# CADHERINS IN ONCOLOGY

THERAPEUTIC OPPORTUNITIES THROUGH CADHERIN TARGETING

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## THE CADHERIN SUPERFAMILY

Cadherins are cell adhesion molecules crucial to the orderly association of cells in tissues, organs and organisms. Cadherins consist of an extracellular domain, a transmembrane region and, in most cases, a cytoplasmic domain. The presence of at least one "cadherin repeat" in the extracellular domain defines a protein as a cadherin. The cadherin superfamily is large and diverse, consisting of more than 100 distinct but related cell surface glycoproteins. Cadherins are named according to the tissues where they were first identified, e.g., Epithelial (E)-cadherin, Neural (N)-cadherin, Vascular Endothelial (VE)-cadherin and Osteoblast (OB)-cadherin[1].

**Phylogenetic Tree of the Cadherin Superfamily**

The members of the cadherin superfamily are grouped according to the similarity by comparing the amino acids of the first extracellular domain.

Human cadherin sequences are used except where indicated as Bs, *Botryllus schlosseri*; Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster*; Mm, *Mus musculus*; Rn, *Rattus norvegicus*; Xl, *Xenopus laevis*.

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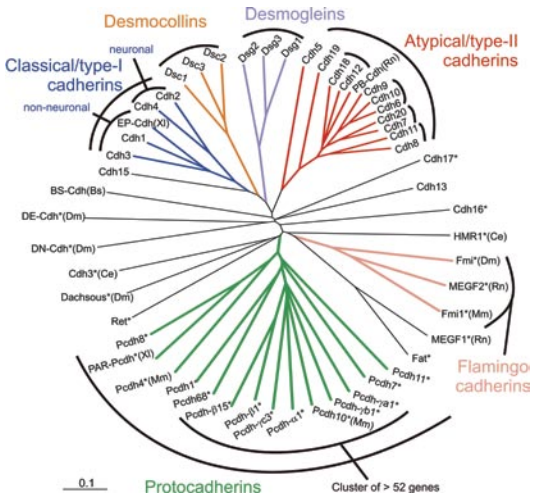


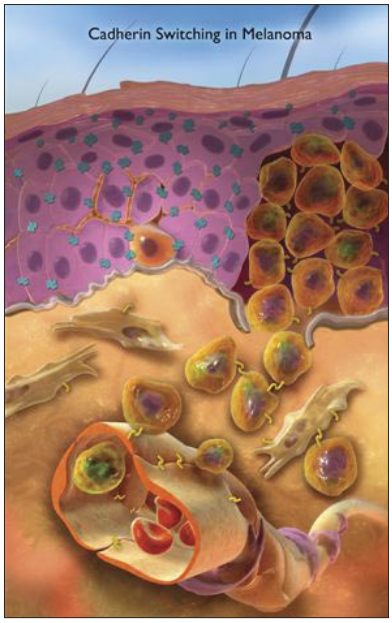

## CADHERINS AS INTEGRATORS OF CELL ADHESION AND SIGNALING

Cadherins on adjacent cells interact with one another to mediate calcium-dependent cell-cell adhesion. Cadherin-mediated adhesion is predominantly homophilic, meaning, for example, that E-cadherin on one cell binds to E-cadherin on an adjacent cell[2]. Cadherin-mediated adhesion is essential for the establishment of tight cell-cell adhesion, and cadherins define the adhesive specificities of cells[3].

The influence of cadherins extends beyond simple cohesiveness. Cadherins are a critical part of signaling pathways[4]. Cross talk between cadherins and components of intracellular signaling complexes regulates cellular behaviors including cell stability and movement, and cell growth, differentiation and cell survival[5–11]. Information transmitted by cell-cell contacts is integrated with cell-matrix and growth factor signaling, to define cell phenotype and function. Loss or altered expression of cadherins has major consequences on many aspects of biology and disease progression, including tumorigenesis[3, 4].

Direct interaction between cadherins and growth factor receptors leads to cadherin-mediated ligand-independent activation of signaling pathways. An example is the interaction between N-cadherin and fibroblast growth factor receptor. This phenomenon occurs during the outgrowth of neurites from neurons[5]. A similar pathway may be subverted in N-cadherin-expressing cancer cells where migratory

properties are advantageous for escape from the primary tumor mass[6, 7]. Cadherin-mediated adhesion is also important for the regulation of the intracellular signals that control apoptosis. For example, cell culture studies have shown that disruption of cadherin-based adhesion regulates survival of endothelial cells, ovarian cells, and neurons[8–10]. In endothelial cells, association between VE-cadherin and vascular endothelial growth factor (VEGF) receptor-2 is required for VEGF-dependent cell survival signaling[11]. Thus, cadherins play a role in cross talk with both ligand-dependent and ligand-independent intracellular signaling pathways.




