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A Role for Cadherins in Cellular Signaling and Differentiation

Karen A. Knudsen,[2] Christy Frankowski,[1] Keith R. Johnson,[1] and Margaret J. Wheelock[1]*

[1]Department of Biology, University of Toledo, Toledo, OH 43606
[2]Lankenau Medical Research Center, Wynnewood, PA 19096

Abstract Cadherins form a family of cell-cell adhesion proteins that are critical to normal embryonic development. Expression of the various family members is regulated in a complex pattern during embryogenesis. Both reduced and inappropriate expression of cadherins have been associated with abnormal tissue formation in embryos and tumorigenesis in mature organisms. Evidence is accumulating that signals unique to individual members of the cadherin family, as well as signals common to multiple cadherins, contribute to the differentiated phenotype of various cell types. While a complete understanding of the regulation of cadherin expression of the molecular nature of intracellular signaling downstream of cadherin adhesion is essential to an understanding of embryogenesis and tumorigenesis, our knowledge in both areas is inadequate. Clearly, elucidating the factors and conditions that regulate cadherin expression and defining the signaling pathways activated by cadherins are frontiers for future research. J. Cell. Biochem. Suppls. 30/31:168–176, 1998. © 1998 Wiley-Liss, Inc.

Key words: cadherin; catenin; differentiation

Classical cadherins are important morphoregulatory molecules whose expression is precisely controlled in time and space during embryogenesis as they participate in the formation of organs and tissues [reviewed in Gumbiner, 1996]. Their importance to development is evidenced by embryonic lethality or abnormal tissue maturation in mice null for specific cadherins [reviewed in Hynes, 1996]. Moreover, altered expression or function of cadherins is implicated in the progression of cancer [reviewed in Takeichi, 1993].

Cadherins comprise a family of Ca^{2+}-dependent transmembrane glycoproteins that specifically self-associate through their extracellular domains, providing cellular recognition. The cadherin intracellular domain interacts with several proteins collectively called catenins that link cadherins to the actin cytoskeleton [reviewed in Wheelock et al., 1996]. This linkage is required for full cadherin adhesive activity. Either β-catenin or plakoglobin binds directly to the cadherin and to α-catenin, while α-catenin links directly and indirectly to actin. Their ability to self-associate and link to the actin cytoskeleton simultaneously enables cadherins to mediate both the cell recognition required for cell sorting and the strong cell-cell adhesion needed to form tissues. A model of cadherin linkage to the cytoskeleton is presented in Figure 1.

In addition to its structural role in the adherens junction, β-catenin also is found in the cytosol, where it interacts with other proteins and is an integral part of the Wnt/Wingless signaling pathway [reviewed in Cadigan and Nusse, 1997]. The cytoplasmic level of β-catenin is regulated in a complex fashion by cadherins, kinases, the APC tumor suppressor protein, and axin. Tight regulation of the level of cytoplasmic β-catenin is important, since it can interact with members of the high mobility group family of transcription factors, such as LEF-1, and alter transcription and cell fate [reviewed in Bienz, 1998]. Because of its role in both cadherin-mediated cell adhesion and transcriptional regulation, β-catenin is in a key position to coordinate morphogenesis and cellular differentiation.

There is ample evidence establishing both morphoregulatory and signaling roles for

*Correspondence to: Margaret J. Wheelock, Department of Biology, University of Toledo, Toledo, OH 43606.
E-mail: mwheelo@uoft02.utoledo.edu

