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# INSIGHTS FROM MODEL SYSTEMS
# The Tumor-Suppressor Function of E-Cadherin

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## Introduction

The transition from benign tumors to invasive, metastatic cancer cells involves changes in the extracellular matrix environment, cell motility, and cell-cell adhesion. Because cell-cell–adhesion molecules are dynamically regulated during human carcinogenesis, they have been implicated in tumorigenesis, especially during the later stages of tumor progression—that is, tumor-cell invasion and metastasis (Thiery 1996). In this review, we focus on the evidence from genetics and cell-culture studies that support a functional role of E-cadherin/catenin adhesion complexes in carcinogenesis. We discuss both the potential downstream signals that changes in E-cadherin–mediated cell-cell adhesion may elicit during tumorigenesis and a possible connection between E-cadherin function and Wnt signaling.

Many studies have implicated E-cadherin in the development of human cancers. The great interest in this protein may, in part, reflect its role as the major cell-cell–adhesion molecule in epithelial cells, the cell type from which ~80% of human cancers derive. Epithelial cells are tightly interconnected through a junctional complex, consisting of tight junctions, adherens junctions, and desmosomes, structures that are intimately associated with the actin and intermediate cytoskeletal filament systems. E-cadherin mediates $Ca^{2+}$-dependent homophilic interactions, as the major adhesion receptor in adherens junctions. This function is essential both to establish and to maintain cell-cell junctions (reviewed in Takeichi 1995; Aberle et al. 1996), and mice deficient for E-cadherin die in utero, because of defective formation of the first epithelium, the trophectoderm (Larue et al. 1994; Riethmacher et al. 1995).

Intracellularly, E-cadherin is linked to the catenins ($\alpha$-, $\beta$-, and $\gamma$-catenin/plakoglobin), which connect E-cadherin to the actin cytoskeleton (fig. 1). Failure either to assemble the E-cadherin/catenin complex or to properly connect to the actin cytoskeleton results in the loss of cell adhesion. Hence, mutations in numerous genes other than *CDH1,* which encodes the human E-cadherin, may also affect cell adhesion. Finally, although this view is supported mainly by circumstantial evidence, E-cadherin–mediated cell adhesion may also participate in the transduction of transmembrane signals that regulate gene expression and cell fate (reviewed in Huber et al. 1996; Gumbiner 1997; Bullions and Levine 1998).

Like the classical cadherins, desmosomal cadherins have been implicated in human disease. Dsg1 (desmoglein I), which is a major adhesive component in suprabasal epidermal and lingual desmosomes, is the antigenic target of autoantibodies found in patients with the blistering disease pemphigus foliaceus (Eyre and Stanley 1987). Another desmoglein, Dsg3, is recognized by sera from patients with a more severe form of this disease, known as "pemphigus vulgaris" (Amagai et al. 1991).

## E-Cadherin Function Is Suppressed during Carcinogenesis

In recent years, a large number of studies have revealed that E-cadherin function is frequently inactivated during the development of human carcinomas, including those of the breast, colon, prostate, stomach, liver, esophagus, skin, kidney, and lung (for a more extensive list, see Birchmeier and Behrens 1994; Bracke et al. 1996). Abrogation of E-cadherin function may occur by any of several mechanisms, but it frequently involves deletion or mutation of the *CDH1* gene (reviewed in Birchmeier and Behrens 1994; Bracke et al. 1996). Remarkably, germ-line mutations in *CDH1* have been identified in cases of familial gastric cancers, indicating that aberrations in this gene are sufficient to predispose to the development of malignant cancer (Guilford et al. 1998). Furthermore, changes in the expression of proteins that are part of the E-cadherin–adhesion complex have also been found to impair E-cadherin–mediated cell-cell adhesion. For example, down-regulation of $\alpha$-catenin or $\beta$-catenin expression (De Leeuw et al. 1997), as well as the expression of mutant forms of either $\alpha$-catenin (Bullions et al. 1997) or $\beta$-catenin (Oyama et al.

