CADHERIN EXPRESSION IN THE EYE

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SUMMARY

E(pithelial)-cadherin and N(eural)-cadherin are transmembrane cell-cell adhesion molecules, belonging to the subfamily of classical cadherins. The expression of E- and N-cadherin is spatiotemporally regulated and associated with a variety of normal morphogenetic events. The expression of E- and N- cadherin is also involved in carcinogenesis. E-cadherin functions as a tumor-suppressor. N-cadherin, however, is associated with cancer progression. The study of the expression pattern of E- and N-cadherin in the normal and tumorous eye is the aim of our research.

SAMENVATTING

E(pitheliaal)-cadherine en N(euronaal)-cadherine zijn transmembranaire cel-cel adhesie moleculen, behorend tot de subfamilie van de klassieke cadherines. E- en N-cadherine expressie verschilt in plaats en tijd en is geassocieerd met een reeks normale morfogenetische veranderingen. E- en N-cadherine spelen ook een rol in de carcinogenese. Het E-cadherine is een tumor-suppressor. Het N-cadherine daarentegen kan progressie van kanker in de hand werken. Ons onderzoek behelst de cadherine-expressie in het normale en tumorale oog.

RÉSUMÉ

La E(pitheliale)-cadhérine et la N(eurale)-cadhérine sont des molécules transmembranaires de l'adhésion intercellulaire appartenant à la famille des cadhérines classiques. La E- et la N-cadhérine sont exprimées de façon spatiotemporelle, et de ce fait elles sont impliquées dans de nombreuses étapes de la morphogénèse normale. La E- et la N-cadhérine participent aussi à la carcinogénèse. La E-cadhérine est un puissant suppresseur de l'invasion. La N-cadhérine, en revanche, peut favoriser la progression tumorale. L'optique de notre recherche est l'expression des cadhérines dans l'oeil normal et cancéreux.

KEY WORDS

cadherin, retinoblastoma

MOTS CLES

cadhérine, rétinoblastome

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INTRODUCTION

Cadherins are a superfamily of related transmembrane glycoproteins that require calcium for their structure and function. The first direct evidence that cadherins are cell-cell adhesion molecules was provided by transfection experiments (29). The cadherins cluster in adherens junctions, producing a honeycomb pattern by immunocytochemistry. E(pithelial)- and N(eural)-cadherin belong to the ''classical'' or ''type I'' cadherins, that are characterized by a conserved Histidine-Alanine-Valine (HAV) cell adhesion recognition sequence in their first extracellular domain and by a highly conserved cytoplasmic domain, which associates to the actin cytoskeleton via the catenins. E-cadherin is a prototype molecule for non-neuronal cadherins and is expressed by all types of epithelial cells; N-cadherin is a prototype molecule for the neuronal cadherins (23). They stimulate differentiation into respectively epithelial and neuronal tissues (16).

Morphogenesis involves dynamic rearrangements of cells and cell layers. The classical cadherins are considered to be regulators of these processes. Most information about these phenomena comes from immunohistochemistry of chick and mouse tissues. At the implantation stage, E-cadherin is expressed in all cells of the embryo. According to the type of differentiation, E-cadherin either remains on the cell surface or disappears. As a rule, E-cadherin is expressed in those ectodermal and endodermal cells that undergo epithelial differentiation, except for terminally differentiated epithelial cells such as the lens cells which will eventually lose their E-cadherin (29). Downregulation of E-cadherin occurs in mesodermal cells migrating through the primitive streak into the space between the ectoderm and the endoderm, in neural cells invaginating from the neural plate and in cells forming the lens vesicle. In all three cases, cells invaginate from the surface ectoderm (29). As the expression of N-cadherin coincides with the segregation of cells from other cell layers, invaginating cells transiently express both E-cadherin and N-cadherin. After this stage, E-cadherin and N-cadherin are expressed on a mutually exclusive basis (10) at least in normal cells.

Invasion of cells into the surrounding tissues and migration is not restricted to embryogenesis, but also occurs during tumor progression. The E-cadherin gene not only functions as an invasion-suppressor gene (the cancer cells are inhibited to invade into the extracellular matrix) (1), but also as a tumor-suppressor gene (the cancer cells are inhibited to grow) (8). By contrast, N-cadherin may have a promoter role in cancer progression as it increases the motility of the cancer cells (14) as well as their interaction with the stroma (10).

It is our goal to determine the expression-pattern of E- and N-cadherin in the normal and in the tumorous eye.