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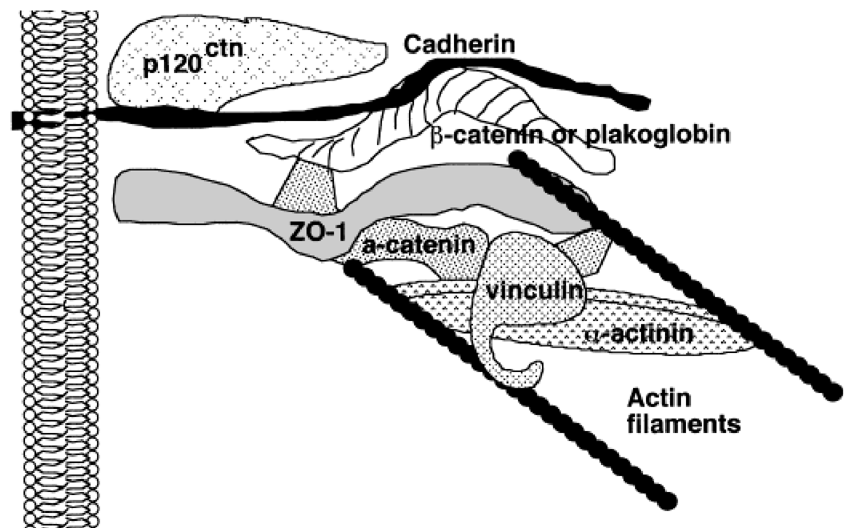


Fig. 1. Model for the linkage of cadherins to the actin cytoskeleton, based on a compilation of published information. The cadherin cytoplasmic domain interacts with either α-catenin or plakoglobin, which interacts with α-catenin. α-Catenin interacts with α-actinin, vinculin, ZO-1, and actin filaments. ZO-1, α-actinin and vinculin also interact with actin filaments, and vinculin interacts with α-actinin. P120^{ctn} binds directly to the cadherin and, although it has not been implicated in linkage to the cytoskeleton, it has been suggested to regulate the interaction of the complex with the cytoskeleton.

β-catenin and abundant information that cadherins are adhesion molecules and important morphoregulators. By contrast, a role for cadherins as signaling molecules is less well established, although evidence is growing that they are more than just biological glues. A number of studies indicate that cadherins play an active role in regulating cellular differentiation. How cadherins affect differentiation and what signaling pathway(s) connect cadherin activity at the membrane to gene transcription in the nucleus represent a scientific frontier.

CADHERINS AND CELLULAR DIFFERENTIATION

Several lines of evidence using function perturbing antibodies, forced expression of exogenous protein, or genetic manipulation indicate that cadherins play an important role in cellular differentiation. The phenotype of a cell can be altered by the loss or gain of cadherin. In cultures of epiblast cells from primitive streak-stage mouse embryos, treatment with function perturbing anti-E-cadherin antibodies causes the epithelial epiblast cells to become flatter, fibroblast-like, and migratory [Burdsal et al., 1993]. In addition, the antibody-treated cells cease to express an epiblast marker (SSEA-1); downregulate E-cadherin; upregulate vimentin, a marker of primitive streak mesoderm; and assume cell-matrix adhesion properties characteristic of mesodermal cells. In other words, the loss of functional E-cadherin alters cellular differentiation and shifts the cells from an epithelial to mesenchymal phenotype.

Cultured epiblast cells isolated from primitive streak-stage chick embryos, undergo an E-cadherin to N-cadherin conversion, similar to epiblast cells entering the primitive streak. N-cadherin, but not E-cadherin, expressing cells differentiate into skeletal muscle and function perturbing antibodies to N-cadherin block their differentiation, suggesting that N-cadherin promotes skeletal muscle differentiation [George-Weinstein et al., 1997]. In agreement, forced expression of N-cadherin in cadherin-negative skeletal myogenic cells enhances their differentiation [Redfield et al., 1997]. Moreover, disrupting cadherin function in 2- to 4-cell *Xenopus* embryos using a dominant negative N-cadherin inhibits MyoD expression [Holt et al., 1994]. Adhesion mediated by N-cadherin also appears to affect the differentiation of cardiac muscle cells. Treatment of early chick embryos with function perturbing anti-N-cadherin antibodies inhibits both heart morphogenesis and differentiation of the cardiomyocytes [Linask et al., 1997]. Similarly, treatment of precardiac mesoderm cells in vitro inhibits cardiomyocyte differentiation [Imanaka-Yoshida et al., 1998].

