

N-cadherin in the spotlight of cell-cell adhesion, differentiation, embryogenesis, invasion and signalling

LARA D.M. DERYCKE and MARC E. BRACKE*

Laboratory of Experimental Cancerology, Department of Radiotherapy, Nuclear Medicine and Experimental Cancerology, Ghent University Hospital, Belgium

ABSTRACT Cell migration is a process which is essential during embryonic development, throughout adult life and in some pathological conditions. Cadherins, and more specifically the neural cell adhesion molecule N-cadherin, play an important role in migration. In embryogenesis, N-cadherin is the key molecule during gastrulation and neural crest development. N-cadherin mediated contacts activate several pathways like Rho GTPases and function in tyrosine kinase signalling (for example via the fibroblast growth factor receptor). In cancer, cadherins control the balance between suppression and promotion of invasion. E-cadherin functions as an invasion suppressor and is downregulated in most carcinomas, while N-cadherin, as an invasion promoter, is frequently upregulated. Expression of N-cadherin in epithelial cells induces changes in morphology to a fibroblastic phenotype, rendering the cells more motile and invasive. However in some cancers, like osteosarcoma, N-cadherin may behave as a tumour suppressor. N-cadherin can have multiple functions: promoting adhesion or induction of migration dependent on the cellular context.

KEY WORDS: *N-cadherin, cancer, embryogenesis, invasion, signalling*

Migration and invasion

Cell migration is a process that is essential during embryonic development and throughout further life. In the adult, cell migration is crucial for homeostatic processes, such as effective immune responses and repair of injured tissues. To migrate, the individual cell body must modify its shape to interact with the surrounding tissue structures. The extracellular matrix (ECM) forms a substrate, as well as a barrier for the advancing cell body. Cell migration through tissues results from a continuous cycle of interdependent steps. In general, there are five steps involved in cell migration in the ECM. First comes the protrusion of the leading edge, where growing actin filaments connect to adapter proteins and push the cell membrane in an outward direction. In a second step cell-matrix interactions and focal contacts are formed. After that, surface proteases such as matrix metalloproteinases (MMP) are recruited and focal proteolysis takes place. Then the cell contracts by actomyosin activation, and finally the tail of the cell is detached from its substrate (Friedl and Wolf, 2003).

Border cells of the *Drosophila melanogaster* ovary are nowadays used as a model for migration. There are three recently discovered signalling pathways that control distinct aspects of migration: a global steroid-hormone signal defines the timing of migration, a highly localised cytokine signal that activates the Janus kinase-signal transducer and activator of transcription is

both necessary and sufficient to induce migration, and finally, a growth factor that is analogous to platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) contributes to guiding the cells to their destination (Montell, 2003).

In embryonic morphogenesis two types of collective cell movement can be observed. The first one involves mass migration whereby a tissue moves in a coordinated manner. Gastrulation is an example of mass migration. In the blastocyst large groups of cells migrate collectively as sheets to form the three layers that will eventually form the embryo. Cells within these layers migrate to target locations and form various tissues and organs. The second type of movement requires loss of cell-cell contacts for the migration of individual cells or small groups of cells through the ECM, as seen in neural crest migration. Cells delaminate from the ectodermal layer and acquire migratory properties as they undergo the process of epithelial to mesenchymal transition (EMT). Another example is the migration of muscle precursor cells from the somites to the limbs (Locascio and Nieto, 2001; Horwitz and Webb, 2003).

The failure of cells to migrate to their appropriate locations can result in developmental abnormalities and also in pathological

Abbreviations used in this paper: ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; MMP, matrix metalloproteinase; N-cadherin, Neural cadherin.

*Address correspondence to: Dr. Marc Bracke. Laboratory of Experimental Cancerology, Department of Radiotherapy, Nuclear Medicine and Experimental Cancerology, De Pintelaan 185, Ghent University Hospital, B-9000 Ghent, Belgium. Fax + 32-9240-4991; e-mail: brackemarc@hotmail.com

processes, including vascular and inflammatory diseases, and tumour invasion and metastasis (Lauffenberger and Horwitz, 1996). Aberrant cell migration may play a role in cancer. Cancer is one of the prime causes of human morbidity and mortality, and most of the cancer deaths arise from metastases. Cancer cells have defects in regulatory circuits that govern normal cell proliferation and homeostasis. A cell becomes cancerous because of essential alterations in its physiology: limitless replicative potential due to self-sufficiency in growth signals, insensitivity to growth inhibitory signals or escape from programmed cell death, induction of angiogenesis and acquisition of invasive and metastasising potential (Hanahan and Weinberg, 2000). Of all the processes involved in tumour progression, local invasion and the formation of tumour metastases are clinically the most relevant ones, but the least well understood at the molecular level. They represent one of the great challenges in experimental cancer research.

During the progression of cancer, primary tumour cells move out, invade into adjacent tissues and travel to distant sites. Most important, these processes allow cancer cells to enter the lymphatic and blood vessels for dissemination into the circulation. Invasion is resumed when extravasation occurs in distant organs, and when the secondary tumour contributes to the metastatic cascade. Cancer cells use diverse patterns of migration. They can disseminate as individual cells or expand as solid strands, sheets, files or clusters. Leukemia, lymphoma and most sarcomas disseminate as single cells, while epithelial cells commonly use collective migration. In principle, the lower the differentiation state, the higher the tendency of the tumour to disperse via individual cells (Thiery, 2002; Friedl and Wolf, 2003).

Similarities between the three signalling pathways described for the ovarian border cell migration, and the pathways that are deregulated in human cancer cells indicate that signals that contribute to aberrant proliferation and survival of the tumour cells, can also promote motility, and hence invasion (Montell, 2003).

We will discuss in this review the impact of E- and N-cadherin on migration in embryogenesis and tumour invasion. Epithelial or E-cadherin plays a role in collective migration of epithelial cells. E-cadherin is also an invasion suppressor molecule, and in tumours this molecule can be downregulated in different ways (Mareel and Leroy, 2003). Downregulation of E-cadherin is often correlated with upregulation of neural or N-cadherin, an invasion promoter molecule (Tomita *et al.*, 2000; Li and Herlyn, 2000). However, both the regulation of N-cadherin expression and its molecular contribution to invasion are incompletely understood.

Cadherins

In humans there are more than 80 members of the cadherin superfamily. Sequencing the genome of *C. elegans* and *Drosophila* revealed the existence of 14 and 16 different genes, respectively. Cadherins are composed of an extracellular part, that mediates calcium-dependent homophilic interactions between cadherin molecules, a transmembrane and a cytoplasmic part. The extracellular part consists of several cadherin repeats (EC) of ± 110 amino-acids, which are characterised by a number of conserved amino acid sequences such as PE, LDRE, DXNDN and DXD. These motifs can bind 3 calcium ions at each interdomain boundary in a cooperative manner. Classification of cadherins into subfamilies is based on domain layout of individual cadherins,

which include the number and sequence of EC repeats, and the presence of other conserved domains and sequence motifs, like tyrosine kinase and EGF domains. There are four cadherin subfamilies conserved between *C. elegans*, *Drosophila* and humans: classic cadherins, fat-like cadherins, seven-pass transmembrane cadherins and a new subfamily of cadherins that is related to *Drosophila* Cad 102F. Classic cadherins consist of four subgroups: vertebrate type I classic cadherins like E-, placental (P)-, N- and retinal (R)-cadherin, with an HAV sequence in the first cadherin repeat, vertebrate type II classic cadherins which have no HAV in the first repeat, for example vascular endothelial (VE)-cadherin, ascidian classic cadherins and the non-chordate classic cadherins for example D (*Drosophila*) E- and D (*Drosophila*) N-cadherin (Tepass *et al.*, 2000). The molecular mechanism of type I cadherin interaction has recently been unravelled. The model was proposed after elucidation of the crystal structure of the C-cadherin ectodomain: the trans-interaction is formed by a strand dimer (EC1-EC1) where association is found between the side chain of Tryptophan 2 (Trp²) in one molecule and a pocket in the hydrophobic core of another molecule. The cis interaction occurs between EC1 of one molecule and EC2 of another molecule, resulting in the formation of a lattice of a supramolecular complex (Boggon *et al.*, 2002).

It is now known that alterations in the expression and function of cell-cell and cell-matrix adhesion molecules correlate with progression to malignancy. E-cadherin, a homotypic cell-cell adhesion molecule is expressed on most epithelial cells and is an invasion suppressor. E-cadherin expression or function is lost in most of the carcinomas. This may be by mutational inactivation of the E-cadherin gene, hypermethylation of the promoter, transcriptional repression by SIP1 or snail, loss of transactivators like RB, Myc and WT1, transactivation of other cadherins, phosphorylation of Armadillo proteins by tyrosine kinases, sterical hindrance by mucin 1 (MUC-1) or by ectodomain shedding of E-cadherin by matrix metalloproteinases (MMP) (Van Aken *et al.*, 2001). The proof of principle that the loss of E-cadherin is involved in the progression of tumour malignancy came from a transgenic mouse model of pancreatic β cell carcinogenesis (Rip1Tag2). In these mice, the SV40 large T antigen was expressed under the control of the rat insulin promoter, thus inducing neoplastic transformation from differentiated adenoma to invasive carcinoma selectively in the β cells of the islets of Langerhans. In these tumours E-cadherin was downregulated. Forced expression of E-cadherin in the β cell tumours resulted in an arrest in tumour development at the adenoma stage. Conversely, expression of a dominant-negative form of E-cadherin resulted in early invasion and metastasis (Perl *et al.*, 1998). These results show that E-cadherin suppresses tumour invasion, and that loss of E-cadherin can actively participate in the induction of tumour invasion (Cavallaro and Christofori, 2001).

N-cadherin

N-cadherin was first identified in 1982 (Grunwald *et al.*, 1982) as a 130 kD molecule in the chick neural retina that was protected by calcium from proteolysis, and in 1984 A-CAM was identified (now called N-cadherin) as a molecule that was localised at the adherens junctions (Volk and Geiger, 1984). The N-cadherin gene in mice was located on chromosome 18 (Miyatani *et al.*, 1989). Via Yeast Artificial Chromosome (YAC) analysis the structure of the human N-cadherin gene was determined. The entire N-cadherin gene was

