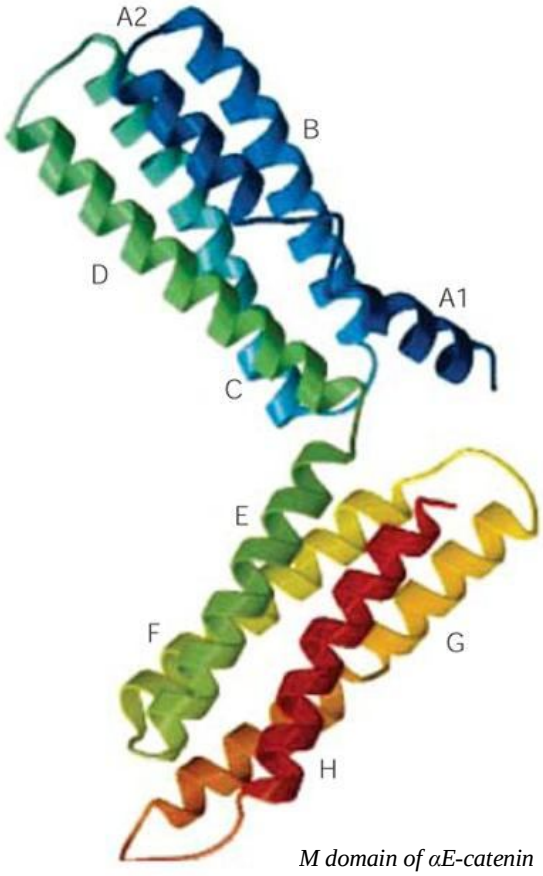
α-catenin in the adherens junction complex

and the possible implications in cancer



M domain of αE-catenin

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Content

1. Introduction

Epithelial cells line organs, glands and surfaces of structures throughout the whole organism. A key property of epithelial tissues is structural integrity. External and internal epithelia form a protective barrier, while internal epithelia also create physiologically controlled subdomains within an organism (Radisky, 2005). Epithelia have an apical upper surface exposed to the body exterior or cavity of an internal organ, and basal lower surface, that is attached to the basal lamina. Cellular binding structures near the apical surface differ in form and function from those regions near the basal surface. Most epithelia contain microvilli, fingerlike extensions of the plasma membrane that functionally increase the exposed surface area. Adjacent to the basal surface is the basal lamina, a thin supporting sheet consisting of glycoproteins that are secreted by epithelial cells. Epithelial cells are held together in intimate contact with each other and the underlying connective tissue (Figure 1).