Interestingly, cadherins other than N-cadherin also appear be able to support muscle differ-entiation. Somite cells isolated from N-cadherin-null mice before death at midgestation differentiate into skeletal muscle in vitro, but express another cadherin, perhaps cadherin-11 [Radice et al., 1997a]. In addition, R-cadherin is expressed by skeletal muscle and its forced expression in E-cadherin-null embryonic stem cells results in the formation of stri-

ated muscle in teratomas in vivo [Rosenberg et al., 1997]. Moreover, forced expression of E-cadherin in the myogenic BHK cell line enhances their differentiation to skeletal muscle [Redfield et al., 1997]. Considering the essential role N-cadherin plays in strong cell-cell interactions in the heart, it was surprising that the N-cadherin-null mouse formed a recognizable heart, albeit with a disorganized myocardium [Radice et al., 1997a]. However, as with skeletal muscle, it is possible that alternative adhesion molecules can compensate for a chronic loss of N-cadherin. Thus, it is likely that similar intracellular signaling events can arise from adhesion mediated by multiple members of the cadherin family. The result of such signaling likely depends on the cell type and its unique internal and external environments.

Muscle is not the only cell type whose differentiation is affected by cadherin mediated adhesion. Function perturbing anti-E-cadherin antibodies disrupt thymocyte differentiation [Müller et al., 1997] and maturation of the erythroid lineage [Armeanu et al., 1995], suggesting that E-cadherin is involved in differentiation of these cells. In embryonic stem cells that are negative for E-cadherin, forced expression of E-cadherin results exclusively in the formation of epithelia, whereas forced expression of N-cadherin results in neuroepithelium and cartilage [Larue et al., 1996] implying that, in these cells, different members of the cadherin family send unique signals.

Experiments that make use of transgenic mice illustrate the importance of cadherin function in regulating the differentiation state of intact tissues. Targeted inhibition of E-cadherin function in the mouse intestinal epithelium by expression of a dominant negative cadherin results in loss of the differentiated polarized phenotype and precocious apoptosis of enterocytes [Hermiston and Gordon, 1995]. Although a precise function has not been ascribed to P-cadherin, global loss of this protein in mice results in precocious mammary gland development [Radice et al., 1997b], suggesting that P-cadherin plays a role in maintaining the undifferentiated state in this tissue.

Skin is another tissue whose differentiation is affected by cadherin activity. Both E-cadherin and P-cadherin are expressed by the keratinocytes of the epidermis. Keratinocyte cultures have been used as a model system to investigate the role of cadherins in epidermal differentiation. Keratinocytes propagated in calcium-deficient medium exhibit minimal cell-cell interactions and express the phenotype of undifferentiated basal cells. Upon addition of calcium, the cells organize junctional complexes, express differentiation markers, and begin to stratify. Function-perturbing antibodies against the cadherins have been used to demonstrate that these molecules play critical roles in the formation of adherens junctions and desmosomes, the reorganization of the cytoskeleton and stratification, and the expression of differentiation-specific markers [reviewed in Jensen and Wheelock, 1996].

Similar to keratinocytes of the skin, squamous epithelial cells of the oral cavity express both E-cadherin and P-cadherin. These cells also form a multi-layered epithelium and express specific differentiation markers as they stratify. Some oral squamous cell carcinomas express N-cadherin rather than E-cadherin or P-cadherin. In cultures derived from these tumors, the cells no longer display an epithelial morphology, but rather appear as fibroblastic cells. Indeed, forced expression of N-cadherin in oral squamous epithelial cells results in decreased E-cadherin expression and an epithelial to mesenchyme transition that is characterized by increased cell motility and invasion [Islam et al. 1996]. One interpretation of these data is that oral squamous epithelial cells have cadherin-specific signal transduction pathways. E-cadherin mediates a signal to maintain an epithelial cell phenotype, whereas N-cadherin signals the cell to convert to the motile, fibroblastic phenotype of a mesenchymal cell.

