# MOLECULAR ASPECTS OF CANCER METASTASIS : EXTRACELLULAR REGULATION OF THE E-CADHERIN/CATENIN COMPLEX.

Marc MAREEL, Tom BOTERBERG, Veerle NOË, Eric BRUYNEEL and Marc BRACKE
*Laboratory of Experimental Cancerology, University Hospital, De Pintelaan, 185, B-9000 Ghent, Belgium.*

Cancer is a disease of growth and differentiation. The respective activation or inactivation of oncogenes and tumor-suppressor genes transforms a normal tissue into a benign, i.e. noninvasive tumor. Invasion-promoter and invasion-suppressor genes are related to differentiation more than to growth regulation. Their respective activation or inactivation transforms a benign tumor into an invasive, i.e. malignant, one (Mareel et al., 1993). Invasive tumors are malignant because they escape therapeutic control through local extension or formation of distant metastases. We consider cancer invasion within the frame of a microecosystem that functions through the molecular crosstalk between cancer cells and host cells. Such crosstalks establish invasion-suppressor and invasion-promoter complexes, implicated in cell-cell and cell-substrate adhesion, migration and proteolysis. It is crucial that the elements of such complexes are produced by both the cancer cells and the host cells.

The epithelial cell-cell adhesion molecule E-cadherin forms a signal transducing complex with the catenins, with other cytoplasmic proteins as well as with transmembrane receptors (Figure 1). This complex may act as an invasion-suppressor as evidenced by observations on experimental and clinical cancers (Bracke et al., 1996). The complex can be regulated at multiple levels through its different elements: mutations in cadherin or catenin genes, mRNA stability, phosphorylation of ß-catenin, intracellular and extracellular associations with other proteins (Takeichi et al., 1995).

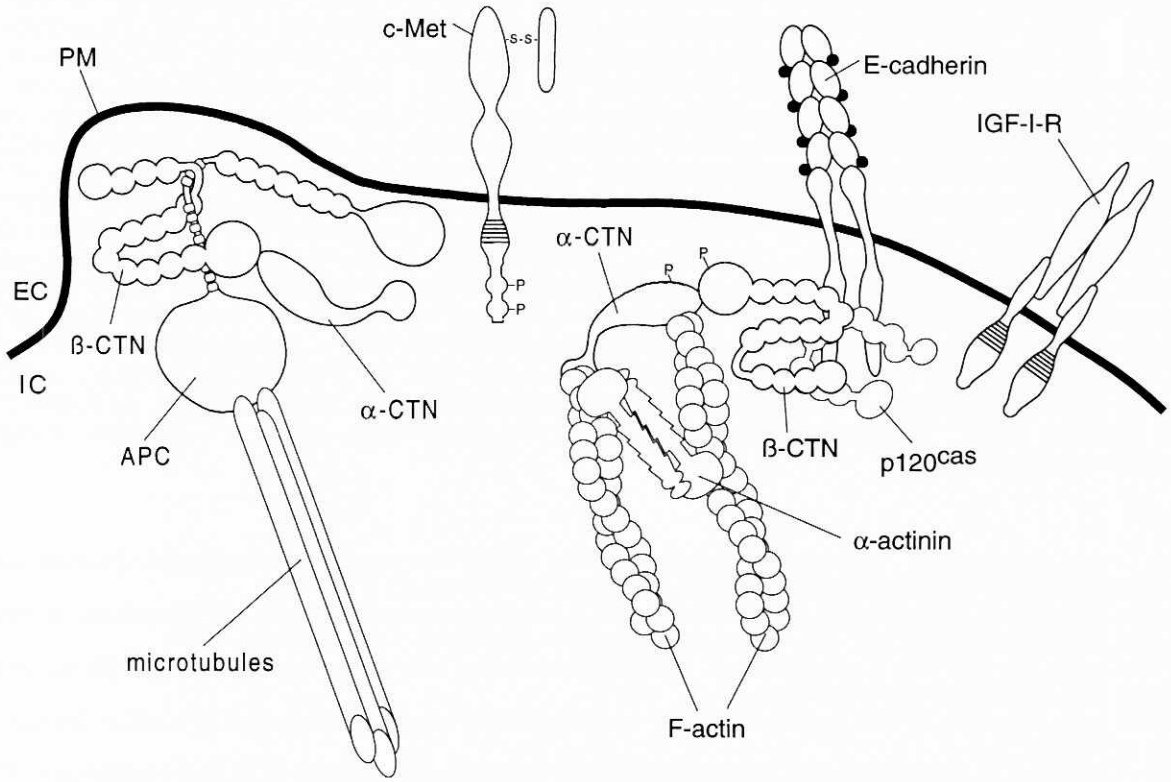


Figure 1. Schematic representation of the E-cadherin/catenin and catenin/APC complexes and their relationship with the cytoskeleton. EC, extracellular; PM, plasmamembrane; IC, intracellular; α-CTN, α-catenin; ß-CTN, ß-catenin; p120$^{cas}$; cadherin-associated *src* substrate; APC, adenomatous polyposis coli protein; P, phosphorylation site; c-Met, scatter factor/hepatocyte growth factor receptor; IGF-I-R, insulin-like growth factor I receptor. Filled circles indicate $Ca^{2+}$-binding domains ions at the cell surface proximal end of cadherin protomers. Tandemly repeated circles in ß-catenin, p120$^{cas}$ and APC indicate armadillo domains in the respective molecules. Stripes symbolize kinase domains. Modified after Mareel et al. (1996).