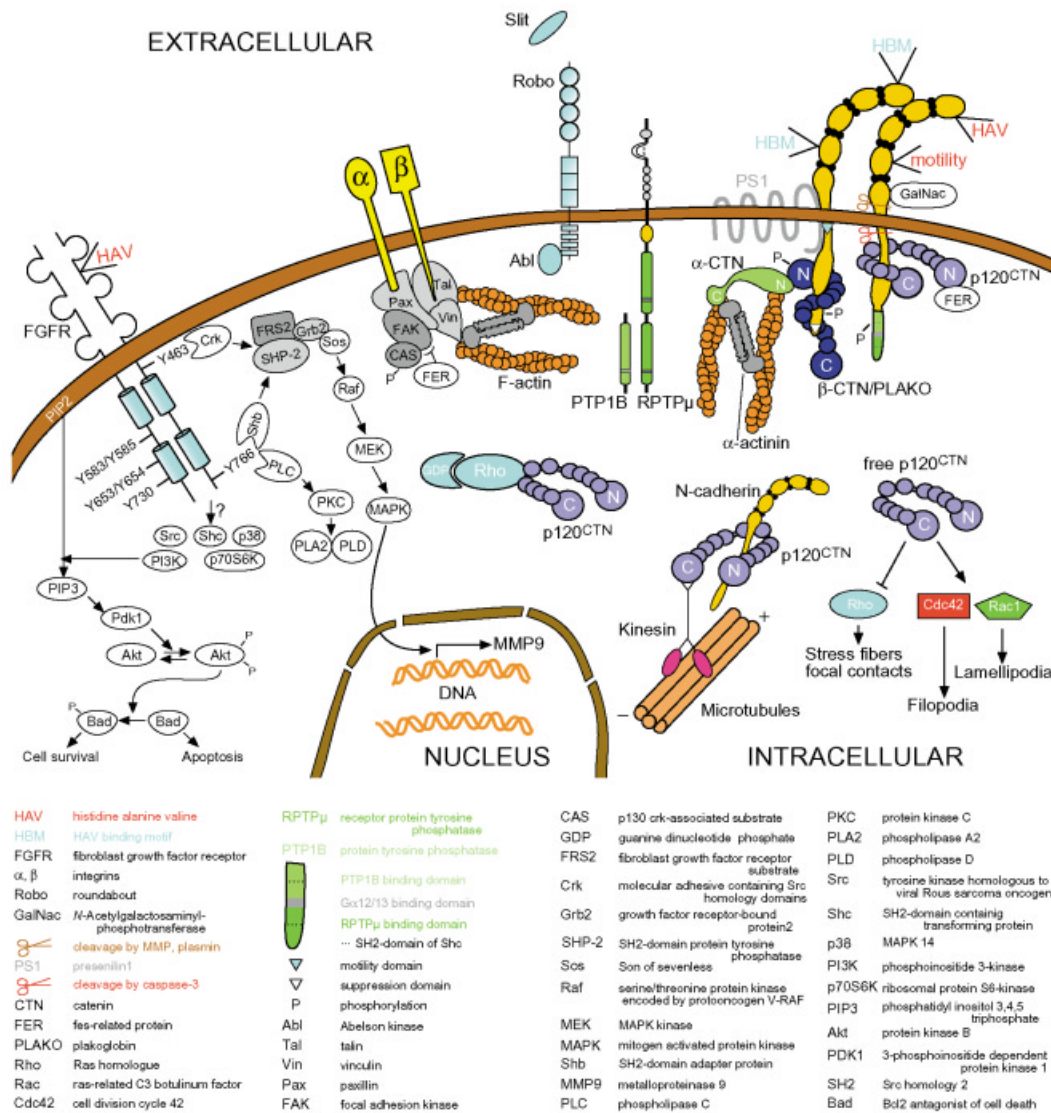


Fig. 1. Schematic overview of the N-cadherin/catenin complex and the multiple proteins which associate and influence the complex. N-cadherin associates via its HBM motif with the HAV sequence of the FGFR. N-cadherin also has a HAV sequence in the first extracellular domain (EC). Activation of FGFR can lead to activation of MAPK and transcription of MMP9, inducing invasion and metastasis. In EC4, a domain is present which is responsible for N-cadherin's pro-migratory behaviour. However, in the cytoplasmic part there are also domains which can stimulate or inhibit migration. N-cadherin mediates also survival of cells via the PI3K-AKT pathway. Many molecules associate directly with the complex, such as GalNacPtase, p120^{ctn}, β-catenin, PTP1B, RPTPμ and α12, or indirectly like FER (implicated in the cadherin/catenin complex, but also in the integrin complex) and α-catenin. Many molecules can change the complex like proteases (MMP, caspase-3, PS 1) or the Robo-Abl kinase. When free cytoplasmic p120^{ctn} is present, it changes the morphology of the cell by blocking Rho in the GDP state or activating Rac1 and Cdc42. N-cadherin is already associated with the catenins in the ER and a microtubular kinesin-driven mechanism is involved in the trafficking to the plasma membrane (Adapted from Van Aken et al., 2001 and Anastasiadis et al., 2001).

mapped to a 250-kb region on chromosome 18q11.2. The gene is composed of 16 exons, and homology was found not only between human and mouse, but also between N-cadherin and other cadherins (Wallis *et al.*, 1994). The protein exists of five extracellular cadherin repeats (EC1 to EC5), a transmembrane and a cytoplasmic part that are encoded by exons 4 to 13, 13 and 14, and 14 to 16, respectively. Eight sequence polymorphisms were identified in a Japanese population: three CCT or GCC-type trinucleotide repeat polymorphisms adjacent to the initiation codon and five other novel single-nucleotide polymorphisms in the coding

region (Harada *et al.*, 2002). The promoter of N-cadherin does not contain CCATT or TATA boxes, but showed a high overall GC content, high CpG dinucleotide content, and several consensus Sp1 and Ap2 binding sequences (Li *et al.*, 1997).

N-cadherin domains and associated proteins

In this part we will discuss the molecules associated with the extracellular and intracellular part of N-cadherin, their influence on N-cadherin function and the induction of signalling pathways (Fig. 1).

Although N-cadherin typically forms homotypic homophilic interactions, also heterotypic homophilic and heterophilic interactions have been described. Examples include the interaction between N-cadherin molecules of Sertoli cells and spermatides, and between N- and R-cadherin in transfected L cells and in neurons at certain neural synapses (Shan *et al.*, 2000).

The fibroblast growth factor receptor (FGFR) is implicated in N-cadherin function. In the nervous system, N-cadherin function is involved in a number of key events that range from the control of axonal growth and guidance to synapse formation to synaptic plasticity (Doherty and Walsh, 1996). Neurite outgrowth stimulated by N-cadherin is inhibited by a wide variety of agents that block the FGFR function, including the expression of a dominant negative FGFR (Williams *et al.*, 1994). In addition, N-cadherin can promote contact-dependent survival of ovarian granulosa cells in an FGFR-dependent manner (Trolice *et al.*, 1997). More recently it has been illustrated that both N-cadherin and the FGFR are necessary to increase cell motility and induce metastasising capacity (Suyama *et al.*, 2002). N-cadherin and the FGFR interact directly: the HAV sequence present in the FGFR associates with the IDPVNGQ sequence present in EC4 of N-cadherin (Williams *et al.*, 2001). This motif was already previously described as a candidate for interaction with FGFR, based on sequence homology of the motifs within N-cadherin (INPISGQ in EC1) and R-cadherin (IDPVSGR in EC1) that interact with the HAV region in N-cadherin (Doherty and Walsh, 1996; Williams *et al.*, 2000b). Peptides used to investigate N-cadherin function were found to have opposite effects on neurite outgrowth: whereas INP (Williams *et al.*, 2000b) and a cyclic HAV peptide (Williams *et al.*, 2000a) antagonize its function, the cyclic dimeric version of the HAV and the INPISG sequence have an agonistic effect on neurite outgrowth (Williams *et al.*, 2002). The latter peptides act by binding to and clustering N-cadherin in the cells, thereby activating the N-cadherin/FGFR signalling cascade. After stimulation with FGF 2, invasion of breast carcinoma cells was demonstrated in the same degree as in cells transfected with N-cadherin, suggesting that N-cadherin and FGFR synergize to generate signals that affect the invasive behaviour. As a consequence of N-cadherin binding, internalisation of the FGFR is inhibited. This is causing a sustained cell surface expression of FGFR, leading to a persistent MAPK-ERK (mitogen activated protein kinase-extracellular signal regulated kinase) activation, MMP-9 expression and tumour invasion (Suyama *et al.*, 2002). Thus, N-cadherin may be involved in both ligand-dependent and ligand-independent interactions with the FGFR (Wheelock and Johnson, 2003).

Transfection of epithelial cells with N-cadherin influences the morphology and the behaviour of these cells: it induces a "motile phenotype" (Islam *et al.*, 1996; Hazan *et al.*, 1997). By transfection of chimeras of E- and N-cadherin in squamous epithelial cells, a 69 amino acid portion of EC4 was identified that is necessary for epithelial to mesenchymal transition and an increase in motility by N-cadherin. The motile phenotype induced by N-cadherin is independent of cell-cell adhesion because an antibody, recognizing the 69 amino acid sequence, inhibited cell motility without inhibiting cell-cell aggregation, providing evidence that adhesion and motility can be two separate features (Kim *et al.*, 2000).

Recently, an S (suppression of movement) -domain (a C-terminal domain: AA699-710 of E-cadherin) was identified in both E- and N-cadherin, though N-cadherin lacked the capacity to

suppress motility, presumably because its domain is masked or latent. This inability of N-cadherin to suppress movement required the presence of the modulation-of-movement-domain (M-domain), consisting of the juxtamembrane domain. The authors suggested several ways in which diversity in cadherin function might arise in different cell types. Variations could be expected if cells differ in expression of molecules that interact with the S and M-domain (Fedor-Chaiken *et al.*, 2003). For example, N-cadherin has no influence on the movement of MDA-MB-435, but the same molecule inhibits the migration of LM8 mouse osteosarcoma cells (Kashima *et al.*, 2003). So, the effect of cadherins can be cell type specific.

The cytoplasmic part of N-cadherin is complexed with a multitude of molecules, such as the catenins p120 catenin (p120^{ctn}), β -catenin and α -catenin, which are possible regulators of cadherin function. p120^{ctn} binds to the juxtamembrane domain and is a key molecule in the regulation of the adhesive or motile phenotype. When p120^{ctn} is phosphorylated, its binding to N-cadherin is increased, reducing the adhesive activity of the latter. Cadherin adhesive activity is also subject to regulation by Rho GTPases. Overexpression of p120^{ctn} in fibroblasts or cadherin-deficient cells causes a branching phenotype, whereas in epithelial cells an increasing lamellipodia formation is observed. In fibroblast, this cytoplasmic p120^{ctn} inhibits RhoA, resulting in an increase in cell motility and activation of Rac1 and Cdc42. In line with the direct binding of RhoA and p120^{ctn} in *Drosophila* (Magie *et al.*, 2002), one hypothesis says that a direct interaction of p120^{ctn} with RhoA keeps RhoA in the inactive GDP state. According to another hypothesis the association of p120^{ctn} with vav2, a Rho-GEF (guanine nucleotide exchange factor) explains the activation of Rac1 and Cdc42 (Anastasiadis *et al.*, 2001).

Fer (fes -related protein; fes: feline sarcoma), a nonreceptor tyrosine kinase, interacts via its coiled-coil domain with the coiled-coil domain of p120^{ctn}. Fer is implicated in the regulation of adherens junctions and focal adhesions. Trojan peptides, recognizing the juxtamembrane domain of N-cadherin, caused Fer to dissociate from N-cadherin, rendering Fer available for complex formation with FAK (Arregui *et al.*, 2000). This correlated with disruption of focal adhesion and reduced tyrosine phosphorylation of the docking protein p130Cas. These observations indicate that Fer has a role in the regulation of cell adhesion and migration through effects on both adherens junctions and focal adhesions (Greer, 2002). Fer and Fyn kinase phosphorylate Tyrosine 142 of β -catenin, and this (unphosphorylated) tyrosine is necessary for the association of β -catenin with α -catenin. In contrast, phosphorylation of tyrosine residues of p120^{ctn} increases the binding of the Fer/Fyn-p120^{ctn} complex to cadherin (Piedra *et al.*, 2003).

P120^{ctn} not only modulates the function of cadherins but is also important in the trafficking and maturation of the cadherin-catenin complex. Wahl *et al.*, have shown that p120^{ctn} readily associates to the cytoplasmic part of N-cadherin in the endoplasmic reticulum (ER). Later on, the cytoplasmic part is phosphorylated, leading to additional binding of β - and α -catenin. The proregion is then removed by furin protease and the complex is transported to the plasma membrane (Wahl *et al.*, 2003). N-cadherin trafficking is mediated by a microtubular kinesin-driven mechanism (Mary *et al.*, 2002) and recent papers elucidated that p120^{ctn} is the link with the microtubule network by direct association of p120^{ctn} with kinesin (Chen *et al.*, 2003; Yanagisawa *et al.*, 2003). Presenilin 1 (PS1),

playing a role in the pathogenesis of early onset familial Alzheimer disease, also binds to the juxtamembrane domain and modulates the adhesive capacity. When dominant negative PS1 is expressed, cell-cell contacts are suppressed, and N-cadherin is localised perinuclearly at the ER and Golgi apparatus. So, PS1 is essential for the trafficking of N-cadherin to the plasma membrane (Uemura *et al.*, 2003).

Another point where the cadherin/catenin complex can be regulated is at its interaction with β -catenin, which is responsible for association with α -catenin and hence for linking the complete complex to the actin network. The interaction of β -catenin with N-cadherin is regulated by multiple proteins (Lilien *et al.*, 2002). The proteoglycan neurocan can inhibit N-cadherin- and β 1-integrin-mediated adhesion and neurite outgrowth. Neurocan interaction with its receptor GalNAcPTase leads to tyrosine hyperphosphorylation of β -catenin and uncoupling of β -catenin from the complex (Lilien *et al.*, 1999; Li *et al.*, 2000). Hyperphosphorylation of β -catenin has consistently been correlated with loss of adhesive function. The nonreceptor protein tyrosine phosphatase PTP1B regulates the phosphorylation of β -catenin (Balsamo *et al.*, 1998). PTP1B needs to be phosphorylated on tyrosine-152 for its association with N-cadherin (Rhee *et al.*, 2001). PTP1B binds to the cytoplasmic part, specifically to the amino acids 872-891 of N-cadherin, and this domain partially overlaps with the β -catenin binding domain. Despite the partial overlap of binding domains, β -catenin and PTP1B do not compete with each other for binding (Xu *et al.*, 2002). The interaction of N-cadherin with PTP1B is essential for its association with β -catenin, its stable expression at the cell surface, and consequently, its function. $G\alpha_{12/13}$, a $G\alpha$ subunit of the heterodimeric G proteins, associates with the cytoplasmic part of N-cadherin, overlapping the binding site of PTP1B, so binding of $G\alpha$ may displace PTP1B and vice versa (Kaplan *et al.*, 2001). The phosphatase PTP μ directly interacts with the carboxy-terminal domain of the cadherins, potentially dephosphorylating these. The absence of PTP μ is correlated with increased phosphorylation of the cadherin itself, but not of β -catenin (Brady-Kalnay *et al.*, 1998). On its turn, increased tyrosine phosphorylation of N-cadherin has been associated with increased turnover of N-cadherin, releasing a 90 kD extracellular fragment (Lee *et al.*, 1997). N-cadherin phosphorylated by Src on tyrosine 851 and 883, associates with the SH2 domain of the adapter protein Shc (Xu and Carpenter, 1999), opening the door to different signalling pathways.