What role cadherins play in cellular motility is a complex question. Forced expression of E-cadherin in rat astrocytoma cells [Chen et al., 1997] and increased N-cadherin in skeletal muscle cells [Huttenlocher et al., 1998] suppress motility, whereas forced expression of E-cadherin in other cells does not suppress motility. Our studies of oral squamous carcinoma cells suggest that an increase in cell motility not only may be due to decreased expression of E-cadherin but also may depend on the expression of another cadherin, such as N-cadherin [Islam et al., 1996]. To further analyze the role cadherins play in cell motility, we employed the A431D cadherin-negative cell line. The A431D cell line was derived from the A431 cervical epidermoid carcinoma cell line by continuous treatment with dexamethasone which

permanently shut off expression of both E-cadherin and P-cadherin [Lewis et al., 1997]. A431D cells were transfected with E-cadherin, P-cadherin, or N-cadherin and motility rates of the cells were compared to one another and to the cadherin-negative A431D cells using the transwell motility assay. Cells traversing the filter during a 24-h period were counted and the numbers plotted in Figure 2. N-cadherin expressing cells were significantly more motile than E- or P-cadherin expressing cells or the parent A431D cells. These experiments indicate that expression of N-cadherin promotes cell motility, at least in the context of the A431D cells.

Together, the results of the above studies indicate that cadherin mediated adhesion at the cell surface affects gene expression and that cadherins play important roles in regulating cellular differentiation. Moreover, the work suggests that both signals unique to a particular cadherin family member and signals common to multiple cadherins will be found in cells. Which signaling pathways are active is likely to depend on the cell type and perhaps its stage of development. For cells that respond uniquely to a particular cadherin, abnormal expression of an inappropriate cadherin may have dire consequences, both for developing embryos and for the progression of cancer.

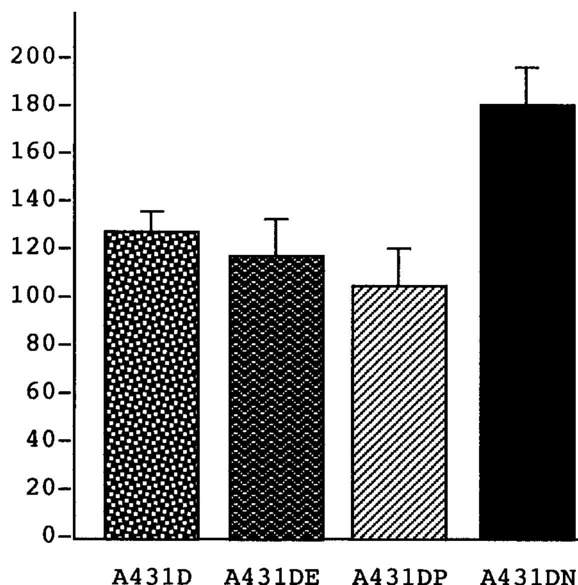


Fig. 2. N-cadherin promotes cell motility in A431D cells. Equal numbers of A431D cells and cells transfected with E-cadherin, P-cadherin or N-cadherin were plated in transwell motility chambers. After 24 h, the filters were processed and the number of cells transversing the filter in 10 random fields was averaged. Assays were performed in triplicate and the data was plotted as the number of motile cells. The standard error of each data set is indicated. Both E-cadherin and P-cadherin slightly decreased the motility of A431D cells, while N-cadherin significantly increased the motility of these cells.

CADHERIN REGULATION

Given the great importance of cadherins to both morphogenesis and cellular differentiation, regulation of their activity becomes critical. There is evidence in the literature that cadherin activity can be regulated in multiple ways that may reflect the cell type and local environment. One way that cadherin function can be regulated is at the level of protein expression and due to space restrictions this will be our focus here. However, it is important to keep in mind that there are additional ways that cadherin function might be modulated, including composition of the cadherin/catenin complex, linkage to the cytoskeleton, phosphorylation state of the cadherin and/or catenins, clustering of the cadherin in the plane of the membrane, presence of a mutated nonfunctional cadherin, proteolytic processing of the cadherin to its active form, proteolytic cleavage of the extracellular cadherin domain, and influence by other membrane proteins or proteoglycans.

