of transcriptional activator [18].

The complex then translocates to the nucleus and binds to the promoters of target genes, resulting in the activation of those genes [19]. Mutations in the adenomatous polyposis coli gene result in loss of the ability to degrade β-catenin or mutations in exon 3 of the β-catenin gene that prevent phosphorylation, resulting in activation of this pathway because of increased cytoplasmic β-catenin [16,20]. Therefore, cytoplasmic and nuclear localization of β-catenin is not necessarily owing to mutations of the β-catenin gene.

To the best of our knowledge, there are only a few reports investigating what role the cadherins and their undercoat proteins play in pituitary adenomas, and whether or not mutations of exon 3 of the β-catenin gene are frequent molecular events in pituitary adenomas is still controversial [21–24]. Therefore, to clarify the role of classic cadherin adhesion molecules in pituitary adenomas, we examined expression of the three major type I classic cadherins—E-, N-, and P-cadherin—and their undercoat proteins—α-, β-, and γ-catenin, and pp120—in various types of pituitary adenomas. Further, we investigated mutations of exon 3 of the β-catenin gene and methylation of the E-cadherin gene promoter region in a part of pituitary tumors with aberrant expression of β-catenin and/or E-cadherin.

**Materials and Methods**

**Tumor Specimens and Histologic Analysis**

Pituitary adenomas were removed during transsphenoidal surgery from patients after full endocrine preoperative evaluations at the University Hospital of Tokushima (Tokushima, Japan) and Toranomon Hospital (Tokyo, Japan) between 1990 and 1999. The resected tumors were fixed in 10% buffered formalin and subsequently