N-cadherin function and signalling

N-cadherin promotes survival in melanoma and prostate carcinoma cells. N-cadherin ligation recruits phosphatidylinositol 3-kinase (PI3K) which activates Akt, resulting in inactivation of the pro-apoptotic molecule Bad (Bcl2 antagonist of cell death, Bcl2 is the acronym for B cell lymphoma) (Li *et al.*, 2001; Tran *et al.*, 2002). However, N-cadherin can also have an inhibitory effect on cell proliferation. Overexpression of N-cadherin in cells suppresses cell proliferation by prolonging the G2/M phase and inducing β -catenin dependent expression of p21 (inhibitor of cyclin dependent kinase, cdk) which inhibit Cdc2 activity (Kamei *et al.*, 2003). P27, another cdk inhibitor is involved in N-cadherin mediated contact inhibition of cell growth and cell cycle arrest in the G1 phase (Levenberg *et al.*, 1999).

N-cadherin stimulates migration and invasion of cells. Different groups demonstrated that aberrant expression of N-cadherin in cancer cells makes the cells more motile and invasive. Our laboratory has demonstrated that retinal pigment epithelial cells (RPE) are invasive in collagen type I. RPE cells have a polarised epithelial phenotype *in vivo* but become rapidly fibroblastic and invasive when explanted *in vitro*. In these conditions they undergo a switch from E- to N-cadherin expression. Such a switch was already seen in the epiblast cells of the chick embryo when the cells were treated with hepatocyte growth factor (HGF) (Deluca *et al.*, 1999). We found indications for an autocrine HGF/c-Met loop stimulating RPE cell invasion via focal adhesion kinase (FAK). N-cadherin activates FAK in invasive RPE cells (Van Aken *et al.*, 2003).

In order to mimic and control the formation of cadherin mediated cell-cell contacts, N-cad-Fc chimera, comprising the N-cadherin ectodomain linked to an IgG Fc fragment, have been used. These chimera form dimers by inter-chain disulfide bridges of the Fc domains. Chimera-loaded beads bound specifically to various cells expressing N-cadherin, inducing a rapid recruitment of cadherin/catenin complexes, followed by a strong anchorage of actin filaments, leading to cytoskeletal reorganisation and activation of intracellular signalling pathways (Lambert *et al.*, 2000). Rac1 is required for the anchoring of the cadherin/catenin complex to the actin filaments in the myogenic C2 cells (Lambert *et al.*, 2002). Further studies demonstrated that for the formation of lamellipodia a p120^{ctn}-PI3K-Rac1 pathway is triggered, while for the organisation of the cadherin complex and the actin cytoskeleton only p120^{ctn} and Rac1 are needed (Gavard *et al.*, 2004). In addition, N-cadherin also controls crucial steps in myogenic differentiation, and addition of N-cad-Fc beads triggered myogenesis in isolated myoblasts. Here, inactivation of Rac1 and Cdc42 was observed, while RhoA was activated. The RhoA GTPase activity is important for myogenic differentiation since it controls the expression and the activity of the transcription factor SRF (serum response factor) which binds to motifs present in the promoter of muscle-specific genes. As a result the promoter of muscle-determining factor MyoD is stimulated by N-cadherin-dependent contact formation (Charasse *et al.*, 2002). A balance between Rac1-Cdc42 and RhoA activity determines the cellular phenotype and biological behaviour of various cell systems: actin cytoskeleton organisation, formation of focal adhesions, neurite extension and myogenesis. In fibroblasts the activation of RhoA leads to assembly of stress fibers and focal contacts, which mediate adhesion to ECM. Activation of Rac1 and Cdc42, however, results in the formation of filopodia and lamellipodia. In mouse fibroblasts, Rac1 signalling is able to antagonize Rho activity. Activation of Rac1 by the GEF Tiam1 in these cells induces an epithelial-like morphology with functional cadherin-based adhesion and inhibition of migration (Sander *et al.*, 1999; Yap and Kovacs, 2003).

Full length N-cadherin and its 90 kD N-terminal fragment have been shown to promote cell-matrix adhesion and neurite outgrowth when presented as a substratum (Paradies and Grunwald, 1993; Bixby *et al.*, 1994). Soluble N-cad-Fc can also stimulate FGFR dependent neurite outgrowth (Utton *et al.*, 2001).

N-cadherin is expressed in human endothelial cells, but its function in angiogenesis is not fully elucidated. Literature data demonstrated that N-cadherin is expressed during early neuroectoderm vascularization where it probably establishes interactions

between neuroectoderm and endothelium, followed by a downregulation of N-cadherin in endothelia when the cells differentiate to a blood-retina and blood-brain barrier (Gerhardt *et al.*, 1999). N-cadherin has also been indicated as an angiogenic factor in non-small-cell lung cancer because biopsies positive for N-cadherin were hypervascular (Nakashima *et al.*, 2003). In our laboratory we could find that plasmin cleaved a 90 kD ectodomain fragment from N-cadherin, coined soluble N-cadherin. Soluble N-cadherin induced angiogenesis in the chick chorioallantoic membrane and the rabbit cornea. The 10-mer HAV peptide (LRAHAVDING) had the same pro-angiogenic effect as soluble N-cadherin (our unpublished data).

N-cadherin up- and downregulation

The N-cadherin/catenin functions are influenced by multiple intracellular and extracellular factors (Table 1). We will discuss a few factors more into detail. The upregulation of N-cadherin at the transcription level has been explored. In *Drosophila* development

the transcription factor twist initiates DN-cadherin expression during early mesoderm formation. Another transcription factor, snail, is required for an increase in the level of N-cadherin (Oda *et al.*, 1998). In biopsies of gastric carcinoma, a correlation was demonstrated between the expression of N-cadherin and twist (Rosivatz *et al.*, 2002). Growth factors as EGF and HGF are able to induce a switch from E- to N-cadherin. An example is found in breast carcinoma cells co-expressing E- and N-cadherin. When treated with EGF they undergo epithelial-mesenchymal transition-like changes, including upregulation of vimentin, downregulation of E-cadherin and upregulation of N-cadherin (Ackland *et al.*, 2003).

P120^{ctn} is an important regulator of the turnover of cadherins. Upon p120^{ctn} knockdown with siRNA (small interfering RNA), the cadherins are rapidly degraded, probably via ubiquitination (Davis *et al.*, 2003). Also, proteases like MMP (Paradies and Grunwald, 1993), caspase-3 (Hunter *et al.*, 2001) and presenilin (Marambaud *et al.*, 2003) may cleave N-cadherin, giving rise to different fragments. MMPs shed a 90 kD ectodomain fragment, soluble N-cadherin, that is still functional while the role and the fate of the

TABLE 1

MECHANISMS OF REGULATION OF THE N-CADHERIN/CATENIN COMPLEX

Factor	Context	Properties	Reference
UPREGULATION			
twist and snail	<i>Drosophila</i> gastric cancer	correlation between twist and N-cadherin expression	Oda <i>et al.</i> , 1998 Rosivatz <i>et al.</i> , 2002
GATA-4	heart	binding to N-cadherin promoter	Zang <i>et al.</i> , 2003
SOX9	chondrocytes	enhancing N-cadherin promoter activity	Panda <i>et al.</i> , 2001
Pax6	Lens placode	induction of N-cadherin expression	Van Raamsdonk <i>et al.</i> , 2000
HOXD3	Lung cancer cells	induction of N-cadherin expression	Hamada <i>et al.</i> , 2001
HGF	epiblast cells	when cells ingress the primitive streak	Deluca <i>et al.</i> , 1999
phorbol ester	osteoblasts	PKC dependent	Delannoy <i>et al.</i> , 2001
EGF	breast carcinoma cells	induction of EMT	Ackland <i>et al.</i> , 2003
gonadal steroids	hippocampus testis Sertoli cells Sertoli cells granulosa cells ovary	mRNA levels increased mRNA levels increased protein levels increased mRNA levels increased	Monks <i>et al.</i> , 2001 Pötter <i>et al.</i> , 1999 MacCalman <i>et al.</i> , 1997 Perryman <i>et al.</i> , 1996 Blaschuk and Farookhi, 1989 MacCalman <i>et al.</i> , 1995
DOWNREGULATION/ FUNCTIONAL INHIBITION			
IL-6	melanoma	mRNA and protein level decreased	Gil <i>et al.</i> , 2002
dexamethasone	osteoblasts	inhibition of expression	Lecanda <i>et al.</i> , 2000
caspase 3	osteoblasts	Proteolysis at the juxtamembrane domain	Hunter <i>et al.</i> , 2001
plasmin		producing a 90 kD ectodomain fragment	Our unpublished data
MMP	retina	producing a 90 kD ectodomain fragment	Paradies and Grunwald, 1993
presenilin	neurons	ε-cleavage produces an intracellular domain peptide CBP	Marambaud <i>et al.</i> , 2003
<i>Porphyromonas gingivalis</i>	epithelial cells	loss of cell-cell adhesion and apoptosis	Chen <i>et al.</i> , 2001
Bismuth/ cadmium	proximal tubule epithelium	nephrotoxicity	Leussink <i>et al.</i> , 2001 Prozialeck <i>et al.</i> , 2003
siRNA of p120 ^{CTN}		rapid turnover of cadherin by proteasome/lysosome	Davis <i>et al.</i> , 2003
thalidomide		binds to N-terminal domain mimicking a tryptophan residue	Thiele <i>et al.</i> , 2000
Robo	axons	activation of the receptor by Slit: complex formation of Robo/Abi/N-cadherin resulting in β-catenin phosphorylation	Rhee <i>et al.</i> , 2002
<i>Chlamydia trachomatis</i>	cervical epithelial cells	Breakdown of the N-cadherin/β-catenin complex	Prozialeck <i>et al.</i> , 2002
N-acetylglucosaminyl transferase V	neural retina cells	loss of cell-cell adhesion and uncoupling of the N-cadherin /transferase complex from actin	Balsamo and Lillien, 1990 Balsamo <i>et al.</i> , 1991 Balsamo <i>et al.</i> , 1995 Guo <i>et al.</i> , 2003

Abbreviations used: GATA-4, zinc finger transcription factor recognizes the consensus motif (A/T)GATA(A/G); SOX9, DNA binding SRY box found in SOX family member; Pax6, paired box protein 6; HOXD3, Homeobox D3; HGF, hepatocyte growth factor; EGF, epidermal growth factor; IL-6, interleukin 6; siRNA, small interfering RNA; TF, transcription factor; PKC, protein kinase C; CBP, CREB binding protein; CREB, cyclic AMP response element binding protein.

residual transmembrane/ intracellular part is not clear. Only for the intracellular peptide fragment of N-cadherin, produced after PS1 cleavage, a role is described. It forms a complex with transcriptional coactivator CBP (CREB binding protein) in the cytoplasm and promotes the proteasomal degradation of CBP, via the ubiquitin-proteasome pathway. N-cadherin has an important role during embryogenesis. Thalidomide, a drug that causes teratogenicity, affects mostly organs originating from neural crest cells. Thalidomide was found to bind at the N-terminal domain of N-cadherin, mimicking a tryptophan residue which is critical for its homodimerization, and thus functionally inhibiting homodimerisation (Thiele *et al.*, 2000). In axon trajectories, the Robo transmembrane receptor forms a complex with N-cadherin. After activation with Slit, a complex between Robo, Abl and N-cadherin is formed, followed by tyrosine phosphorylation of β -catenin and resulting in loss of the critical N-cadherin-actin connection (Rhee *et al.*, 2002).