Cadherins are expressed in precise and dynamic ways during embryonic development. For example, E-cadherin is downregulated and N-cadherin upregulated as epiblast cells enter the primitive streak to form mesoderm during gastrulation [Takeichi, 1988]. Another example is presented in an extensive study by Cho et al. [1998] defining the changing pattern of cadherin expression during development of the renal epithelium. As these cells undergo a mesenchyme to epithelial transition, the newly formed epithelium switches from expression of cadherin 11 to spatially restricted expression of E-cadherin or cadherin-6. A third example is seen in the developing central nervous system (CNS), where a number of cadherins are expressed in restricted patterns and have been proposed to function in the maintenance of segmentation and in the gradual emergence of functional structures in this tissue [reviewed in Redies and Takeichi, 1996]. Importantly, the level of expression or the particular cadherin expressed can change in tumors compared to normal tissue and may contribute to the tumorigenic characteristics of the cells including in-

creased growth rates, increased cell motility and decreased cell-cell interactions [reviewed in Takeichi, 1993]. Thus, understanding how the expression of cadherins is regulated becomes critical to our understanding of the cellular changes that occur as organisms undergo normal developmental events, as well as those that occur during tumorigenesis. Although there is some information on the regulatory elements present in cadherin genes, this is an area of incomplete knowledge.

The upstream regulatory regions of mouse E- and P-cadherin and chicken N-cadherin genes contain putative Ap2 and Sp1 binding sites [Ringwald et al., 1991; Behrens et al., 1991; Faraldo and Cano, 1993; Li et al., 1997], indicating common themes for the regulation of cadherin expression. The mouse E-cadherin gene contains an E-pal palindromic sequence not found in the P-cadherin gene [Behrens et al., 1991; Faraldo and Cano, 1993]. The E-pal sequence is composed of E boxes, which are also present in the human E-cadherin promoter [Giroldi et al., 1997]. E boxes are recognition sites for the basic helix-loop-helix family of transcription factors and are implicated in the downregulation of E-cadherin in cancer cells [Giroldi et al., 1997]. Also in the upstream region of the E-cadherin gene are putative glucocorticoid receptor and progesterone receptor binding sites [Ringwald et al., 1991].

Work on the L-CAM (chicken E-cadherin) gene has revealed that in addition to upstream promoter sequences, *cis*-acting sequences within the gene itself control spatiotemporal expression of the protein [Sorkin et al., 1993]. An enhancer region within the second intron contains putative binding sites for the transcription factors Ap2, Sp1, and E2A, as well as a consensus binding site for hepatocyte nuclear factor-1 (HNF-1), a liver-enriched POU (Pit-Oct-Unc)-homeodomain transcription factor. In studies with chloramphenicol acetyltransferase (CAT) gene reporter constructs containing the L-CAM promoter and second intron enhancer, CAT activity was activated by *HoxD9* and HNF-1, both of which appeared to act through the HNF-1 binding site [Goomer et al., 1994]. This work suggests that homeobox-containing genes play a role in regulating the spatiotemporal expression of cadherins. In addition, other laboratories have reported the presence of enhancer elements in introns of cadherin genes. Hennig et al. [1996] showed that there are AP-2 sites in the first intron of mouse E-cadherin, while Hatta and Takeichi [1994] reported enhancer elements in intron two of mouse P-cadherin.

Like that of mouse E-cadherin, correct spatiotemporal expression of chicken N-cadherin appears to involve sequences outside of the immediate 5′ upstream region of the gene [Li et al., 1997]. The sequences immediately 5′ of the gene contain a GC rich region with several Sp1 and Ap2 consensus binding motifs, and a high proportion of CpG dinucleotides, but no CCAAT or TATA boxes [Li et al., 1997]. Several additional potential regulatory elements, including E boxes, are found in the upstream 5′ region of the gene.

There is evidence that transcription factors can alter the expression of cadherins. For example, mice carrying a mutation in the *Pax6* gene have altered expression of R-cadherin which causes decreased aggregation of cells in the developing forebrain resulting in a malformed cerebral cortex and failure of the mice to develop eyes and a nasal cavity [Stoykova et al., 1997]. By contrast, mutations in the homeobox gene *Hoxa-4* have no effect on the expression of N-cadherin [Packer et al., 1997]. There also is evidence that β-catenin complexed with LEF-1 can bind to the E-cadherin promoter and thus may be involved in regulating the expression of this gene [reviewed in Bienz 1998].