**Cadherin Switching in Melanoma**

E-cadherin (blue) is expressed by the keratinocytes (normal skin cells) and melanocytes (pigment cells) producing anchoring of the melanocytes in the skin. N-cadherin (yellow) expression is up-regulated and E-cadherin expression is down-regulated (cadherin switch), when the melanocyte starts to become a malignant melanoma cell. The loss of E-cadherin diminishes adhesion of the melanoma cell to keratinocytes, leading to uncontrolled melanoma cell proliferation and the development of an invasive melanoma tumor. N-cadherin expression may also facilitate the progression of melanoma by enabling melanoma cells to invade the dermis and microvasculature and adhere to N-cadherin expressed on fibroblasts and endothelial cells.

## CADHERIN SWITCHING

The expression of a given cadherin on a cell is not necessarily fixed. Its expression may be down-regulated, while the expression of another type of cadherin may be up-regulated. This phenomenon is known as cadherin switching. Cadherin switching is a critical and normal process that occurs during embryonic development when the cadherin expression profile of a cell is changed, reflecting a new function[12].

Cancer progression is also often characterized by one or more cadherin switches. Early-stage, well-differentiated carcinomas express E-cadherin (like the epithelial cells from which they are derived). E-cadherin functions to establish and maintain the integrity of the polarized, tightly adhesive epithelial monolayer and the organization seen in the early tumor. However, poorly differentiated, highly invasive carcinomas frequently lose E-cadherin expression[13]. Cadherin switching in tumors may take the form of the "epithelial to mesenchymal transition" in which cells that express E-cadherin switch to mesenchymal cadherins such as N-cadherin or OB-cadherin. This change in cadherin expression, to N- and OB-cadherin, causes the epithelial cells to lose their tightly adherent, polarized and well-defined shape and to become loosely adherent, flattened and migratory[4, 6, 7, 12, 13].

Further, such cadherin switching promotes properties such as dedifferentiation, local invasion and metastasis, leading to a poor prognosis[12–15].

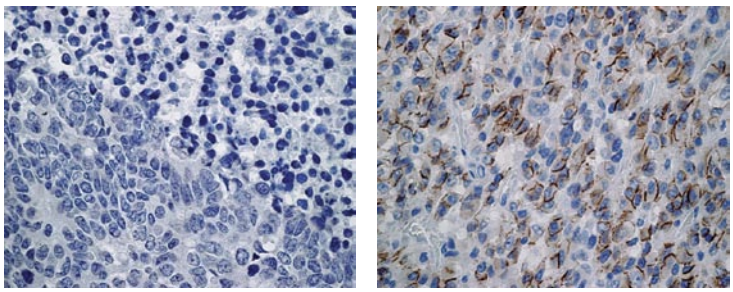
Cadherin switching is well studied in the melanocyte to melanoma cell transition. E-cadherin is expressed by keratinocytes (normal skin cells) and melanocytes (pigment cells) producing anchoring of the melanocytes in the skin[16]. N-cadherin expression is up-regulated and E-cadherin expression is down-regulated when the melanocyte starts to become a malignant melanoma cell. The loss of E-cadherin diminishes adhesion of the melanoma cell to keratinocytes, leading to uncontrolled melanoma cell proliferation and the development of an invasive melanoma tumor[16]. N-cadherin expression may also facilitate the progression of melanoma by enabling melanoma cells to invade the dermis and microvasculature and adhere to N-cadherin expressed on endothelial cells[16]. Similar cadherin switching is also noted in a wide variety of carcinomas—including those of breast, prostate, and bladder, among others[14, 15, 17, 18].

Secondary tumors (metastases) frequently express N- or OB-cadherin. In these circumstances, cadherins may function to promote adhesion of the tumor cells to a new secondary site. For example, OB-cadherin may promote adhesion to bone in bone metastases[15].