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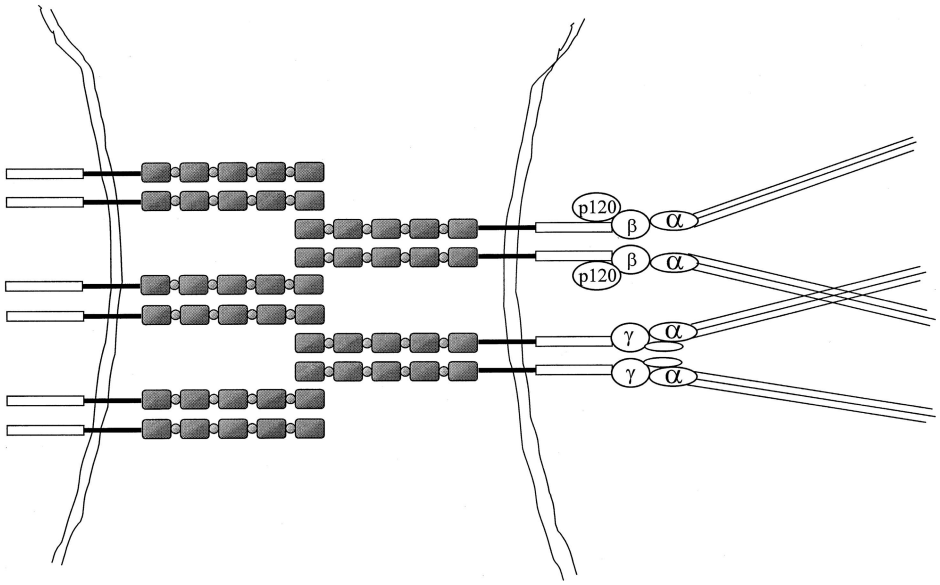



**Figure 1** Schematic representation of the E-cadherin adhesion complex (for details, see the text)

1994), sometimes coincides with malignant transformation. Moreover, mutations of the β-catenin gene are frequently found in colon cancers (Sparks et al. 1998). However, it remains to be determined, for each type of cancer, how changes in the expression of either α-catenin or β-catenin affect E-cadherin function.

In addition to deletions and mutations, several other mechanisms have been identified that directly affect E-cadherin expression and function. For example, chromatin rearrangement, hypermethylation, and loss of transcription-factor binding frequently coincide with suppression of E-cadherin–promoter activity in invasive carcinoma cells (Yoshiura et al. 1995; Hennig et al. 1996). Notably, in some tumor types, inactivation of the E-cadherin gene by hypermethylation appears to be a major mechanism—for example, in papillary thyroid carcinoma, where hypermethylation of the E-cadherin promoter has been found in 83% of the cases examined (Graff et al. 1998).

Other epigenetic events that are required for E-cadherin function during normal developmental processes may also be involved in the misregulation of E-cadherin during tumorigenesis. For example, E-cadherin function can be affected by the Rho family of GTPases (Braga et al. 1997; Jou and Nelson 1998), growth factor–receptor signaling (Hoschuetzky et al. 1994; Shibamoto et al. 1994), integrin-linked kinase (Novak et al. 1998), and matrix metalloproteases (Lochter et al. 1997).

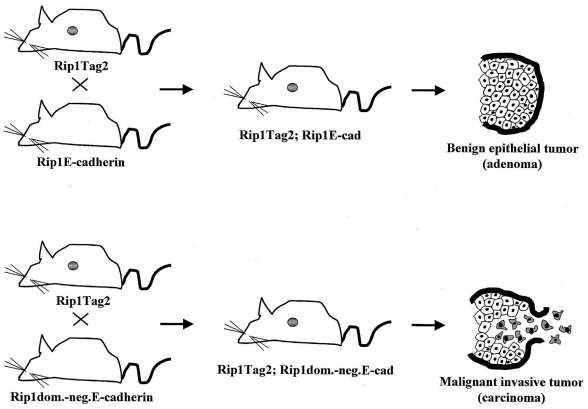
Because decreased E-cadherin function generally correlates with dedifferentiation, infiltrative tumor growth, and metastasis, E-cadherin has been proposed as a marker to indicate poor prognosis (reviewed in Birchmeier and Behrens 1994; Bracke et al. 1996). Although E-cadherin is not the only cadherin that is expressed in epithelial cells, few others have been implicated in cancer. One exception is H-cadherin, which has been found to be lost during the development of breast cancer (Lee 1996).