In some cases, extracellular influences have been implicated in the regulation of cadherin expression. For example, two lines of evidence suggest that integrin mediated cell-matrix adhesion can affect cadherin expression. Forced expression of the α5 integrin subunit in primary quail myoblasts upregulates N-cadherin expression resulting in contact inhibition of cell migration and suggesting that integrins and cadherins coordinately regulate cell motility [Huttenlocher et al., 1998]. In epithelial cells, overexpression of integrin-linked kinase (ILK), which binds to the integrin β1 subunit, simultaneously stimulates fibronectin matrix assembly and downregulates E-cadherin [Wu et al., 1998]. In addition, expression of ILK by epithelial cells results in increased cell motility, translocation of β-catenin to the nucleus, and transcriptional activation of a β-catenin/LEF-1 complex [Novak et al., 1998]. These studies, together with studies implicating cadherins in the regulation of integrin expression [Hodivala and Watt, 1994], provide evidence that there is

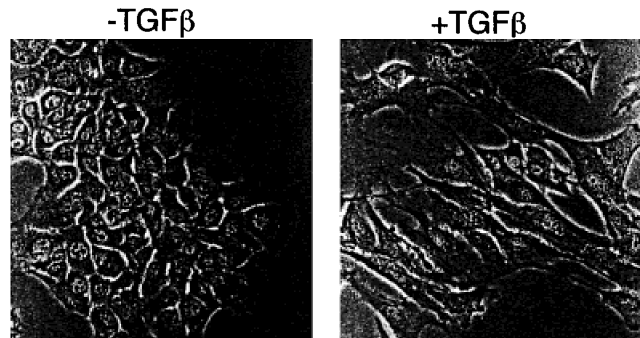
cross-talk between cell-matrix and cell-cell interactions and stimulates interest in the cellular signaling pathways involved in this cross-talk.

There is also growing evidence that hormones, growth factors, and other secreted factors can alter cadherin expression. For example, in mice bearing mutated forms of the Wnt-1 signaling protein, E-cadherin is abnormally expressed [Shimamura et al., 1994], possibly because of the interaction of β-catenin with LEF-1. In addition, insulin has been shown to downregulate N-cadherin during retinal development in vitro [Roark et al., 1992], and other growth factors, such as hepatocyte growth factor (HGF), have been shown to reduce cadherin levels, perhaps by decreasing the stability of the protein.

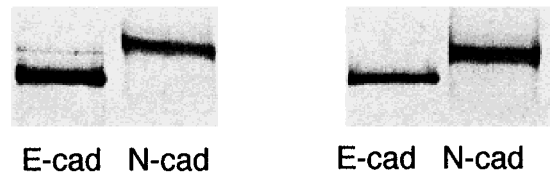
Transforming growth factor-β (TGF-β) is a well-studied growth and differentiation factor that signals a variety of biological effects depending on the cell type. TGF-β is known to increase the expression of integrins and extracellular matrix proteins. It also can alter cadherin/catenin expression. Mouse mammary epithelial cells (NMuMG) treated with TGF-β undergo a striking epithelial to mesenchyme transition that is accompanied by a decrease in E-cadherin [Miettinen et al., 1994](Fig. 3A,B). In addition, TGF-β treatment of NMuMG cells increases N-cadherin expression (Fig. 3B), significantly shifting the E-cadherin/N-cadherin ratio in the cells. This is particularly interesting in light of the experiments discussed above, showing that N-cadherin expression by squamous epithelial cells results in increased cell motility [Islam et al., 1996](Fig. 2).