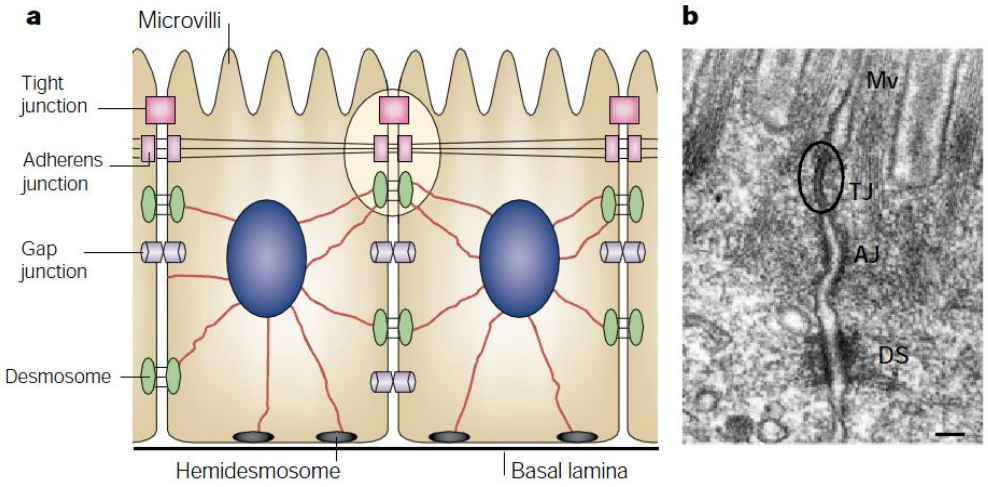


Figure 1: Intestinal epithelial cells with adherens junctions, tight junctions and desmosomes. a | the junctional complex at the most apical region of lateral membranes is circled. **b** | electron micrograph of the junctional complex in mouse intestinal epithelial cells. The junction is circled. Mv, microvilli; TJ, tight junction; AJ, adherens junction; DS, desmosome. Scale bar: 200nm. (Tsukita, Furuse, & Itoh, 2001)

As can be seen in Figure 1, the junctional complex of simple epithelial cells consists of three components; tight junctions, adherens junctions and desmosomes. The adherens junctions (AJ) are the strongest and play an important role in tissue integrity. During cytokinesis or cell migration, cell contacts are broken and re-established, which means that the adhesive state of cells is highly dynamic (Pokutta & Weis, 2000).

Cell-cell junctions are organized by adhesion proteins and the underlying cytoskeleton. They can respond to signals that eminate from changes in cell shape during tube formation and cell movement during convergent extension in gastrulation (Keller, 2002; Lubarsky & Krasnow, 2003). The junctions serve as a permeability barrier between different biological comportments in the body (Drees et al., 2005).

Part of embryonic development in which the junctional complex is critically restructured is the epithelial to mesenchymal transition (EMT) (Thiery, 2002) (Figure 2). It is an essential step to progress beyond the blastula stage of embryonic development. One of the steps in EMT is downregulation of certain proteins of the adherens junction complex, like E-cadherin. This is necessary for the cells to be able to detach from each other (Thiery, 2002). Primitive epithelial cells undergo EMT to from non-epithelial cells that are loosely embedded in an extracellular matrix (Thiery, 2002). For the migration in an extracellular environment and for colonization of areas involved in organ formation, epithelial cells need appropriate morphology.

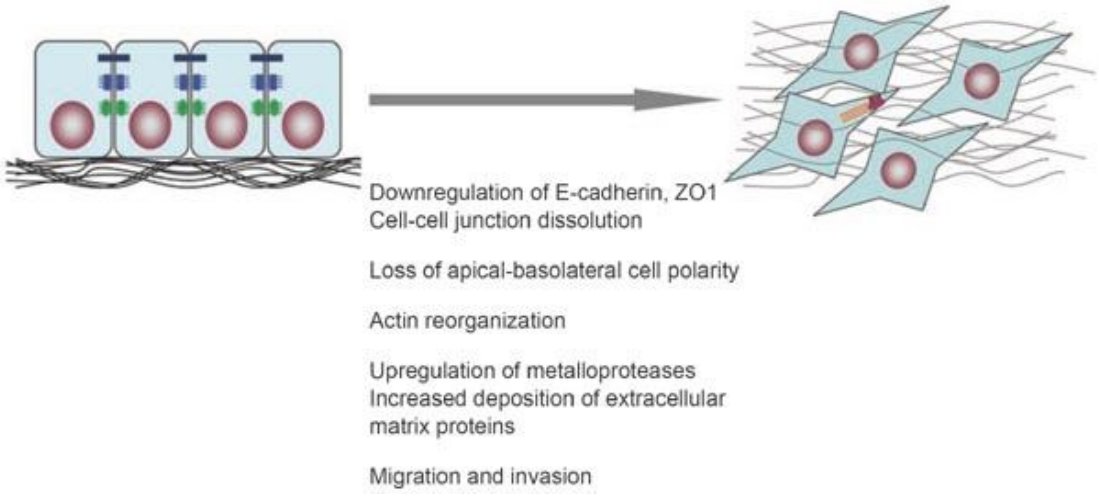


Figure 2: Epithelial mesenchymal transition. Occurs when epithelial cells lose their epithelial characteristics, including dissolution of cell-cell junctions (tight junctions (black), adherens junctions (blue) and desmosomes (green)) and loss of apical-basolateral polarity. The cells acquire a mesenchymal phenotype characterized by actin reorganization and stress fiber formation (red), migration and invasion. (Xu, Lamouille, & Derynck, 2009)

When the integrity of the AJ complex is compromised, which is often the case in cancer, this can lead to invasion and metastasis. Several components of the complex that may play a role in cancer will be described later in this report.