## E-Cadherin Suppresses Tumor-Cell Invasion

Because the loss of E-cadherin function so closely parallels carcinogenesis, many groups have sought to elucidate the role of E-cadherin in tumor progression. In various cell-culture systems, the reestablishment of a functional cadherin/catenin complex causes invasive tumor-cell lines to revert to a benign, epithelial cellular phenotype (reviewed in Frixen et al. 1991; Vleminckx et al. 1991; Birchmeier and Behrens 1994; Bracke et al. 1996), clearly demonstrating that E-cadherin can suppress the overt features of advanced tumor progression. However, it has remained unresolved whether the loss of E-cadherin–mediated cell adhesion is a prerequisite for tumor progression or is a consequence of dedifferentiation during tumor progression in vivo. Using a transgenic mouse model of pancreatic β-cell tumorigenesis (Rip1Tag2; Hanahan 1985), our laboratories have recently addressed this question and have demonstrated that the loss of E-cadherin–mediated cell-cell adhesion is causally involved in the transition from well-differentiated adenoma to invasive carcinoma (Perl et al. 1998; see sidebar). However, the mere loss of E-cad-

### Rip1Tag2—A Versatile Transgenic Mouse Model for Tumor Progression

Rip1Tag2, a prototype of the mouse models for tumorigenesis, is a transgenic mouse line that expresses SV 40 T antigen under the control of the insulin promoter in the β-cells of the pancreatic islets of Langerhans (Hanahan 1985). These mice reproducibly develop β-cell tumors in a multistage tumorigenesis pathway involving islet hyperplasia (~70% of all islets), the formation of new blood vessels (angiogenesis; ~20%), well-differentiated, benign tumors (adenomas; ~2%), and, finally, dedifferentiated, invasive tumors (carcinomas; ~0.5%). Metastases are usually not found in these mice, probably because they succumb to hypoglycemia (i.e., they die before metastasis can happen), with increased tumor mass.




The Rip1Tag2 tumor model has proved a powerful tool with which to study the molecular mechanism of multistage tumor development, including tumor-cell proliferation, apoptosis, and angiogenesis. We have employed the Rip1Tag2 transgenic mouse model to investigate molecular mechanisms of tumor invasion and metastasis. As in the progression of many epithelial cancers, E-cadherin is lost during the transition from well-differentiated adenoma to invasive carcinoma in Rip1Tag2 transgenic mice (Perl et al. 1998). To assess whether loss of E-cadherin–mediated cell adhesion is a cause or a consequence of tumor progression in vivo, we have intercrossed Rip1Tag2 transgenic mice with transgenic mice that express either wild-type E-cadherin or a dominant-negative form of E-cadherin, specifically in the pancreatic β-cells (Perl et al. 1998). Maintenance of E-cadherin expression during β-cell tumorigenesis results in arrest of tumor development at the adenoma stage. In contrast, expression of a dominant-negative E-cadherin induces early invasion and metastasis. The results demonstrate that loss of E-cadherin–mediated cell adhesion is one rate-limiting step in the progression from adenoma to carcinoma in vivo.

herin–mediated adhesion appears not to be sufficient for the tumor cells to penetrate the basal lamina and invade the surrounding tissue (Perl et al. 1998). Thus, we speculate that E-cadherin may also convey signals that actively induce tumor-cell invasion.