Interestingly, TGF-β treatment of NMuMG cells also increases plakoglobin expression and induces a striking increase in the level of plakoglobin associated with N-cadherin in co-immunoprecipitation experiments (Fig. 3C). The composition of the cadherin/catenin complex may influence cadherin activity, which in turn may influence the phenotype of the cell. β-Catenin and plakoglobin appear to be able to substitute for one another in the formation of the adherens junction (Fig. 1). However, they are not identical to one another; our laboratory has shown that junctions composed of E-cadherin/plakoglobin complexes are capable of inducing desmosome formation in epithelial cells,

A. Phase microscopy



B. Immunoblot analysis



C. Association of plakoglobin with cadherins

Fig. 3. Mouse mammary epithelial cells undergo an epithelial to mesenchyme transition when treated with transforming growth factor-βTGF-β. **A:** Subconfluent monolayers of NMuMG cells were treated with TGF-β for 24 h and photographed with a Zeiss Axiophot microscope. The TGF-β-treated cells show a scattered fibroblastic phenotype compared with the epithelial-like nontreated cells. **B:** Treated and nontreated cultures were extracted with nonionic detergent. Equal amounts of protein were resolved by 7% SDS-PAGE, transferred to nitrocellulose and immunoblotted with antibodies against E-cadherin or N-cadherin. The TGF-β-treated cells express decreased levels of E-cadherin and increased levels of N-cadherin. **C:** Protein extracts were immunoprecipitated with antibodies against E-cadherin or N-cadherin, resolved by 7% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotted with antibodies against plakoglobin. Significantly more plakoglobin is associated with N-cadherin in the TGF-β-treated cells.

whereas junctions composed of E-cadherin/β-catenin complexes are not [Lewis et al., 1997]. In addition, Simcha et al. [1998] have shown that β-catenin and plakoglobin have different properties with respect to nuclear localization and transactivation potential, suggesting that even a small change in expression or availability of one of these proteins may dramatically impact the phenotype or behavior of the cell. Thus, the change in cadherin expression, together with the change in the composition of the adherens junction, may contribute to the transition from an epithelial to fibroblastic morphology.

In some cells, cadherin expression appears to be influenced by steroid hormones. N-cadherin expression by granulosa cells isolated from rat ovaries is stimulated by 17β-estradiol and follicle-stimulating hormone (FSH)[reviewed in Blaschuk et al., 1995]. Likewise, FSH plus testosterone increases N-cadherin in Sertoli cells from the testis [Perryman et al., 1996]. In vivo, both testosterone (which is converted to 17β-estradiol) and estradiol stimulate N-cadherin expression in the testis [reviewed in Blaschuk et al., 1995]. Studies from our laboratory have demonstrated that long-term treatment of the A431 epidermoid carcinoma cell line with dexa-