N-cadherin expression from embryo to adult

Members of the cadherin superfamily have distinct expression patterns during embryonic development and in the adult. Changes in cadherin expression are often associated with changes in cellular morphology and tissue architecture. During gastrulation, E-cadherin is downregulated in the primitive streak as cells undergo an epithelial-mesenchymal transition and concomitantly express N-cadherin in the mesoderm (Hatta and Takeichi, 1986). This expression of N-cadherin is initiated by the transcription factor twist in *Drosophila* (Oda *et al.*, 1998). During neurulation, a similar change in expression occurs in the developing neuroepithelium. Different groups analysed the role of N-cadherin in embryogenesis by using knockouts or an artificial system of cytodifferentiation, in which either teratomas or cultured embryoid bodies from genetically manipulated embryonic stem (ES) cells are generated and analysed. When N-cadherin was constitutively expressed in the E-cadherin negative ES cells, the resulting teratomas formed neuroepithelia and cartilage (Larue *et al.*, 1996). N-cadherin knockout mice die at day 10 of gestation. The embryos display major heart defects and malformed neural tubes and somites (Radice *et al.*, 1997). However, all tissues expected to be formed at this stage are apparently present and seem to be normally differentiated. Re-expression of N-cadherin using muscle-specific promoters (α - or β -myosin heavy chain) partially rescues N-cadherin null embryos. These embryos exhibit an increased number of somites, branchial arches and the presence of forelimb buds, however, brain development is still impaired (Luo *et al.*, 2001).

N-cadherin is implicated in several aspects of cardiac development including sorting out of the precardiac mesoderm, establishment of left-right asymmetry, cardiac looping morphogenesis and trabeculation of the myocardial wall. N-cadherin is one of the earliest proteins to be asymmetrically expressed in the chicken embryo and its activity is required during gastrulation for a proper establishment of the left-right axis (Garcia-Castro *et al.*, 2000). In the early embryo N-cadherin is found in the mesoderm and the notochord, while in the late embryo it is present in neural tissue, lens and some other epithelial tissues, cardiac and skeletal muscles, nephric primordium, some mesenchymal tissue, mesothelium and primordial germ cells (Hatta *et al.*, 1987; Takeichi, 1988).

N-cadherin is expressed in early hematopoietic cells (CD34⁺CD19⁺) and is involved in the development and retention of

early hematopoietic cells in the bone marrow (Puch *et al.*, 2001). Cartilage formation in the developing vertebrate embryonic limb consists of highly coordinated and orchestrated series of events involving the commitment, condensation and chondrogenic differentiation of mesenchymal cells and the production of cartilaginous matrix. Here, N-cadherin has a role in the cellular condensation (Tuan, 2003), being a direct target of SOX9, a transcription factor that is essential for chondrocyte differentiation and cartilage formation (Panda *et al.*, 2001). Misexpression of wnt7a (wingless/int, a chondro-inhibitor *in vitro*) in mesenchymal chondrogenic cultures directly led to prolonged expression of N-cadherin, stabilisation of N-cadherin mediated cell-cell adhesion and eventual inhibition of chondrogenesis (Tufan and Tuan, 2001; Tufan *et al.*, 2002). N-cadherin mRNA levels increase during osteogenic and myogenic differentiation and decrease during adipogenic differentiation. N-cadherin is expressed in all stages of osteoblast bone formation: mRNA levels for example increase at the stages of nodule formation and mineralisation, and *in vitro* N-cadherin levels increase concomitantly with osteoblast differentiation (Ferrari *et al.*, 2000). A lot of factors regulate the expression of N-cadherin in osteoblasts: BMP-2, FGF-2 and phorbol ester increase the level of N-cadherin in a PKC-dependent way, while TNF α and IL-1 are responsible for a decrease in expression. However, N-cadherin expression is decreased in primary and metastatic osteosarcoma (see also below) (Marie, 2002).

N-cadherin plays also an important role in skeletal muscle differentiation. Cells with the potential to undergo skeletal myogenesis are present in the epiblast layer. All cells express the skeletal muscle-specific transcription factor MyoD but only the epiblast cells that express N-cadherin but not E-cadherin will differentiate into skeletal muscle (George-Weinstein *et al.*, 1997). So, N-cadherin is involved in myoblast migration and homing as well as in muscle differentiation (Brand-Saberi *et al.*, 1996).

Migratory cells play an important role in embryonic development and disease. A migratory cell population known as neural crest can be defined as a pluripotent population of cells that arise from the dorsal part of the neural tube during or just before closure. After an epithelial-mesenchymal transition (EMT), they migrate over long distances along distinct pathways to many different regions of the embryo and contribute to a diverse array of tissues and cell types, such as the peripheral nervous system, melanocytes, some endocrine cells, craniofacial cartilage and bone. The transcription factor Slug is involved in both the formation of the neural crest precursors and in neural crest migration. Slug downregulates cadherins, leading to a loss of cell-cell contacts and allowing the cells to migrate. Indeed, when neural crest cells are still associated with the neural tube, they express N-cadherin but once they start migrating N-cadherin is downregulated. At the end of the dorso-ventral migration N-cadherin is re-expressed in aggregating cells, just before the formation of the dorsal root and sympathetic ganglia. After the dorso-lateral migration only the dermal melanocytes express N-cadherin and establish contacts with the fibroblasts in the dermis (Nieto, 2001; Pla *et al.*, 2001).

As is evident from the above, N-cadherin is expressed at different time points and tissues in the embryo. In the adult, N-cadherin is restricted to neural tissue, retina, endothelial cells, fibroblasts, osteoblasts, mesothelium, myocytes, limb cartilage, oocytes, spermatids and Sertoli cells.

TABLE 2

**EXPRESSION OF N-CADHERIN IN HUMAN CANCER CELL LINES AND BIOPSIES
AND CORRELATION WITH THE EXPRESSION FOUND IN EMBRYO AND ADULT**

tumour type	embryo	adult	cell line or biopsy	% positivity	observation /properties	reference
DE NOVO EXPRESSION						
Breast carcinoma	-	-	BT549, MDA-MB-436, HS578T, HS578N		invasive, fibroblastic, metastatic	Hazan <i>et al.</i> , 1997
			SUM159PT		motile, invasive	Nieman <i>et al.</i> , 1999
			Biopsies		+ in sarcomatoid metaplastic carcinoma	Han <i>et al.</i> , 1999
			Biopsies	48	no correlation with survival	Peralta Soler <i>et al.</i> , 1999
Prostate carcinoma	-	-	Ectopic expression in MCF-7 cells		motile, invasive	Hazan <i>et al.</i> , 2000
			Biopsies of ductal carcinoma <i>in situ</i>	12.3	no correlation with grade	Paredes <i>et al.</i> , 2002
			Biopsies	30	+ in invasive carcinomas	Kovacs <i>et al.</i> , 2003
			PC3N and JCA1		induction of epithelial-mesenchymal interactions	Tran <i>et al.</i> , 1999
Bladder carcinoma	-	-	TSU-pr1, PPC-1, ALVA-31, PC3, JCA-1		invasive, metastatic	Bussemakers <i>et al.</i> , 2000
			Biopsies	60	when Gleason score above 7	Tomita <i>et al.</i> , 2000
Thyroid carcinoma	-	-	5637, Wmcb2, SW-780, SW-800, SW-1710, J82, T24		fibroblastic	Giroldi <i>et al.</i> , 1999
			T24, RT112, TCCSUP		epithelioid/ fibroblastic	Mialhe <i>et al.</i> , 2000
			Biopsies	39	+ in invasive tumours	Rieger-Christ <i>et al.</i> , 2001
Squamous cell carcinoma	-	-	HTh7, C643, SW1736, HTh74		fibroblastic	Husmark <i>et al.</i> , 1999
			SCC1, UM-SSC-11A, UM-SCC-11B SCC9		fibroblastic	Islam <i>et al.</i> , 1996 Li <i>et al.</i> , 1998
RE-EXPRESSION						
Melanoma	+	-	Biopsies / cell lines	75/ 90		Hsu <i>et al.</i> , 1996
			MeWo, A375		stronger adhesion, invasive, metastatic	Matsuyoshi <i>et al.</i> , 1997
Leukemia	+	-	Biopsies		+ in metastases	Sanders <i>et al.</i> , 1999
			Biopsies	56		Laskin and Miettinen, 2002
			Oh13T, F6T, K3T, Molt-4F, CEM, Jurkat		ATL and T-cell leukemia +	Tsutsui <i>et al.</i> , 1996
Gastric carcinoma	+	-	Hut102	50	ATL cell lines	Matsuyoshi <i>et al.</i> , 1998
			Biopsies of AFP producing carcinoma	100	aggregation and co-aggregation with mesenchymal cells	Kawamura-Kodoma <i>et al.</i> , 1999
Chordomas	+	-	Biopsies	21	correlation with twist	Yanagimoto <i>et al.</i> , 2001
			Biopsies	100		Rosivatz <i>et al.</i> , 2002
Rhabdomyosarcoma	+	-	Biopsies	50		Laskin and Miettinen, 2002
			RD, HS729		no correlation	Horiguchi <i>et al.</i> , 2004 Soler <i>et al.</i> , 1993
UPREGULATION						
Leiomyoma	+	+	Cells		grow irregular compared to normal overexpression	Kobayashi <i>et al.</i> , 1996
Mesothelioma	+	+	Biopsies		+ in pleural mesothelia	Tai <i>et al.</i> , 2003
			Biopsies	70	- in lung adenocarcinoma	Han <i>et al.</i> , 1997
Adrenal tumours	+	+	Biopsies	70 to 100		Laskin and Miettinen, 2002
			Biopsies		up in pheochromocytomas	Ordonéz, 2003
			Biopsies		down in adrenocortical carcinoma	Khorrman-Manesh <i>et al.</i> , 2002
DOWNREGULATION						
Osteosarcoma	+	+	Biopsies		- in metastasis	Kashima <i>et al.</i> , 1999
Ovarian carcinoma	+	+	Dunn and LM8		migration and metastasis inhibited	Kashima <i>et al.</i> , 2003
			Biopsies		+ in benign and borderline tumours	
Glioblastoma	+	+	Biopsies		not in ovarian cancer	Darai <i>et al.</i> , 1997
			Biopsies		- in mucinous cystadenoma	Peralta Soler <i>et al.</i> , 1997
			Biopsies		+ in normal and metaplastic ovarian	Wong <i>et al.</i> , 1999
			Biopsies		aberrant P-cadherin expression	Patel <i>et al.</i> , 2003
Renal cell carcinoma	+	+/-	Biopsies		no differences	Shinoura <i>et al.</i> , 1995
			Biopsies		down at time of recurrence	Asano <i>et al.</i> , 2000
			Biopsies		correlation with histological grade	Utsuki <i>et al.</i> , 2002
			Caki-1, Caki-2, ACHN, A498		- in oncocytomas	Tani <i>et al.</i> , 1995
					+ in renal cell carcinoma	
OTHERS NOT CLASSIFIED						
Small cell carcinoma in cervix			Biopsies	0	no expression compared with 65 % in other small cell carcinoma	Zarka <i>et al.</i> , 2003
Merkel cell carcinoma			Biopsies (neuroendocrine)	63		Han <i>et al.</i> , 2000

Abbreviations used: '+', expression of N-cadherin; '-', no expression of N-cadherin; up, upregulation; down, downregulation; AFP, alpha-foetoprotein; ATL, adult T-cell leukemia; T cell leukemia, human thymus derived cell line.

N-cadherin and cancer

The process of EMT not only occurs under physiological conditions during normal embryonic development, it also takes place in pathological situations, such as the acquisition of an invasive phenotype in tumour cell lines of epithelial origin. This goes together with the first steps of the metastatic process. The EMT associated with tumour progression frequently involves downregulation of E-cadherin expression and the acquisition of migratory properties. Snail is a strong and direct repressor of E-cadherin (Cano *et al.*, 2000), and influencing the levels of N-cadherin expression, a pro-migratory factor. Indeed, in a number of human cancer types which have lost E-cadherin, *de novo* expression of N-cadherin is observed (Tomita *et al.*, 2000).