We are currently interested in the downregulation of the E-cadherin complex by soluble factors in the extracellular milieu. Reversible downregulation of the E-cadherin/catenin complex has raised the question about a possible role for

T. Boterberg and V. Noë are Research Assistants of the N.F.W.O. (Kom op Tegen Kanker).

cytokines in microecosystems of invasion. The human squamous carcinoma cell line COLO 16 is known to secrete several cytokines, including a putative ligand for the c-erbB2 tyrosine kinase receptor. Conditioned medium from COLO 16 cells was tested with two variants of the human MCF-7 breast cancer cell family : the invasive MCF-7/6 and the non-invasive MCF-7/AZ variant, both expressing the E-cadherin/catenin complex in a way that is sensitive to functional modulation by extrinsic agents (Bracke et al., 1991). Conditioned medium from COLO 16 cultures rapidly (after about 1 h) induced scattering of MCF-7/AZ, but not of MCF-7/6 cells, in culture on solid substrate. This medium also diminished fast calcium-dependent E-cadherin-specific aggregation of MCF-7/AZ cells, but did not induce their invasion into precultured heart fragments. Immunocytochemical staining showed loss of the typical honeycomb staining pattern of E-cadherin that is localized at cell-cell contacts. Confocal laser scanning microscopy suggests internalization and/or accumulation of E-cadherin in the cytoplasm. IL(interleukin)-1, IL-6, GM-CSF (granulocyte monocyte-colony stimulating factor), SCF (stem cell factor) or TGF (transforming growth factor) -ß failed to mimic these effects of the COLO 16-conditioned medium. There is little reason to accept the implication of SF/HGF (scatter factor/hepatocyte growth factor), known to inactivate the complex by tyrosine phosphorylation of ß-catenin. MRC-5 supernatants or purified SF/HGF failed to mimic the effects of the conditioned medium (our unpublished results in collaboration with J. Jouanneau). Moreover, MCF-7 is known to be insensitive to scatter factor. We systematically searched for differences in adhesion molecules between MCF-7/AZ and MCF-7/6 cells. So far, no immunocytochemical differences were found for vinculin, perlecan (antibodies provided by G. David) or the $\alpha_2$-integrin subunit. Differences were, however, found with antibodies recognizing both syndecan 1 and 3 or the $\beta_1$-integrin subunit with stronger signals for MCF-7/6 than MCF-7/AZ cells. It is interesting that coexpression of syndecan 1 and E-cadherin has been reported recently (Leppä et al., 1996).

Fragments from the extracellular part of E-cadherin have been implicated in downregulation of the complex (Willems et al., 1995). Regulation of the homophilic interactions of E-cadherin implicates its first extracellular domain, containing an H-A-V sequence. Decapeptides containing the H-A-V sequence as well as cadherin-specific flanking sequences interfere with the antiinvasive function of the complex, possibly because they cause tyrosine phosphorylation of ß-catenin. These observations suggest an autocrine loop of downregulation in which invasion is maintained by breakdown fragments of the E-cadherin extracellular domain. A decapeptide (LFSHAVSSNG) derived from human E-cadherin inhibited fast aggregation of MDCK ts.*src* Cl2 cells expressing E-cadherin. This decapeptide as well as DECMA-1 ( a monoclonal antibody against dog E-cadherin) also specifically stimulated invasion into collagen type I and into precultured heart fragments. Invasion into collagen type I was even more stimulated when the H-A-V peptide was elongated to 20 amino acids. In MDCK ts.*src* Cl2 treated with the human E-cadherin decapeptide and also with DECMA-1, a stimulation of tyrosine phosphorylation of ß-catenin was observed, which correlates with inactivation of the E-cadherin/catenin complex. Herbimycin A inhibited the stimulatory effect of the decapeptide in the collagen type I invasion assay as well as tyrosine phosphorylation of ß-catenin. Our observations indicate that E-cadherin functions, including suppression of cancer cell invasion may be regulated by peptides or breakdown products derived from E-cadherin. The production of such fragments may be promoted by an increased proteolytic activity of invasive cells. Such mechanism underscores the coordinated action of promoter complexes in microecosystems of invasion.

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