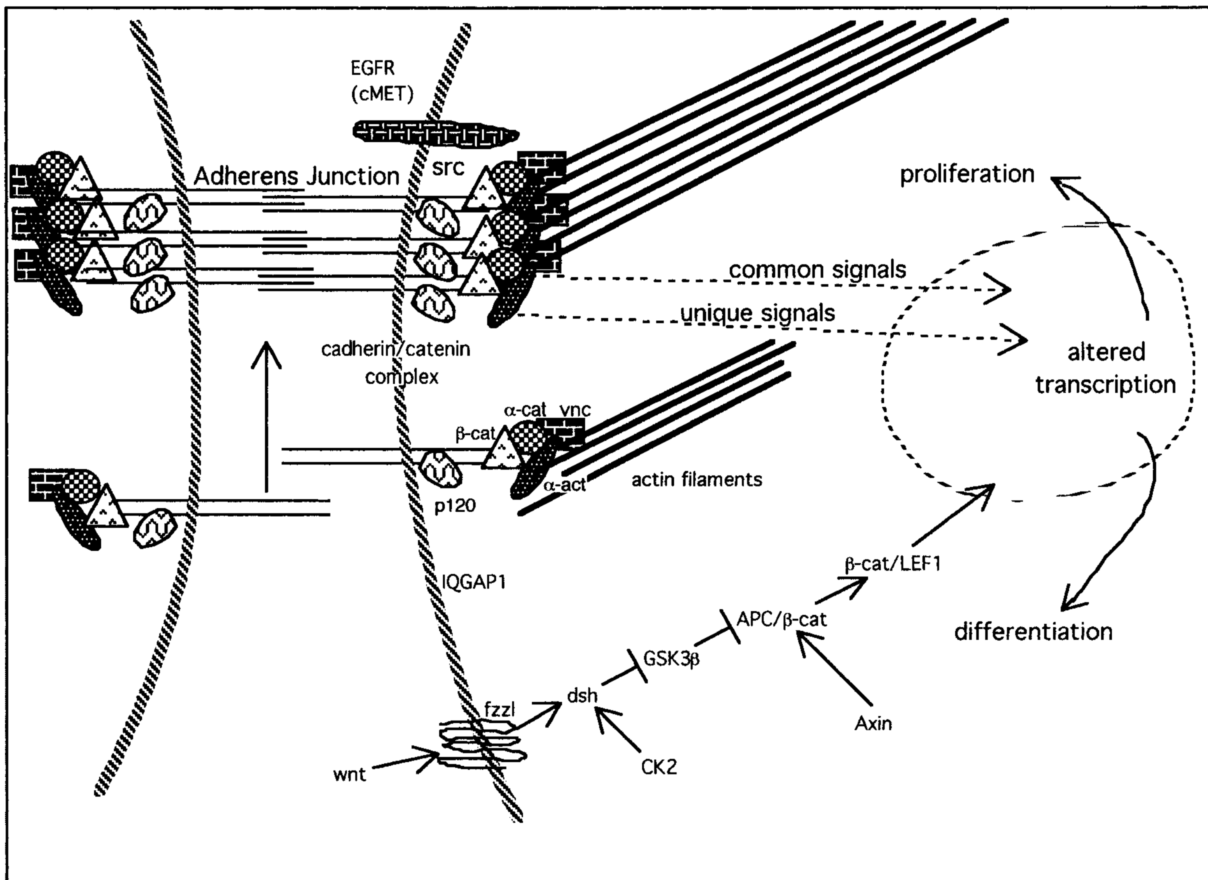


Fig. 4. Model for known and postulated intracellular signaling arising from cadherins and catenins. The model represents both a compilation of published information and conjecture. Cadherins form a complex with $p120^{ctn}$ (p120), β-catenin (β-cat) or plakoglobin, and α-catenin (α-cat) that is linked to the actin cytoskeleton both directly and indirectly through α-actinin (α-act) and vinculin (vnc). The cadherins self-associate in an anti-parallel fashion to promote cell-cell adhesion and are assembled in the plane of the plasma membrane into adherens junctions. The activity of cytoplasmic kinases (e.g., src) and membrane bound receptor kinases (e.g., EGFR and cMET), as well as other proteins, such as IQGAP1, modulate cadherin activity and its linkage to the actin cytoskeleton. Based on our work and that of others, we propose that both signals unique to individual members of the cadherin family and signals common to multiple cadherins arise from the cadherin/catenin complex, and that the presence of these signaling pathways varies according to cell type and developmental state. The molecular nature of the signaling pathways downstream of cadherin/catenin complexes is unknown and represents an underexplored area of research. Signaling from the cadherin/catenin complex is predicted to be coordinated with signaling arising from other modifiers of cellular phenotype, in particular, β-catenin, in the context of the Wnt/Wingless pathway.

methasone results in permanent downregulation of both E-cadherin and P-cadherin [Lewis et al., 1997]. Since A431 cells were derived from a cervical carcinoma and may be hormone responsive, our data present further evidence that steriod hormones can play a role in regulating cadherin expression in cells derived from some tissues.

It is clear from the above examples that the local environment can dramatically or subtly alter cadherin levels in cells. In turn, cadherin-mediated adhesion, together with cell-matrix adhesion, hormones, cytokines, and other secreted factors, can influence cellular behavior in ways that are certain to have an impact on morphogenesis and cell differentiation in embryos or tumors. Figure 4 summarizes our current understanding of the ways by which cadherins, catenins and their respective signaling pathways may influence cellular proliferation and differentiation.

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