### N-cadherin Expression on Tumor Cells

Immunohistochemistry staining of tumor tissue (metastatic adenocarcinoma) with an anti-N-cadherin antibody (left). This sample is N-cadherin negative. The tumor does not express the molecular target N-cadherin.

Immunohistochemistry staining of tumor tissue (adrenocortical carcinoma) with an anti-N-cadherin antibody (right). This sample is N-cadherin positive. The brown color represents where N-cadherin is present on the cell surface.



## MULTIPLE CADHERINS REPRESENT TARGETS IN ONCOLOGY

Agents that target and inhibit cadherin function have the potential to attack the progression of cancer at several vulnerable points:

- **Direct targeting of cadherins expressed on cancer cells may disturb cadherin-mediated signaling, leading to apoptosis of cancer cells**
- **Cadherin inhibitors may exploit the structural weaknesses of the tumor vasculature, causing angiolysis and preventing tumor growth**
- **Inhibiting cadherin-mediated adhesion of tumor cells may block adhesion to secondary sites such as bone**

### N-CADHERIN

N-cadherin was one of the earliest cadherins to be discovered. It mediates cell-cell adhesion in a range of tissues such as brain, muscle and blood vessels. In certain cell types, including neurons and cancer cells, N-cadherin is important for promoting motility, via its interaction with the growth factor receptors such as fibroblast growth factor receptor[5–7]. N-cadherin also plays a central role in the microvasculature where it mediates adhesion between endothelial cells, as well as between endothelial cells and pericytes[19–22]. Mice that lack N-cadherin do not form blood vessels[23].

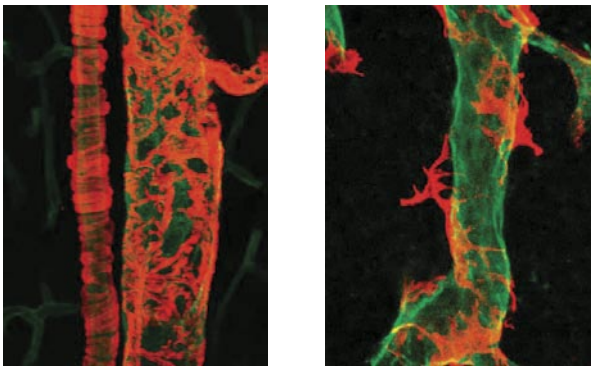
Normal capillaries (microvessels) are composed of two cellular components: endothelial cells and pericytes. The endothelial cells line the lumen of the microvessels, whereas the smooth muscle-like pericytes wrap themselves around the endothelium. N-cadherin mediates adhesion between these two cell types and plays a central role in maintaining stable vascular structure. Tumor blood vessels differ from normal blood vessels. They lack the cellular and structural components that are necessary to form stable vasculature. In particular, tumor microvessels are relatively deficient in pericytes, and those that are present adhere poorly to the endothelium[24–26]. The fragile nature of the tumor microvasculature renders tumor vessels more susceptible to N-cadherin antagonists. Targeting N-cadherin is therefore a promising approach to the development of potentially effective and selective tumor vascular targeting agents.



### Abnormal Tumor Vascular Structure

Pericytes in a healthy venule and capillary (left).
Pericytes (seen here in red) are a type of smooth muscle cells that encircle endothelial cells (seen here in green) of blood vessels and are critical for maintaining the vessels' structural integrity.

Pericytes in tumor blood vessels (right).
In tumors, pericytes (seen here in red) are structurally abnormal and poorly bound to endothelial cells (seen here in green). These defects lead to tumor blood vessels that are weak and more susceptible to rupture than healthy blood vessels.

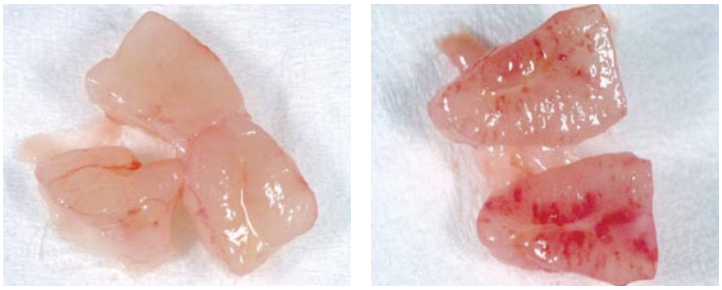


N-cadherin is a classical cadherin, containing the amino acid sequence Histidine-Alanine-Valine (HAV) in the active binding site. Peptides and peptidomimetics that mimic this binding site inhibit N-cadherin-dependent functions[27, 28]. Such agents are being explored in oncology to target the weakly adherent cells of the tumor vasculature, as well as N-cadherin expressed on poorly differentiated cancer cells.