2. αE-catenin in development and homeostasis

Epithelial cells are linked together by adherens junctions, tight junctions and desmosomes (Figure 1). The adherens junctions are the strongest and most important for tissue integrity.

2.1 Adherens junctions

Adherens junctions (AJ) are responsible for the initiation and stabilization of cell-cell adhesion, regulation of the actin cytoskeleton, intracellular signaling and transcriptional regulation (Hartsock & Nelson, 2008). They are found between many different cell types, such as epithelial cells, cardiac myocytes and fibroblasts (Yap et al., 1997). The classical adherens junctions are cadherin-based and mainly function in anchoring the actin cytoskeleton and mediating cell-cell adhesion (Yap et al., 1997). To form cell-cell adhesions, individual cadherins self-associate extracellularly. Classical cadherins are linked to the actin cytoskeleton by interacting with several catenins such as β-catenin and p120 catenin and indirect interaction with α-catenin (Knudsen et al., 1995). The exact biochemical composition and spatio-temporal regulation of the AJ is still largely unknown.

2.2 Tight junctions

The tight junction, or zonula occludens, is a site where two cells are attached closely together in epithelial and endothelial cellular sheets. Cells are linked with each other to maintain structural integrity of the sheets that line fluid comportments. The intercellular space between these adjacent cells is sealed, so there is no diffusion of solutes possible. Tight junctions vary in tightness depending on the cell type and the permeability can be regulated (Madara, 1988). Furthermore, they are thought to function as a fence between apical and basolateral membrane domains and they can function as a gate, controlling paracellular passage of ions and solutes between cells (Schneeberger & Lynch, 1992).

The tight junctions' strands are associated laterally, in the opposing membrane of the adjacent cells, thus forming paired tight junction strands. An integral membrane protein localized at tight junctions is occludin, the first identified protein in chicken and mammals (Ando-Akatsuka et al., 1996). Other transmembrane proteins that are integral components of tight junction strands are claudin-1, -2 and zonula occludens (ZO)-1 and ZO-2 (Tsukita et al., 2001).

2.3 Desmosomes

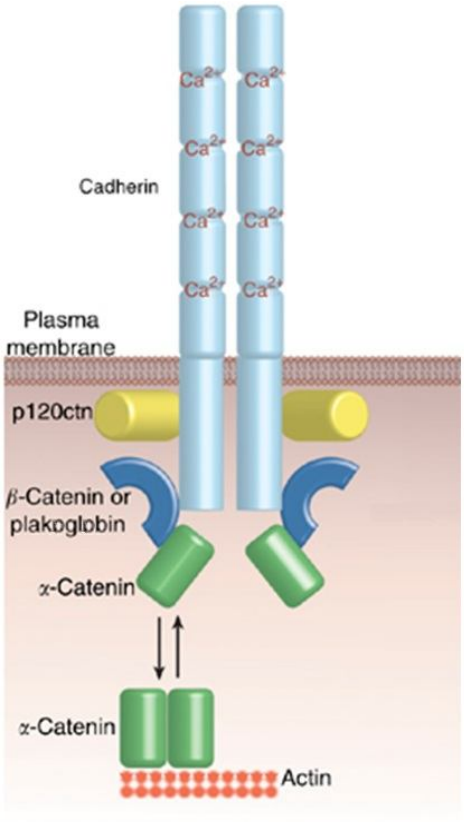
Desmosomes are intercellular junctions involved in adhesion that attach intermediate filaments (keratin) to regions of tight cell-cell adhesion. They are composed of transmembrane proteins of the cadherin family linked to the intermediate filament cytoskeleton. This linkage is probably through γ-catenin (plakoglobin) and desmoplakin (Kowalczyk et al., 1998).