The cadherins have been investigated in different areas of tumour biology. In early neoplasia cadherins play a role in the transformation of cells to an abnormal proliferative phenotype. E and N-cadherin are normally involved in inducing cell cycle arrest. However, N-cadherin also promotes survival in normal granulosa cells (Makrigiannakis *et al.*, 1999) and in melanoma cells (Tran *et al.*, 2002) by distinct mechanisms. In epithelial carcinomas E-cadherin is downregulated in most cases, sometimes accompanied by the upregulation of another cadherin, for example N-cadherin, P-cadherin or cadherin -11. Here, we will focus on the expression of N-cadherin in cancer. We reviewed the literature and present an overview of N-cadherin expression in cancer cells and looked whether this was also the case in their embryonic and adult normal counterparts (Table 2). The table is divided into 4 groups: in the first one (including breast, prostate, bladder, thyroid and squamous cell carcinoma) N-cadherin is 'DE NOVO EXPRESSED' (Table 2) in the cancer cell and N-cadherin is never expressed in the corresponding precursor or adult normal cells. In 1996 the aberrant expression of N-cadherin in squamous cell carcinoma was described. The inappropriate expression of N-cadherin in these cells correlated with a scattered fibroblastic phenotype along with decreased expression of E- and P-cadherin. Transfection with antisense N-cadherin resulted in reversion to a normal appearing squamous epithelial cell morphology, and increased expression of E- and P-cadherin. In addition, transfection of a normal squamous epithelial cell line with N-cadherin induced the scattered fibroblastic phenotype (Islam *et al.*, 1996). Aberrant N-cadherin expression was also found in breast carcinoma cells and biopsies. Breast carcinoma cells expressing N-cadherin are more motile and invasive (Hazan *et al.*, 1997 and 2000). In biopsies N-cadherin was mostly found in invasive carcinoma, but no correlation could be found with grade (Paredes *et al.*, 2002) or patient survival (Peralta Soler *et al.*, 1999). *De novo* expression of N-cadherin was found most frequently in prostate carcinoma: in one series, 60% was positive in carcinomas with a Gleason score above 7 (Tomita *et al.*, 2000). *In vitro* studies show that the expression of N-cadherin mediates an epithelial-mesenchymal transformation, possibly improving the physical interaction with the surrounding stromal fibroblasts and facilitating metastasis (Tran *et al.*, 1999).

In the group 'RE-EXPRESSION' (Table 2) we classified tumours that had embryonic precursor cells expressing N-cadherin. One of the best examples are melanoma cells: melanocytes are derived from neural crest cells, which are N-cadherin positive before they start migrating. N-cadherin was found back in metastasising melanomas (Matsuyoshi *et al.*, 1997; Sanders *et al.*, 1999). In gastric

carcinoma N-cadherin was found in all α -foetoprotein producing tumours (Yanagimoto *et al.*, 2001) and a correlation was found between the expression of twist and N-cadherin expression (Rosivatz *et al.*, 2002). During early development N-cadherin is found in some basal granulated epithelial cells of the stomach, duodenum and jejunum (Gaidar *et al.*, 1998). Another example is the expression of N-cadherin in T-cell leukemia cell lines. Here, N-cadherin is functionally active because it stimulates the co-aggregation and adhesion with mesenchymal cells, which presumably facilitates invasion in mesenchymal tissues of the skin and the central nervous system (Kawamura-Kodama *et al.*, 1999).

A third group, 'UPREGULATION' (Table 2), shows that cells already expressing N-cadherin in embryonic and adult stages can still increase their levels of expression in neoplastic stages. One example is pleural mesothelioma, where a high and homogeneous expression is characteristic (Han *et al.*, 1997; Ordóñez, 2003).

In the last group we collected cancers where N-cadherin levels remain unaltered or are 'DOWNREGULATED' (Table 2). In osteosarcoma, N-cadherin inhibits cell migration and the formation of metastasis (Kashima *et al.*, 1999 and 2003). In glioblastoma no differences were found in N-cadherin expression but at the time of recurrence, decreased N-cadherin expression correlates with dissemination in malignant astrocytic tumours (Asano *et al.*, 2000). In ovarian carcinoma, N-cadherin is expressed in the different stages but one report mentioned that mucinous cystadenomas were N-cadherin negative (Peralta Soler *et al.*, 1997). Recently it was shown that probably P-cadherin is the important aberrantly expressed cadherin in ovarian cancer (Patel *et al.*, 2003).

In summary, multiple *in vitro* and *in vivo* studies showed that aberrant N-cadherin (re-) expression correlates in most cases with a morphological change towards a more fibroblastic phenotype, with cells becoming more motile, invasive and metastatic. There are, however, invasive tumours where N-cadherin is downregulated and where it may play the role of a tumour suppressor molecule.

Nowadays, loss of immunohistochemical E-cadherin expression is sometimes used in surgical pathology to characterize gastric and breast carcinomas. It may be worthwhile to explore also the cases where N-cadherin is aberrantly expressed, and challenge N-cadherin as a candidate prognostic marker. Another ongoing project in our laboratory is the use of circulating soluble N-cadherin, the 90 kD fragment that is released after MMP cleavage, as a potential tumour marker of invasion. Soluble E-cadherin, a 80 kD ectodomain fragment, in the serum or urine of patients with urothelial carcinoma (Griffiths *et al.*, 1996), ovarian carcinoma (Gadducci *et al.*, 1999) and gastric carcinoma (Gofuku *et al.*, 1998) has already been launched as a circulating tumour marker. Yet, we believe that soluble N-cadherin has better chances as a potential circulating tumour marker than soluble E-cadherin, because in general N-cadherin expression is upregulated in invasive tumours.

Conclusion

N-cadherin is associated with a lot of molecules that regulate its function. It is involved in a lot of processes like cell-cell adhesion, differentiation, embryogenesis, migration, invasion and signal transduction. In embryogenesis, during gastrulation, cells undergo an epithelial-mesenchymal transition leading to the expression of N-cadherin and the downregulation of E-cadherin in the mesoderm. This switch is regulated by multiple growth and transcription

factors. A similar situation appears in carcinomas where loss of E-cadherin is correlated with an upregulation of N-cadherin. The aberrant expression (*de novo* or re-expression) of N-cadherin attributes a more fibroblastic phenotype to the cancer cells, and they become more motile and invasive. One of the transcription factors responsible for upregulation is twist. Further research on other possible factors that affect the N-cadherin switch, on the signalling pathways initiated in N-cadherin mediated invasion and on the perspective of N-cadherin as a potential marker of invasion is needed.

Acknowledgments

We gratefully thank Veerle Van Marck, Elisabeth Van Aken and Christophe Stove for the critical reading of the manuscript, Georges De Bruyne for technical assistance and Jean Roels for preparation of the illustration. This work was sponsored by the Fund for Scientific Research (FWO)-Flanders, Brussels, Belgium and the Belgian Association for Cancer Research (BACR), Brussels, Belgium.

References

- ACKLAND, M.L., NEWGREEN, D.F., FRIDMAN, M., WALTHAM, M.C., ARVANITIS, A., MINICHIELLO, J., PRICE, J.T. and THOMPSON, E.W. (2003). Epidermal growth factor-induced epithelio-mesenchymal transition in human breast carcinoma cells. *Lab. Invest.* **83**: 435-448.
- ANASTASIADIS, P.Z. and REYNOLDS, A.B. (2001). Regulation of Rho GTPases by p120-catenin. *Curr. Opin. Cell Biol.* **13**: 604-610.
- ARREGUI, C., PATHRE, P., LILIE, J. and BALSAMO, J. (2000). The nonreceptor tyrosine kinase Fer mediates cross-talk between N-cadherin and β 1-integrins. *J. Cell Biol.* **149**: 1263-1273.
- ASANO, K., KUBO, O., TAJIKA, Y., TAKAKURA, K. and SUZUKI, S. (2000). Expression of cadherin and CSF dissemination in malignant astrocytic tumors. *Neurosurg. Rev.* **23**: 39-44.
- BALSAMO, J. and LILIE, J. (1990). N-cadherin is stably associated with and is an acceptor for a cell surface N-acetylgalactosaminylphosphotransferase. *J. Biol. Chem.* **265**: 2923-2928.
- BALSAMO, J., ARREGUI, C., LEUNG, T. and LILIE, J. (1998). The nonreceptor protein tyrosin phosphatase PTP1B binds to the cytoplasmic domain of N-cadherin and regulates the cadherin-actin linkage. *J. Cell Biol.* **143**: 523-532.
- BALSAMO, J., ERNST, H., ZANIN, M.K.B., HOFFMAN, S. and LILIE, J. (1995). The interaction of the retina cell surface N-acetylgalactosaminylphosphotransferase with an endogenous proteoglycan ligand results in inhibition of cadherin-mediated adhesion. *J. Cell Biol.* **129**: 1391-1401.
- BALSAMO, J., THIBOLDEAUX, R., SWAMINATHAN, N. and LILIE, J. (1991). Antibodies to the retina N-acetylgalactosaminylphosphotransferase modulate N-cadherin-mediated adhesion and uncouple the N-cadherin transferase complex from the actin-containing cytoskeleton. *J. Cell Biol.* **113**: 429-436.
- BIXBY, J.L., GRUNWALD, G.B. and BOOKMAN, R.J. (1994). Ca^{2+} influx and neurite growth in response to purified N-cadherin and laminin. *J. Cell Biol.* **127**: 1461-1475.
- BLASCHUK, O.W. and FAROOKHI, R. (1989). Estradiol stimulates cadherin expression in rat granulosa cells. *Dev. Biol.* **136**: 564-567.
- BOGGON, T.J., MURRAY, J., CHAPPUIS-FLAMENT, S., WONG, E., GUMBINER, B.M. and SHAPIRO, L. (2002). C-cadherin ectodomain structure and implications for cell adhesion mechanisms. *Science* **296**: 1308-1313.
- BRADY-KALNAY, S.M., MOURTON, T., NIXON, J.P., PIETZ, G.E., KINCH, M., CHEN, H., BRACKENBURY, R., RIMM, D.L., DEL VECCHIO, R.L. and TONKS, N.K. (1998). Dynamic interaction of PTP μ with multiple cadherins *in vivo*. *J. Cell Biol.* **141**: 287-296.
- BRAND-SABERI, B., GAMEL, A.J., KRENN, V., MÜLLER, T.S., WILTING, J. and CHRIST, B. (1996). N-cadherin is involved in myoblast migration and muscle differentiation in the avian limb bud. *Dev. Biol.* **178**: 160-173.
- BUSSEMAKERS, M.J.G., VAN BOKHOVEN, A., TOMITA, K., JANSEN, C.F.J. and SCHALKEN, J.A. (2000). Complex cadherin expression in human prostate cancer cells. *Int. J. Cancer* **85**: 446-450.
- CANO, A., PÉREZ-MORENO, M.A., RODRIGO, I., LOCASCIO, A., BLANCO, M.J., DEL BARRIO, M.G., PORTILLO, F. and NIETO, M.A. (2000). The transcription factor Snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nature Cell Biol.* **2**: 76-83.
- CAVALLARO, U. and CHRISTOFORI, G. (2001). Cell adhesion in tumor invasion and metastasis: loss of the glue is not enough. *Biochim. Biophys. Acta* **1552**: 39-45.
- CHARRASSE, S., MERIANE, M., COMUNALE, F., BLANGY, A. and GAUTHIER-ROUVIÈRE, C. (2002). N-cadherin-dependent cell-cell contact regulates Rho GTPases and β -catenin localization in mouse C2C12 myoblasts. *J. Cell Biol.* **158**: 953-965.
- CHEN, X., KOJIMA, S.-i, BORISY, G.G. and GREEN, K.J. (2003). p120 catenin associates with kinesin and facilitates the transport of cadherin-catenin complexes to intercellular junctions. *J. Cell Biol.* **163**: 547-557.
- CHEN, Z., CASIANO, C.A. and FLETCHER, H.M. (2001). Protease-active extracellular protein preparations from *Porphyromonas gingivalis* W83 induce N-cadherin proteolysis, loss of cell adhesion, and apoptosis in human epithelial cells. *J. Periodontol.* **72**: 641-650.
- DARÁI, E., SCOAZEC, J.-Y., WALKER-COMBROUZE, F., MLIKA-CABANNE, N., FELDMANN, G., MADELENAT, P. and POTET, F. (1997). Expression of cadherins in benign, borderline, and malignant ovarian epithelial tumors: a clinicopathologic study of 60 cases. *Hum. Pathol.* **28**: 922-928.
- DAVIS, M.A., IRETON, R.C. and REYNOLDS, A.B. (2003). A core function for p120-catenin in cadherin turnover. *J. Cell Biol.* **163**: 525-534.
- DELANNOY, P., LEMONNIER, J., HAÏ, E., MODROWSKI, D. and MARIE, P.J. (2001). Protein kinase C-dependent upregulation of N-cadherin expression by phorbol ester in human calvaria osteoblasts. *Exp. Cell Res.* **269**: 154-161.
- DELUCA, S.M., GERHART, J., COCHRAN, E., SIMAK, E., BLITZ, J., MATTIACCI-PAESSLER, M., KNUDSEN, K. and GEORGE-WEINSTEIN, M. (1999). Hepatocyte growth factor/scatter factor promotes a switch from E- to N-cadherin in chick embryo epiblast cells. *Exp. Cell Res.* **251**: 3-15.
- DOHERTY, P. and WALSH, F.S. (1996). CAM-FGF receptor interactions: a model for axonal growth. *Mol. Cell. Neurosci.* **8**: 99-111.
- FEDOR-CHAIKEN, M., MEIGS, T.E., KAPLAN, D.D. and BRACKENBURY, R. (2003). Two regions of cadherin cytoplasmic domains are involved in suppressing motility of a mammary carcinoma cell line. *J. Biol. Chem.* **278**: 52371-52378.
- FERRARI, S.L., TRAIANEDES, K., THORNE, M., LAFAGE-PROUST, M.-H., GENEVER, P., CECCHINI, M.G., BEHAR, V., BISELLO, A., CHOREV, M., ROSENBLATT, M. and SUVA, L.J. (2000). A role for N-cadherin in the development of the differentiated osteoblastic phenotype. *J. Bone Miner. Res.* **15**: 198-208.
- FRIEDL, P. and WOLF, K. (2003). Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat. Rev. Cancer* **3**: 362-374.
- GADDUCCI, A., FERDEGHINI, M., COSIO, S., ANNICCHIARICO, C., CIAMPI, B., BIANCHI, R. and GENAZZANI, A.R. (1999). Preoperative serum E-cadherin assay in patients with ovarian carcinoma. *Anticancer Res.* **19**: 769-772.
- GAIDAR, Y.A., LEPEKHIN, E.A., SHEICHETOVA, G.A. and WITT, M. (1998). Distribution of N-cadherin and NCAM in neurons and endocrine cells of the human embryonic and fetal gastroenteropancreatic system. *Acta Histochem.* **100**: 83-97.
- GARCÍA-CASTRO, M.I., VIELMETTER, E. and BRONNER-FRASER, M. (2000). N-Cadherin, a cell adhesion molecule involved in establishment of embryonic left-right asymmetry. *Science* **288**: 1047-1051.
- GAVARD, J., LAMBERT, M., GROSHEVA, I., MARTHIENS, V., IRINOPOULOU, T., RIOU, J.-F., BERSHADSKY, A. and MÈGE, R.-M. (2004). Lamellipodium extension and cadherin adhesion: two cell responses to cadherin activation relying on distinct signalling pathways. *J. Cell Sci.* **117**: 257-270.
- GEORGE-WEINSTEIN, M., GERHART, J., BLITZ, J., SIMAK, E. and KNUDSEN, K.A. (1997). N-cadherin promotes the commitment and differentiation of skeletal muscle precursor cells. *Dev. Biol.* **185**: 14-24.
- GERHARDT, H., LIEBNER, S., REDIES, C. and WOLBURG, H. (1999). N-cadherin expression in endothelial cells during early angiogenesis in the eye and brain of the chicken: relation to blood-retina and blood-brain barrier development. *Eur. J. Neurosci.* **11**: 1191-1201.
- GIL, D.G., LITYNSKA, A. and LAIDLER, P.M. (2002). Cancer detection and prevention on line. Interleukin 6 downregulates N-cadherin and α 3 β 1 integrin expression in human melanoma cells. <http://www.cancerprev.org/Meetings/2002/Abstracts/1194/91>.