Administration of N-cadherin antagonists to tumor-bearing animals leads to specific vascular disruption, including decreased adhesion between the endothelial cells and pericytes, rupture of the blood vessels (angiolysis), and disruption of blood supply to the tumor.

### Tumor Vascular Disruption Following N-cadherin Antagonism

Tumor tissue samples from animals treated intravenously with an N-cadherin antagonist (right), or its vehicle control (left). The treated tumor displays ruptured blood vessels, while the blood supply to the control tumor remains intact.

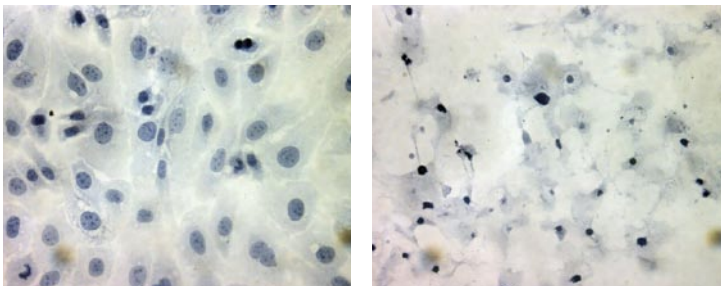


In cell culture studies, HAV peptides cause apoptosis of endothelial cells by disrupting cadherin-regulated cell survival signals[8].

### Apoptosis and N-cadherin Antagonism

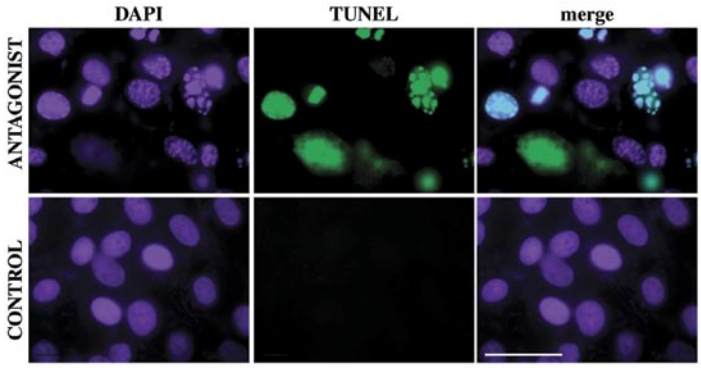
Treatment of confluent cultures of N-cadherin expressing SKOV3 cancer cells with N-cadherin antagonist causes extensive cell death (right) compared with untreated cells (left). Apoptosis was confirmed by TUNEL assay, induction of caspase-3 and alteration of genes expression associated with apoptosis. (Stained hematoxylin.)

Adherex expresses gratitude to Dr. Orest Blaschuk and Normand Lavoie, McGill University, for providing these images.





Induction of apoptosis as a therapeutic mechanism in response to the administration of N-cadherin antagonism is under investigation.




### Apoptosis of Vascular Endothelial Cells Treated with N-cadherin Antagonist

Treatment of confluent cultures of bovine capillary endothelial cells with N-cadherin antagonist (top) induces apoptosis, shown here by the fragmented DNA (blue) and TUNEL reactivity (green) compared with endothelial cells treated with a control compound (bottom).(Stained terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) and 4',6-Diamidino-2-phenylindole (DAPI).)

Reprinted from *Experimental Cell Research*, Vol 294, Erez N., Zamir E., Gour B.J., Blaschuk O.W. and Geiger, B., Induction of apoptosis in cultured endothelial cells by a cadherin antagonist peptide: involvement of fibroblast growth factor receptor-mediated signalling, 366–378, Copyright (2004), with permission from Elsevier. Adherex expresses gratitude to Dr. Benjamin Geiger and Noam Erez, Weizmann Institute, for providing these images.

Adherex is currently developing N-cadherin antagonist molecules in the preclinical and clinical settings.