DISTRIBUTION OF THE CADHERINS IN THE NORMAL EYE

The corneal epithelium shows E-cadherin expression in all cell layers (21,28). Corneal endothelial cells, stromal fibroblasts (keratocytes) and myofibroblasts express N-cadherin (25). Stromal swelling of preserved corneas is dependent on the integrity of endothelial barrier function, the activity of the endothelial basolateral Na/K ATPase pump, the osmotic gradient between the stroma and the storage media, and to a lesser extent, the epithelial barrier function. Transplant failure can be caused by long storage of the cornea at 4°C, leading to disruption of the interendothelial contacts mediated by N-cadherin and to corneal swelling (12).

Lens cells gradually lose E-cadherin and gain N-cadherin expression (29), making N-cadherin the main adhesion protein in the adult lens (9).

The developing neuroretina has been used as a model for neurite outgrowth. Expression of N-cadherin during neural development is under dynamic spatiotemporal regulation. N-cadherin is initially expressed in all undifferentiated retinal cells in mouse (11) and chick (17) embryos. It stimulates the axonal outgrowth from retinal neurons (15). This neurite growth is mediated by activation of the fibroblast growth factor receptor 1 (FGFR1), possibly by direct interaction between HAV-motifs, present on both FGFR1 and N-cadherin (6). Through later development, N-cadherin expression is steadily downregulated until it is localized only at the

outer limiting membrane in avian species (20,29) or at the inner nuclear layer in mouse (11). The downregulation of N-cadherin is modulated by a decrease in mRNA level (26) and by dephosphorylation/phosphorylation, the balance of which determines whether N-cadherin remains stably expressed or is targeted for proteolytic production of N-cadherin fragments (17). N-cadherin-mediated neurite outgrowth does not stop because of the proteolytic breakdown of N-cadherin, as one might expect, since the N-cadherin fragments substitute for the intact molecule (24). A cell-surface N-acetylgalactosaminylphosphotransferase (GalNAcPTase) specifically associates with N-cadherin and is a critical component of one regulatory circuit that coordinates the inhibitory activity of b1 integrin on neurite outgrowth. Extracellular binding of the ligand neurocan on its receptor GalNAcPTase results in intracellular disassembly of the tyrosine kinase Fer from N-cadherin. Fer gains the opportunity to bind to and consequently inhibit function of another transmembrane cell adhesion molecule, b1-integrin. GalNAcPTase is present in the inner nuclear and ganglion cell layer, the two forming a sandwich around the inner plexiform layer where neurocan is localized (18). N-cadherin is also transiently expressed on vascular endothelial cells and pericytes of the developing chick eye, where they may be involved in the commitment of early blood vessels to form the blood-retina barrier (7).

RPE cells were reported to express N-cadherin in vitro (5). Burke et al. (1999) however showed that islands of epithelioid cells in postconfluent cultures of RPE expressed both N- and E-cadherin (2). Upon transfection in a rat RPE cell line, E-cadherin is capable of inducing a basal distribution of the Na/K ATPase pump (19). Accordingly, Burke et al. (2000) found an inverse correlation between the level of E-cadherin expression and the apical distribution of the Na/K ATPase pump in the RPE in vivo (3).

DISTRIBUTION OF THE CADHERINS IN THE DISEASED EYE

There are few reports in literature about the implication of cadherins in eye diseases.

No differences in E-cadherin expression were found between epithelial cells of healthy cornea as compared to wounded cornea (21). Reduced expression of VE-cadherin in the retinal vessels of a diabetic eye was reported by Davidson et al. (2000) in one case (4). Downregulation of E- and upregulation of N-cadherin is already described for cutaneous melanomas (13,22), but there are no reports discussing cadherin expression in ocular melanoma.

Cadherin expression has been determined for most non-ocular tumors, but retinoblastoma was one of the rare tumors lacking on the list (1) Schiffman and Grunwald (1991) showed N-cadherin expression in two retinoblastoma cell lines, but not in retinoblastoma tissue (27). We examined the expression of E-cadherin and N-cadherin in retinoblastoma tissue (5 snap-frozen samples of the operating theatre, provided by Prof. Dr. P. De Potter, UCL, Brussels, Belgium) and in 3 non-tumorous neuroretinas. E-cadherin was expressed in none of the 5 samples. Gelectrophoresis followed by Western blotting showed N-cadherin to be present in both retinoblastoma and normal neuroretina as one band at 135 kDa. Immunohistochemistry revealed two major features:

1) N-cadherin expression is not only restricted to the outer limiting membrane of the neuroretina (20, 29) or the inner nuclear layer (11) but is also present in the outer nuclear layer. An explanation could be that the structure of human neuroretina differs from avian but also from other vertebrate neuroretina.

2) There is a clear difference in N-cadherin pattern between the retinoblastoma specimens and neuroretina: the regular honeycomb pattern of N-cadherin, a- and b-catenin was disturbed in retinoblastoma, showing a dotted pattern at the cell-cell contacts.

Currently, we are investigating the structure and function of the whole N-cadherin/catenin complex in retinoblastoma as compared to normal neuroretina.

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