- GIROLDI, L.A., BRINGUIER, P.-P., SHIMAZUI, T., JANSEN, K. and SCHALKEN, J.A. (1999). Changes in cadherin-catenin complexes in the progression of human bladder carcinoma. *Int. J. Cancer* 82: 70-76.
- GOFUKU, J., SHIOZAKI, H., DOKI, Y., INOUE, M., HIRAO, M., FUKUCHI, N. and MONDEN, M. (1998). Characterization of soluble E-cadherin as a disease marker in gastric cancer patients. *Br. J. Cancer* 78: 1095-1101.
- GREER, P. (2002). Closing in on the biological functions of Fps/Fes and Fer. *Nat. Rev. Mol. Cell Biol.* 3: 278-289.
- GRIFFITHS, T.R.L., BROTHERRICK, I., BISHOP, R.I., WHITE, M.D., MCKENNA, D.M., HORNE, C.H.W., SHENTON, B.K., NEAL, D.E. and MELLON, J.K. (1996). Cell adhesion molecules in bladder cancer: soluble serum E-cadherin correlates with predictors of recurrence. *Br. J. Cancer* 74: 579-584.
- GRUNWALD, G.B., PRATT, R.S. and LILIEN, J. (1982). Enzymic dissection of embryonic cell adhesive mechanisms. III. Immunological identification of a component of the calcium-dependent adhesive system of embryonic chick neural retina cells. *J. Cell Sci.* 55: 69-83.
- GUO, H.-B., LEE, I., KAMAR, M. and PIERCE, M. (2003). N-acetylglucosaminyltransferase V expression levels regulate cadherin-associated homotypic cell-cell adhesion and intracellular signaling pathways. *J. Biol. Chem.* 278: 52412-52424.
- HAMADA, J.-i., OMATSU, T., OKADA, F., FURUUCHI, K., OKUBO, Y., TAKAHASHI, Y., TADA, M., MIYAZAKI, Y.J., TANIGUCHI, Y., SHIRATO, H., MIYASAKA, K. and MORIUCHI, T. (2001). Overexpression of homeobox gene *HOXD3* induces coordinate expression of metastasis-related genes in human lung cancer cells. *Int. J. Cancer* 93: 516-525.
- HAN, A.C., PERALTA-SOLER, A., KNUDSEN, K.A., WHEELLOCK, M.J., JOHNSON, K.R. and SALAZAR, H. (1997). Differential expression of N-cadherin in pleural mesotheliomas and E-cadherin in lung adenocarcinomas in formalin-fixed, paraffin-embedded tissues. *Hum. Pathol.* 28: 641-645.
- HAN, A.C., SOLER, A.P., KNUDSEN, K.A. and SALAZAR, H. (1999). Distinct cadherin profiles in special variant carcinomas and other tumors of the breast. *Hum. Pathol.* 30: 1035-1039.
- HAN, A.C., SOLER, A.P., TANG, C.-K., KNUDSEN, K.A. and SALAZAR, H. (2000). Nuclear localization of E-cadherin expression in Merkel cell carcinoma. *Arch. Pathol. Lab. Med.* 124: 1147-1151.
- HANAHAN, D. and WEINBERG, R.A. (2000). The hallmarks of cancer. *Cell* 100: 57-70.
- HARADA, H., KIMURA, A., FUKINO, K., YASUNAGA, S., NISHI, H. and EMI, M. (2002). Genomic structure and eight novel exonic polymorphisms of the human N-cadherin gene. *J. Hum. Genet.* 47: 330-332.
- HATTA, K. and TAKEICHI, M. (1986). Expression of N-Cadherin adhesion molecules associated with early morphogenetic events in chick development. *Nature* 320: 447-449.
- HATTA, K., TAKAGI, S., FUJISAWA, H. and TAKEICHI, M. (1987). Spatial and temporal expression pattern of N-cadherin cell adhesion molecules correlated with morphogenetic processes of chicken embryos. *Dev. Biol.* 120: 215-227.
- HAZAN, R.B., KANG, L., WHOOLEY, B.P. and BORGAN, P.I. (1997). N-cadherin promotes adhesion between invasive breast cancer cells and the stroma. *Cell Adhesion Commun.* 4: 399-411.
- HAZAN, R.B., PHILLIPS, G.R., FANG QIAO, R., NORTON, L. and AARONSON, S.A. (2000). Exogenous expression of N-cadherin in breast cancer cells induces cell migration, invasion, and metastasis. *J. Cell Biol.* 148: 779-790.
- HORIGUCHI, H., SANO, T., QIAN, Z.R., HIROKAWA, M., KAGAWA, N., YAMAGUCHI, T., HIROSE, T. and NAGAHIRO, S. (2004). Expression of cell adhesion molecules in chordomas: an immunohistochemical study of 16 cases. *Acta Neuropathol. (Berl.)* 107: 91-96.
- HORWITZ, R. and WEBB, D. (2003). Cell migration. *Curr. Biol.* 13: R756-R759.
- HSU, M.-Y., WHEELLOCK, M.J., JOHNSON, K.R. and HERLYN, M. (1996). Shifts in cadherin profiles between human normal melanocytes and melanomas. *J. Invest. Dermatol. Symp. Proc.* 1: 188-194.
- HUNTER, I., MCGREGOR, D. and ROBINS, S.P. (2001). Caspase-dependent cleavage of cadherins and catenins during osteoblast apoptosis. *J. Bone Miner. Res.* 16: 466-477.
- HUSMARK, J., HELDIN, N.-E. and NILSSON, M. (1999). N-cadherin-mediated adhesion and aberrant catenin expression in anaplastic thyroid-carcinoma cell lines. *Int. J. Cancer* 83: 692-699.
- ISLAM, S., CAREY, T.E., WOLF, G.T., WHEELLOCK, M.J. and JOHNSON, K.R. (1996). Expression of N-cadherin by human squamous carcinoma cells induces a scattered fibroblastic phenotype with disrupted cell-cell adhesion. *J. Cell Biol.* 135: 1643-1654.
- KAMEI, J., TOYOFUKU, T. and HORI, M. (2003). Negative regulation of p21 by β -catenin/TCF signaling: a novel mechanism by which cell adhesion molecules regulate cell proliferation. *Biochem. Biophys. Res. Commun.* 312: 380-387.
- KAPLAN, D.D., MEIGS, T.E. and CASEY, P.J. (2001). Distinct regions of the cadherin cytoplasmic domain are essential for functional interaction with α_{12} and β -catenin. *J. Biol. Chem.* 276: 44037-44043.
- KASHIMA, T., KAWAGUCHI, J., TAKESHITA, S., KURODA, M., TAKANASHI, M., HORIUCHI, H., IMAMURA, T., ISHIKAWA, Y., ISHIDA, T., MORI, S., MACHINAMI, R. and KUDO, A. (1999). Anomalous cadherin expression in osteosarcoma. Possible relationships to metastasis and morphogenesis. *Am. J. Pathol.* 155: 1549-1555.
- KASHIMA, T., NAKAMURA, K., KAWAGUCHI, J., TAKANASHI, M., ISHIDA, T., ABURATANI, H., KUDO, A., FUKAYAMA, M. and GRIGORIADIS, A.E. (2003). Overexpression of cadherins suppresses pulmonary metastasis of osteosarcoma *in vivo*. *Int. J. Cancer* 104: 147-154.
- KAWAMURA-KODAMA, K., TSUTSUI, J.-i., SUZUKI, S.T., KANZAKI, T. and OZAWA, M. (1999). N-cadherin expressed on malignant T cell lymphoma cells is functional, and promotes heterotypic adhesion between the lymphoma cells and mesenchymal cells expressing N-cadherin. *J. Invest. Dermatol.* 112: 62-66.
- KHORRAM-MANESH, A., AHLMAN, H., JANSSON, S. and NILSSON, O. (2002). N-cadherin expression in adrenal tumors: upregulation in malignant pheochromocytoma and downregulation in adrenocortical carcinoma. *Endocr. Pathol.* 13: 99-110.
- KIM, J.-B., ISLAM, S., KIM, Y.J., PRUDOFF, R.S., SASS, K.M., WHEELLOCK, M.J. and JOHNSON, K.R. (2000). N-Cadherin extracellular repeat 4 mediates epithelial to mesenchymal transition and increased motility. *J. Cell Biol.* 151: 1193-1206.
- KOBAYASHI, Y., NIKAIDO, T., ZHAI, Y.L., IINUMA, M., SHIOZAWA, T., SHIROTA, M. and FUJII, S. (1996). In-vitro model of uterine leiomyomas: formation of ball-like aggregates. *Hum. Reprod.* 11: 1724-1730.
- KOVACS, A., DHILLON, J. and WALKER, R.A. (2003). Expression of P-cadherin, but not E-cadherin or N-cadherin, relates to pathological and functional differentiation of breast carcinomas. *J. Clin. Pathol.: Mol. Pathol.* 56: 318-322.
- LAMBERT, M., CHOQUET, D. and MÈGE, R.-M. (2002). Dynamics of ligand-induced, Rac1-dependent anchoring of cadherins to the actin cytoskeleton. *J. Cell Biol.* 157: 469-479.
- LAMBERT, M., PADILLA, F. and MÈGE, R.M. (2000). Immobilized dimers of N-cadherin-Fc chimera mimic cadherin-mediated cell contact formation: contribution of both outside-in and inside-out signals. *J. Cell Sci.* 113: 2207-2219.
- LARUE, L., ANTOS, C., BUTZ, S., HUBER, O., DELMAS, V., DOMINIS, M. and KEMLER, R. (1996). A role for cadherins in tissue formation. *Development* 122: 3185-3194.
- LASKIN, W.B. and MIETTINEN, M. (2002). Epithelial-type and neural-type cadherin expression in malignant noncarcinomatous neoplasms with epithelioid features that involve the soft tissues. *Arch. Pathol. Lab. Med.* 126: 425-431.
- LAUFFENBURGER, D.A. and HORWITZ, A.F. (1996). Cell migration: a physically integrated molecular process. *Cell* 84: 359-369.
- LECANDA, F., CHENG, S.-L., SHIN, C.S., DAVIDSON, M.K., WARLOW, P., AVIOLI, L.V. and CIVITELLI, R. (2000). Differential regulation of cadherins by dexamethasone in human osteoblastic cells. *J. Cell. Biochem.* 77: 499-506.
- LEE, M.M., FINK, B.D. and GRUNWALD, G.B. (1997). Evidence that tyrosine phosphorylation regulates N-cadherin turnover during retinal development. *Dev. Genet.* 20: 224-234.
- LEUSSINK, B.T., LITVINOV, S.V., DE HEER, E., SLIKKERVEER, A., VAN DER VOET, G.B., BRUIJN, J.A. and DE WOLFF, F.A. (2001). Loss of homotypic epithelial cell adhesion by selective N-cadherin displacement in bismuth nephrotoxicity. *Toxicol. Appl. Pharmacol.* 175: 54-59.
- LEVENBERG, S., YARDEN, A., KAM, Z. and GEIGER, B. (1999). p27 is involved in N-cadherin-mediated contact inhibition of cell growth and S-phase entry. *Oncogene* 18: 869-876.
- LI, B., PARADIES, N.E. and BRACKENBURY, R.W. (1997). Isolation and characterization of the promoter region of the chicken *N-cadherin* gene. *Gene* 191: 7-13.
- LI, G. and HERLYN, M. (2000). Dynamics of intercellular communication during melanoma development. *Mol. Med. Today* 6: 163-169.