## VE-CADHERIN

VE-cadherin (also known as cadherin-5) is a member of the Type II or atypical cadherin sub-family[1, 29]. VE-cadherin is a cell-cell adhesion molecule present in the endothelium of blood vessels and is responsible for maintaining vascular integrity and remodeling the vascular tree. Mice that lack this adhesion molecule form unstable blood vessels and die, highlighting the key role played by VE-cadherin in the vasculature[11]. Function-blocking antibodies to VE-cadherin disrupt endothelial cell adhesion, cause angiolysis and inhibit angiogenesis,[30–32].

Antagonists to VE-cadherin could be utilized in oncology as angiolytics (vascular targeting agents), antiangiogenics, or vascular permeability regulators.

VE-cadherin antagonists exert their angiolytic effect by targeting interactions between endothelial cells. As previously stated, the structural integrity of tumor microvessels differs from that of normal tissue vessels. Tumor vessels are fragile, leaky, lack pericyte coverage, and exhibit poor cell-cell adhesion[24–26]. The compromised cell-cell contacts between tumor endothelial cells makes tumor vessels susceptible to attack by VE-cadherin antagonists.

VE-cadherin-mediated cell contact also regulates angiogenesis by controlling endothelial cell adhesion, migration, proliferation, and survival. This is mediated in part by interaction at the cell surface between the VE-cadherin molecules and VEGF receptors[11]. VE-cadherin is also an important determinant of vascular permeability. Signals from growth factors such as VEGF lead to changes in the phosphorylation of VE-cadherin and associated catenins, weakening endothelial cell-cell adhesion and increasing vascular permeability[33]. By controlling VE-cadherin-mediated changes in vascular permeability, the delivery of chemotherapeutics to the tumor could potentially be regulated.

Adherex is currently developing VE-cadherin antagonists in the preclinical setting.

## OB-CADHERIN

OB-cadherin (also known as cadherin 11) is also classified as a Type II or atypical cadherin[1, 29]. OB-cadherin is located at the cell surface and mediates homophilic calcium-dependent cell-cell adhesion. OB-cadherin expression is a feature of loosely adherent cells such as those of mesenchymal tissues. It also mediates adhesion between osteoblasts (where it was originally identified)[34].

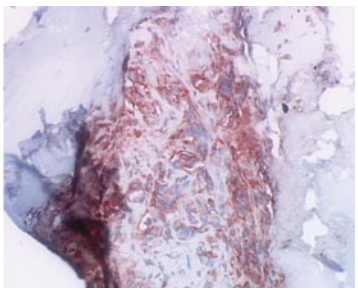
Accumulating evidence suggests that OB-cadherin plays a significant role in cancer. A well-characterized example is the progression of malignant carcinomas. Early-stage carcinomas do not express OB-cadherin, but express E-cadherin, like the epithelial cells from which they are derived. However, cadherin switching may occur, and OB-cadherin is up-regulated as the tumor dedifferentiates and facilitates metastatic dissemination by promoting motility of the tumor cells[15, 35].

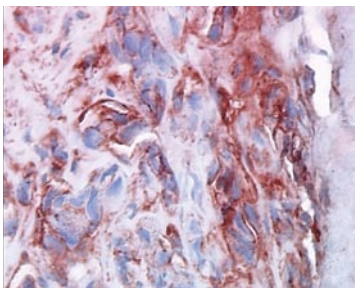
In the case of secondary tumor growth in bone, the presence of OB-cadherin on both cancer cells and osteoblasts may promote adhesion, survival and expansion of metastases.



**OB-cadherin Expression in Breast Metastases to Bone**

OB-cadherin is expressed in bone metastases. Samples of breast metastases taken from human tumors display OB-cadherin expression (brown) when stained with specific antibodies. (Stained anti-OB-cadherin (brown) and hematoxylin (blue)).





Adherex expresses gratitude to Dr. Stephen Byers and Dr. Carolyn Feltes, Georgetown University, for providing these images.

OB-cadherin-mediated cell-cell contact stimulates expression of VEGF family members[36]. VEGFs are stimulators of angiogenesis and lymphangiogenesis, processes that are important to the growth of primary tumors and their metastatic spread. *In vitro*, OB-cadherin antagonists block the up-regulation of VEGF growth factors and also inhibit OB-cadherin-dependent cell adhesion, migration, and invasion. The multifunctional roles of OB-cadherin in the progression of metastatic disease would seem to make this cadherin an ideal target for development of potential agents to inhibit tumor metastasis.

Adherex is currently investigating OB-cadherin antagonists in preclinical studies.

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*Leading the exploration of therapeutic opportunities through cadherin targeting*

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