### β-Catenin—A Potential Mediator of E-Cadherin–Mediated Downstream Signals

β-Catenin is a multifunctional protein that performs many functions in a cell—for example, in E-cadherin–mediated adhesion, in membrane extensions, and in Wnt signaling (reviewed in Barth et al. 1997; Gumbiner 1997). β-Catenin that is not sequestered in the E-cadherin cell-adhesion complex is rapidly phosphorylated by glycogen synthetase kinase-3β (GSK-3β) in the adenomatous polyposis coli (APC)/GSK-3β complex and subsequently is degraded by the ubiquitin-proteasome pathway (fig. 2). Several mechanisms are able to block degradation of β-catenin, culminating in an accumulation of free β-catenin in the cytoplasm; these mechanisms include mutations in the phosphorylation sites that are important for β-catenin's degradation, mutations in APC that render it nonfunctional, as well as activation of GSK-3β activity, which blocks signaling by Wnt. Following translocation into the nucleus, β-catenin binds to members of the TCF/LEF-1 family of transcription factors, possibly modulating the expression of target genes (reviewed in Behrens et al. 1996; Huber et al. 1996; Molenaar et al. 1996; Korinek et al. 1997; Morin et al. 1997; Rubinfeld et al. 1997; Gumbiner 1998).

Several lines of evidence indicate that E-cadherin-mediated signaling could feed into the β-catenin/TCF/LEF-1 pathway. Although β-catenin clearly performs distinct functions in E-cadherin-mediated cell adhesion and in Wnt signaling (Orsulic and Peifer 1996; Sanson et al. 1996), there appears to be some cross-talk between the adhesive and the signaling pathways. Thus, the expression of excess cadherin interferes with Wnt signaling by competing for β-catenin binding (Fagotto et al. 1996; Sanson et al. 1996). It remains to be determined whether the opposite situation occurs—that is, whether loss of cadherin leads to the accumulation of β-catenin free to bind TCF/LEF-1 and to modulate transcription (fig. 2). However, recent experimental evidence supports this possibility: mouse ES cells that lack functional *E-cadherin* genes (i.e., cells that are −/−) express LEF-1 mRNA, whereas wild-type ES cells (i.e., those that are +/+) do not (Huber et al. 1996). Moreover, forced expression of E-cadherin in E-cadherin–deficient ES cells leads to the repression of LEF-1. In return, LEF-1/β-catenin is able to bind to the E-cadherin promoter and to suppress E-cadherin expression (Huber et al. 1996). These results support a connection between E-cadherin function and β-catenin/TCF transcriptional activity, but it remains uncertain whether this link is physiologically relevant and whether the connection is direct or indirect.

More-detailed information regarding the roles of APC and β-catenin during carcinogenesis recently has been obtained from investigation of the function of these pro-