- LI, G., SATYAMOORTHY, K. and HERLYN, M. (2001). N-cadherin-mediated intercellular interactions promote survival and migration of melanoma cells. *Cancer Res.* **61**: 3819-3825.
- LI, H., LEUNG, T.-C., HOFFMAN, S., BALSAMO, J. and LILIE, J. (2000). Coordinate regulation of cadherin and integrin function by the chondroitin sulfate proteoglycan neurocan. *J. Cell Biol.* **149**: 1275-1288.
- LI, Z., GALLIN, W.J., LAUZON, G. and PASDAR, M. (1998). L-CAM expression induces fibroblast-epidermoid transition in squamous carcinoma cells and down-regulates the endogenous N-cadherin. *J. Cell Sci.* **111**: 1005-1019.
- LILIE, J., ARREGUI, C., LI, H. and BALSAMO, J. (1999). The juxtamembrane domain of cadherin regulates integrin-mediated adhesion and neurite outgrowth. *J. Neurosci. Res.* **58**: 727-734.
- LILIE, J., BALSAMO, J., ARREGUI, C. and XU, G. (2002). Turn-off, drop-out: functional state switching of cadherins. *Dev. Dyn.* **224**: 18-29.
- LOCASCIO, A. and NIETO, M.A. (2001). Cell movements during vertebrate development: integrated tissue behaviour versus individual cell migration. *Curr. Opin. Genet. Dev.* **11**: 464-469.
- LUO, Y., FERREIRA-CORNWELL, M.C., BALDWIN, H.S., KOSTETSKII, I., LENOX, J.M., LIEBERMAN, M. and RADICE, G.L. (2001). Rescuing the N-cadherin knockout by cardiac-specific expression of N- or E-cadherin. *Development* **128**: 459-469.
- MACCALMAN, C.D., FAROOKHI, R. and BLASCHUK, O.W. (1995). Estradiol regulates N-cadherin mRNA levels in the mouse ovary. *Dev. Genet.* **16**: 20-24.
- MACCALMAN, C.D., GETSIOS, S., FAROOKHI, R. and BLASCHUK, O.W. (1997). Estrogens potentiate the stimulatory effects of follicle-stimulating hormone on N-cadherin messenger ribonucleic acid levels in cultured mouse Sertoli cells. *Endocrinology* **138**: 41-48.
- MAGIE, C.R., PINTO-SANTINI, D. and PARKHURST, S.M. (2002). Rho1 interacts with p120^{cas} and α -catenin, and regulates cadherin-based adherens junction components in *Drosophila*. *Development* **129**: 3771-3782.
- MAKRIGIANNAKIS, A., COUKOS, G., CHRISTOFIDOU-SOLOMIDOU, M., GOUR, B.J., RADICE, G.L., BLASCHUK, O. and COUTIFARIS, C. (1999). N-cadherin-mediated human granulosa cell adhesion prevents apoptosis. A role in follicular atresia and luteolysis?. *Am. J. Pathol.* **154**: 1391-1406.
- MARAMBAUD, P., WEN, P.H., DUTT, A., SHIOI, J., TAKASHIMA, A., SIMAN, R. and ROBAKIS, N.K. (2003). A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations. *Cell* **114**: 635-645.
- MAREEL, M. and LEROY, A. (2003). Clinical, cellular, and molecular aspects of cancer invasion. *Physiol. Rev.* **83**: 337-376.
- MARIE, P.J. (2002). Role of N-cadherin in bone formation. *J. Cell. Physiol.* **190**: 297-305.
- MARY, S., CHARRASSE, S., MERIANE, M., COMUNALE, F., TRAVO, P., BLANGY, A. and GAUTHIER-ROUVIÈRE, C. (2002). Biogenesis of N-cadherin-dependent cell-cell contacts in living fibroblasts is a microtubule-dependent kinesin-driven mechanism. *Mol. Biol. Cell* **13**: 285-301.
- MATSUYOSHI, N., TANAKA, T., TODA, K.-I. and IMAMURA, S. (1997). Identification of novel cadherins expressed in human melanoma cells. *J. Invest. Dermatol.* **108**: 908-913.
- MATSUYOSHI, N., TODA, K.-I. and IMAMURA, S. (1998). N-cadherin expression in human adult T-cell leukemia cell line. *Arch. Dermatol. Res.* **290**: 223-225.
- MIALHE, A., LEVACHER, G., CHAMPELOVIER, P., MARTEL, V., SERRES, M., KNUDSEN, K. and SEIGNEURIN, D. (2000). Expression of E-, P-, N-cadherins and catenins in human bladder carcinoma cell lines. *J. Urol.* **164**: 826-835.
- MIYATANI, S., SHIMAMURA, K., HATTA, M., NAGAFUCHI, A., NOSE, A., MATSUNAGA, M., HATTA, K. and TAKEICHI, M. (1989). Neural cadherin: role in selective cell-cell adhesion. *Science* **245**: 631-635.
- MONKS, D.A., GETSIOS, S., MACCALMAN, C.D. and WATSON, N.V. (2001). N-cadherin is regulated by gonadal steroids in the adult hippocampus. *Proc. Natl. Acad. Sci. USA* **98**: 1312-1316.
- MONTELL, D.J. (2003). Border-cell migration: the race is on. *Nat. Rev. Mol. Cell Biol.* **4**: 13-24.
- NAKASHIMA, T., HUANG, C., LIU, D., KAMEYAMA, K., MASUYA, D., KOBAYASHI, S., KINOSHITA, M. and YOKOMISE, H. (2003). Neural-cadherin expression associated with angiogenesis in non-small-cell lung cancer patients. *Br. J. Cancer* **88**: 1727-1733.
- NIEMAN, M.T., PRUDOFF, R.S., JOHNSON, K.R. and WHEELLOCK, M.J. (1999). N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. *J. Cell Biol.* **147**: 631-643.
- NIETO, M.A. (2001). The early steps of neural crest development. *Mech. Dev.* **105**: 27-35.
- ODA, H., TSUKITA, S. and TAKEICHI, M. (1998). Dynamic behavior of the cadherin-based cell-cell adhesion system during *Drosophila* gastrulation. *Dev. Biol.* **203**: 435-450.
- ORDÓÑEZ, N.G. (2003). Value of E-cadherin and N-cadherin immunostaining in the diagnosis of mesothelioma. *Hum. Pathol.* **34**: 749-755.
- PANDA, D.K., MIAO, D., LEFEBVRE, V., HENDY, G.N. and GOLTZMAN, D. (2001). The transcription factor SOX9 regulates cell cycle and differentiation genes in chondrocytic CFK2 cells. *J. Biol. Chem.* **276**: 41229-41236.
- PARADIES, N.E. and GRUNWALD, G.B. (1993). Purification and characterization of NCAD90, a soluble endogenous form of N-cadherin, which is generated by proteolysis during retinal development and retains adhesive and neurite-promoting function. *J. Neurosci. Res.* **36**: 33-45.
- PAEDES, J., MILANEZI, F., VIEGAS, L., AMENDOEIRA, I. and SCHMITT, F. (2002). P-cadherin expression is associated with high-grade ductal carcinoma in situ of the breast. *Virchows Arch.* **440**: 16-21.
- PATEL, I.S., MADAN, P., GETSIOS, S., BERTRAND, M.A. and MACCALMAN, C.D. (2003). Cadherin switching in ovarian cancer progression. *Int. J. Cancer* **106**: 172-177.
- PERALTA SOLER, A., KNUDSEN, K.A., SALAZAR, H., HAN, A.C. and KESHGEGIAN, A.A. (1999). P-Cadherin expression in breast carcinoma indicates poor survival. *Cancer* **86**: 1263-1272.
- PERALTA SOLER, A., KNUDSEN, K.A., TECSON-MIGUEL, A., MCBREARTY, F.X., HAN, A.C. and SALAZAR, H. (1997). Expression of E-cadherin and N-cadherin in surface epithelial-stromal tumors of the ovary distinguishes mucinous from serous and endometrioid tumors. *Hum. Pathol.* **28**: 734-739.
- PERL, A.-K., WILGENBUS, P., DAHL, U., SEMB, H. and CHRISTOFORI, G. (1998). A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature* **392**: 190-193.
- PERRYMAN, K.J., STANTON, P.G., LOVELAND, K.L., MCLACHLAN, R.I. and ROBERTSON, D.M. (1996). Hormonal dependency of neural cadherin in the binding of round spermatids to Sertoli cells *in vitro*. *Endocrinology* **137**: 3877-3883.
- PIEDRA, J., MIRAVET, S., CASTAÑO, J., PÁLMEZ, H.G., HEISTERKAMP, N., GARCÍA DE HERREROS, A. and DUÑACH, M. (2003). p120 Catenin-associated Fer and Fyn tyrosine kinases regulate β -catenin Tyr-142 phosphorylation and β -catenin- α -catenin interaction. *Mol. Cell Biol.* **23**: 2287-2297.
- PLA, P., MOORE, R., MORALI, O.G., GRILLE, S., MARTINOZZI, S., DELMAS, V. and LARUE, L. (2001). Cadherins in neural crest cell development and transformation. *J. Cell. Physiol.* **189**: 121-132.
- PÖTTER, E., BERGWITZ, C. and BRABANT, G. (1999). The cadherin-catenin system: implications for growth and differentiation of endocrine tissues. *Endocr. Rev.* **20**: 207-239.
- PROZIALECK, W.C., FAY, M.J., LAMAR, P.C., PEARSON, C.A., SIGAR, I. and RAMSEY, K.H. (2002). *Chlamydia trachomatis* disrupts N-cadherin-dependent cell-cell junctions and sequesters β -catenin in human cervical epithelial cells. *Infect. Immun.* **70**: 2605-2613.
- PROZIALECK, W.C., LAMAR, P.C. and LYNCH, S.M. (2003). Cadmium alters the localization of N-cadherin, E-cadherin, and β -catenin in the proximal tubule epithelium. *Toxicol. Appl. Pharmacol.* **189**: 180-195.
- PUCH, S., ARMEANU, S., KIBLER, C., JOHNSON, K.R., MÜLLER, C.A., WHEELLOCK, M.J. and KLEIN, G. (2001). N-cadherin is developmentally regulated and functionally involved in early hematopoietic cell differentiation. *J. Cell Sci.* **114**: 1567-1577.
- RADICE, G.L., RAYBURN, H., MATSUNAMI, H., KNUDSEN, K.A., TAKEICHI, M. and HYNES, R.O. (1997). Developmental defects in mouse embryos lacking N-cadherin. *Dev. Biol.* **181**: 64-78.
- RHEE, J., LILIE, J. and BALSAMO, J. (2001). Essential tyrosine residues for interaction of the non-receptor protein-tyrosine phosphatase PTP1B with N-cadherin. *J. Biol. Chem.* **276**: 6640-6644.
- RHEE, J., MAHFOOZ, N.S., ARREGUI, C., LILIE, J., BALSAMO, J. and VANBERKUM, M.F.A. (2002). Activation of the repulsive receptor Roundabout inhibits N-cadherin-mediated cell adhesion. *Nat. Cell Biol.* **4**: 798-805.