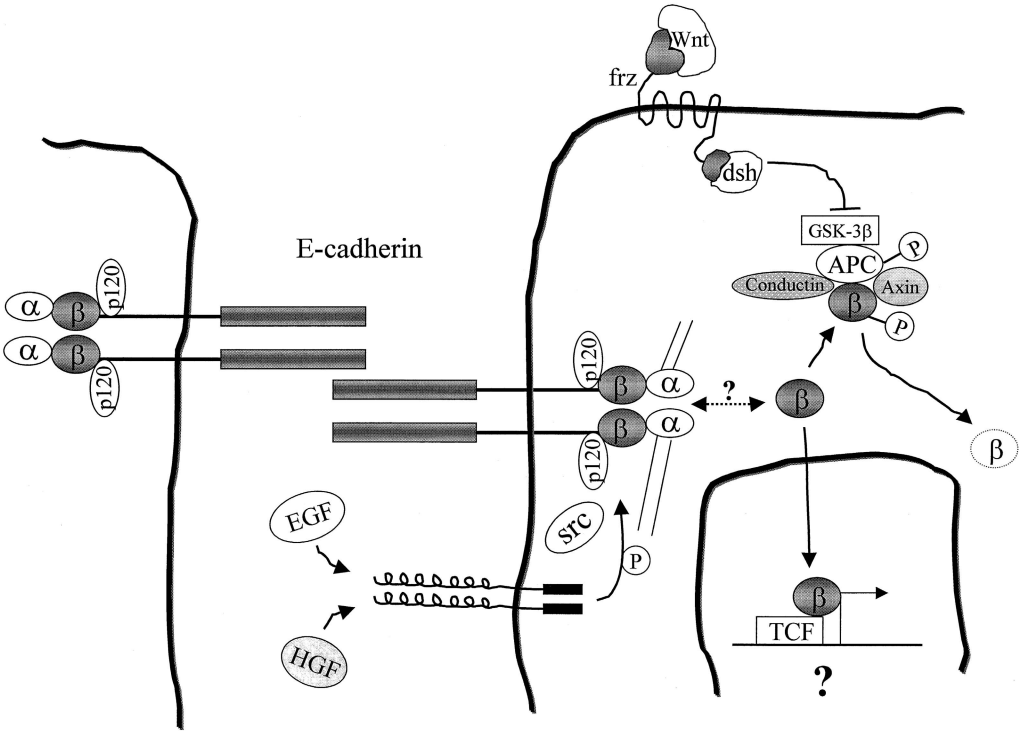



**Figure 2** Schematic representation of the possible link between E-cadherin–mediated cell-cell adhesion and the Wnt-signaling pathway (for details, see the text). Question marks indicate future goals in cancer research: whether E-cadherin competes directly with the Wnt/β-catenin–signaling pathway; whether it affects TCF/β-catenin signaling, independent of the Wnt-signaling pathway; and what additional TCF/β-catenin target genes are relevant to tumorigenesis.

teins in the etiology of colon carcinoma and melanoma (Korinek et al. 1997; Morin et al. 1997; Rubinfeld et al. 1997). Mutations of either β-catenin or APC have the same effect on β-catenin stability and TCF transactivation, consistent with the notion that both genes act in the same signaling pathway (Sparks et al. 1998). These results raise the interesting possibility that β-catenin can act as an oncogene, a notion that Wong et al. (1998) have tested recently by expressing a mutated, protease-resistant form of β-catenin in the small intestine of mice. Although an increase in proliferation and apoptosis of crypt cells was observed, β-catenin appeared not to be sufficient to induce tumor formation (Wong et al. 1998).

The recent identification of *c-MYC* as a direct target gene of TCF/β-catenin–mediated transactivation has added new, important information as to how TCF/β-catenin signaling may affect tumor-cell proliferation (He et al. 1998). By means of serial analysis of gene expression (SAGE), *c-MYC* was identified as a target for APC function in colon cells. Cells without a functional APC gene exhibited high c-MYC expression, whereas reexpression of a functional APC protein resulted in down-regulation of c-MYC. The findings demonstrate that c-MYC activation is a direct consequence of APC loss and, therefore, could induce tumor-cell proliferation.

Finally, it is conceivable that the transcriptional activity of each TCF/LEF-1 family member will depend on both the particular family member in whom the gene is expressed and the cellular context. Thus, each TCF may modulate different target genes in different cell types or in varying combinations with other transcription factors (Hsu et al. 1998).

The notion that cadherin-mediated cell adhesion might transduce signals to the nucleus raises the intriguing possibility that changes in cell adhesion may modulate gene expression and, thus, cell fate. Indeed, the experimental findings that gain of E-cadherin function is able to block the transition from adenoma to carcinoma and that loss of cadherin-mediated cell adhesion can induce tumor-cell invasion (Perl et al. 1998) indicate that cell adhesion-mediated signals may govern progression from adenoma to carcinoma during carcinogenesis. Whether similar signals may govern complete genetic programs during nonpathological conditions, such as mesenchymal-epithelial conversion during embryonic development, remains unclear.

The frequent loss of E-cadherin–mediated cell adhe-

sion in epithelial cancers, together with its function as a repressor of tumor progression, nominates E-cadherin as an important tumor-suppressor gene. However, despite the significant progress in the field, the exact molecular mechanisms underlying E-cadherin–mediated tumor suppression remains unknown. Future research will have to address whether E-cadherin exerts its tumor-suppressing function through its cell-cell adhesion capabilities or through potential downstream signaling pathways.

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