- RIEGER-CHRIST, K.M., CAIN, J.W., BRAASCH, J.W., DUGAN, J.M., SILVERMAN, M.L., BOUYOUNES, B., LIBERTINO, J.A. and SUMMERHAYES, I.C. (2001). Expression of classic cadherins type I in urothelial neoplastic progression. *Hum. Pathol.* 32: 18-23.
- ROSIVATZ, E., BECKER, I., SPECHT, K., FRICKE, E., LUBER, B., BUSCH, R., HÖFLER, H. and BECKER, K.-F. (2002). Differential expression of the epithelial-mesenchymal transition regulators Snail, SIP1, and Twist in gastric cancer. *Am. J. Pathol.* 161: 1881-1891.
- SANDER, E.E., TEN KLOOSTER, J.P., VAN DELFT, S., VAN DER KAMMEN, R.A. and COLLARD, J.G. (1999). Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. *J. Cell Biol.* 147: 1009-1021.
- SANDERS, D.S.A., BLESSING, K., HASSAN, G.A.R., BRUTON, R., MARSDEN, J.R. and JANKOWSKI, J. (1999). Alterations in cadherin and catenin expression during the biological progression of melanocytic tumours. *J. Clin. Pathol.: Mol. Pathol.* 52: 151-157.
- SHAN, W.-S., TANAKA, H., PHILLIPS, G.R., ARNDT, K., YOSHIDA, M., COLMAN, D.R. and SHAPIRO, L. (2000). Functional cis-heterodimers of N- and R-cadherins. *J. Cell Biol.* 148: 579-590.
- SHINOURA, N., PARADIES, N.E., WARNICK, R.E., CHEN, H., LARSON, J.J., TEW, J.J., SIMON, M., LYNCH, R.A., KANAI, Y., HIROHASHI, S., HEMPERLY, J.J., MENON, A.G. and BRACKENBURY, R. (1995). Expression of N-cadherin and α -catenin in astrocytomas and glioblastomas. *Br. J. Cancer* 72: 627-633.
- SOLER, A.P., JOHNSON, K.R., WHEELLOCK, M.J. and KNUDSEN, K.A. (1993). Rhabdomyosarcoma-derived cell lines exhibit aberrant expression of the cell-cell adhesion molecules N-CAM, N-cadherin, and cadherin-associated proteins. *Exp. Cell Res.* 208: 84-93.
- SUYAMA, K., SHAPIRO, I., GUTTMAN, M. and HAZAN, R.B. (2002). A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. *Cancer Cell* 2: 301-314.
- TAI, C.-T., LIN, W.-C., CHANG, W.-C., CHIU, T.-H. and CHEN, G.T.C. (2003). Classical cadherin and catenin expression in normal myometrial tissues and uterine leiomyomas. *Mol. Reprod. Dev.* 64: 172-178.
- TAKEICHI, M. (1988). The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development* 102: 639-655.
- TANI, T., LAITINEN, L., KANGAS, L., LEHTO, V.-P. and VIRTANEN, I. (1995). Expression of E- and N-cadherin in renal cell carcinomas, in renal cell carcinoma cell lines *in vitro* and in their xenografts. *Int. J. Cancer (Pred. Oncol.)* 64: 407-414.
- TEPASS, U., TRUONG, K., GODT, D., IKURA, M. and PEIFER, M. (2000). Cadherins in embryonic and neural morphogenesis. *Nat. Rev. Mol. Cell Biol.* 1: 91-100.
- THIELE, A., THORMANN, M., HOFMANN, H.-J., NAUMANN, W.W., EGER, K. and HAUSCHILDT, S. (2000). A possible role of N-cadherin in thalidomide teratogenicity. *Life Sci.* 67: 457-461.
- THIERY, J.P. (2002). Epithelial-mesenchymal transitions in tumour progression. *Nat. Rev.* 2: 442-454.
- TOMITA, K., VAN BOKHOVEN, A., VAN LEENDERS, G.J.L.H., RUIJTER, E.T.G., JANSEN, C.F.J., BUSSEMAKERS, M.J.G. and SCHALKEN, J.A. (2000). Cadherin switching in human prostate cancer progression. *Cancer Res.* 60: 3650-3654.
- TRAN, N.L., ADAMS, D.G., VAILLANCOURT, R.R. and HEIMARK, R.L. (2002). Signal Transduction from N-cadherin Increases Bcl-2. Regulation of the phosphatidylinositol 3-kinase/Akt pathway by homophilic adhesion and actin cytoskeletal organization. *J. Biol. Chem.* 277: 32905-32914.
- TRAN, N.L., NAGLE, R.B., CRESS, A.E. and HEIMARK, R.L. (1999). N-cadherin expression in human prostate carcinoma cell lines. An epithelial-mesenchymal transformation mediating adhesion with stromal cells. *Am. J. Pathol.* 155: 787-798.
- TROLICE, M.P., PAPPALARDO, A. and PELUSO, J.J. (1997). Basic fibroblast growth factor and N-cadherin maintain rat granulosa cell and ovarian surface epithelial cell viability by stimulating the tyrosine phosphorylation of the fibroblast growth factor receptors. *Endocrinology* 138: 107-113.
- TSUTSUI, J., MORIYAMA, M., ARIMA, N., OHTSUBO, H., TANAKA, H. and OZAWA, M. (1996). Expression of cadherin-catenin complexes in human leukemia cell lines. *J. Biochem.* 120: 1034-1039.
- TUAN, R.S. (2003). Cellular signaling in developmental chondrogenesis: N-cadherin, Wnts, and BMP-2. *J. Bone Joint Surg. Am.* 85-A Suppl 2: 137-141.
- TUFAN, A.C. and TUAN, R.S. (2001). Wnt regulation of limb mesenchymal chondrogenesis is accompanied by altered N-cadherin-related functions. *FASEB J.* 15: 1436-1438.
- TUFAN, A.C., DAUMER, K.M., DELISE, A.M. and TUAN, R.S. (2002). AP-1 transcription factor complex is a target of signals from both Wnt-7a and N-cadherin-dependent cell-cell adhesion complex during the regulation of limb mesenchymal chondrogenesis. *Exp. Cell Res.* 273: 197-203.
- UEMURA, K., KITAGAWA, N., KOHNO, R., KUZUYA, A., KAGEYAMA, T., CHONABAYASHI, K., SHIBASAKI, H. and SHIMOHAMA, S. (2003). Presenilin 1 is involved in maturation and trafficking of N-cadherin to the plasma membrane. *J. Neurosci. Res.* 74: 184-191.
- UTSUKI, S., SATO, Y., OKA, H., TSUCHIYA, B., SUZUKI, S. and FUJII, K. (2002). Relationship between the expression of E-, N-cadherins and beta-catenin and tumor grade in astrocytomas. *J. Neurooncol.* 57: 187-192.
- UTTON, M.A., EICKHOLT, B., HOWELL, F.V., WALLIS, J. and DOHERTY, P. (2001). Soluble N-cadherin stimulates fibroblast growth factor receptor dependent neurite outgrowth and N-cadherin and the fibroblast growth factor receptor co-cluster in cells. *J. Neurochem.* 76: 1421-1430.
- VAN AKEN, E., DE WEVER, O., CORREIA DA ROCHA, A.S. and MAREEL, M. (2001). Defective E-cadherin/catenin complexes in human cancer. *Virchows Arch.* 439: 725-751.
- VAN AKEN, E.H., DE WEVER, O., VAN HOORDE, L., BRUYNEEL, E., DE LAEY, J.-J. and MAREEL, M.M. (2003). Invasion of retinal pigment epithelial cells: N-cadherin, hepatocyte growth factor, and focal adhesion kinase. *Invest. Ophthalmol. Vis. Sci.* 44: 463-472.
- VAN RAAMSDONK, C.D. and TILGHMAN, S.M. (2000). Dosage requirement and allelic expression of *PAX6* during lens placode formation. *Development* 127: 5439-5448.
- VOLK, T. and GEIGER, B. (1984). A 135-kd membrane protein of intercellular adherens junctions. *EMBO J.* 3: 2249-2260.
- WAHL, J.K.III., KIM, Y.J., CULLEN, J.M., JOHNSON, K.R. and WHEELLOCK, M.J. (2003). N-cadherin-catenin complexes form prior to cleavage of the proregion and transport to the plasma membrane. *J. Biol. Chem.* 278: 17269-17276.
- WALLIS, J., FOX, M.F. and WALSH, F.S. (1994). Structure of the human N-cadherin gene: YAC analysis and fine chromosomal mapping to 18q11.2. *Genomics* 22: 172-179.
- WHEELLOCK, M.J. and JOHNSON, K.R. (2003). Cadherin-mediated cellular signaling. *Curr. Opin. Cell Biol.* 15: 509-514.
- WILLIAMS, E., FURNESS, J., WALSH, F.S. and DOHERTY, P. (1994). Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, NCAM and N-cadherin. *Neuron* 13: 583-594.
- WILLIAMS, E., WILLIAMS, G., GOUR, B.J., BLASCHUK, O.W. and DOHERTY, P. (2000a). A novel family of cyclic peptide antagonists suggests that N-cadherin specificity is determined by amino acids that flank the HAV motif. *J. Biol. Chem.* 275: 4007-4012.
- WILLIAMS, E.-J., WILLIAMS, G., GOUR, B., BLASCHUK, O. and DOHERTY, P. (2000b). INP, a novel N-cadherin antagonist targeted to the amino acids that flank the HAV motif. *Mol. Cell. Neurosci.* 15: 456-464.
- WILLIAMS, E.-J., WILLIAMS, G., HOWELL, F.V., SKAPER, S.D., WALSH, F.S. and DOHERTY, P. (2001). Identification of an N-cadherin motif that can interact with the fibroblast growth factor receptor and is required for axonal growth. *J. Biol. Chem.* 276: 43879-43886.
- WILLIAMS, G., WILLIAMS, E.-J. and DOHERTY, P. (2002). Dimeric versions of two short N-cadherin binding motifs (HAVDI and INPISG) function as N-cadherin agonists. *J. Biol. Chem.* 277: 4361-4367.
- WONG, A.S.T., MAINES-BANDIERA, S.L., ROSEN, B., WHEELLOCK, M.J., JOHNSON, K.R., LEUNG, P.C.K., ROSKELLEY, C.D. and AUERSPERG, N. (1999). Constitutive and conditional cadherin expression in cultured human ovarian surface epithelium: influence of family history of ovarian cancer. *Int. J. Cancer* 81: 180-188.
- XU, G., ARREGUI, C., LILIE, J. and BALSAMO, J. (2002). PTP1B modulates the association of β -catenin with N-cadherin through binding to an adjacent and partially overlapping target site. *J. Biol. Chem.* 277: 49989-49997.

- XU, Y. and CARPENTER, G. (1999). Identification of cadherin tyrosine residues that are phosphorylated and mediate Shc association. *J. Cell. Biochem.* 75:264-271.
- YANAGIMOTO, K., SATO, Y., SHIMOYAMA, Y., TSUCHIYA, B., KUWAO, S. and KAMEYA, T. (2001). Co-expression of N-cadherin and α -fetoprotein in stomach cancer. *Pathol. Int.* 51:612-618.
- YANAGISAWA, M., KAVERINA, I.N., WANG, A., FUJITA, Y., REYNOLDS, A.B. and ANASTASIADIS, P.Z. (2004). A novel interaction between kinesin and p120 modulates p120 localization and function. *J. Biol. Chem.* 279: 9512-9521 (DOI: 10.1074/jbc.M310895200).
- YAP, A.S. and KOVACS, E.M. (2003). Direct cadherin-activated cell signaling: a view from the plasma membrane. *J. Cell Biol.* 160:11-16.
- ZARKA, T.A., HAN, A.C., EDELSON, M.I. and ROSENBLUM, N.G. (2003). Expression of cadherins, p53, and BCL2 in small cell carcinomas of the cervix: potential tumor suppressor role for N-cadherin. *Int. J. Gynecol. Cancer* 13: 240-243.
- ZHANG, H., TOYOFUKU, T., KAMEI, J. and HORI, M. (2003). GATA-4 regulates cardiac morphogenesis through transactivation of the N-cadherin gene. *Biochem. Biophys. Res. Commun.* 312: 1033-1038.