2.4 Construction of the adherens junction

2.4.1 Cadherins

Cadherins are Ca^{2+} dependent cell surface glycoproteins involved in cell-cell adhesion with an N-terminal extracellular region, or ectodomain, a transmembrane anchor and a C-terminal intracellular region. There are two kinds of adhesion; homophilic (homotypic) adhesion, the intercellular contact of cells expressing the same cadherin, and heterophilic (heterotypic) adhesion, where intercellular contact of cells expressing different cadherins takes place. Strand exchange is a mode of cadherin dimerization in which a β-strand of one cadherin domain binds another domain and vice versa (Pokutta & Weis, 2007).

E (epithelial)-cadherin is a single-pass, transmembrane glycoprotein and a member of the classical cadherin family, just like N (neuronal)-, P (placental)-, and R (retinal)-cadherin (Menger & Vollmar, 1996). There are five characteristic extracellular cadherin repeat domains for the classical cadherins (Halbleib & Nelson, 2006). E-cadherin is known as uvomorulin, L-CAM, cell-CAM 120/80, or Arc-1 (Wijnhoven, Dinjens, & Pignatelli, 2000).



Cadherin-based adhesion is controlled by intracellular linkage to catenins through various mechanisms. The catenin p120 is an inhibitor of cadherin endocytosis by inhibiting clathrin mediated endocytosis. This way it stabilizes cadherins at the cell surface (Davis et al., 2003; Yap et al., 2007).

2.4.2 Catenins

Except for α-catenin, all catenins (β-catenin, plakoglobin and p120-catenin) share sequence similarity and they belong to the armadillo family of proteins. α-catenin has a different sequence and structural organization. Figure 3 depicts the different catenins of the adherens junction complex.

Figure 3: Adherens junction complex. E-cadherin (light blue), p120-cat/δ-catenin (yellow), plakoglobin/γ-catenin or β-catenin (blue) α-catenin (green). (Niessen, 2007)

p120-catenin (p120ctn) was first identified as a substrate for Src-tyrosine receptor kinase and has sequence homology to the armadillo domain of β-catenin. It has been proposed that p120ctn plays a role in E-cadherin stabilization at the plasma membrane during the formation of cell-cell contacts (Davis et al., 2003). The interaction between p120ctn and E-cadherin is needed to increase adhesiveness of cells. p120ctn interacts with Rho family GTPases and works as a regulator of cell motility through the actin cytoskeleton (Noren et al., 2000).

γ-catenin or plakoglobin (Figure 3) is the only known common cytoplasmic protein between adherens junctions and in desmosomes. It serves as a regulatory protein for desmosome organization (Lewis et al., 1997).

β-catenin (Figure 3) contains 13 repeats of a characteristic armadillo domain of ~42 amino acids that form a triple α-helix. It interacts with E-cadherin by binding the C-terminal cytoplasmic domain of E-cadherin in a phosphor-regulated manner (Perez-Moreno & Fuchs, 2006). It may be the case that the interaction between E-cadherin and β-catenin occurs in the endoplasmic reticulum (ER) and that it is required for cadherin exit from the ER (Chen et al., 1999). Excess β-catenin is targeted to the proteosome, which keeps the cytosolic levels normally low. BCL9-2, a transcription factor involved in epithelial-mesenchymal transition, might have a role in mediating a switch between the adhesive and transcriptional functions of β-catenin. This switch is caused by phosphorylation of Y142 on β-catenin (Brembeck et al., 2004).

β-catenin can bind IQGAP, fascin and α-catenin. Phosphorylation of β-catenin by GSK3β leads to ubiquitination and degradation of β-catenin in proteasomes. The Wnt/Wingless signaling pathway stabilizes β-catenin by inhibiting GSK-3β. The pools of GSK3β are constantly active, but upon Wnt activation they are inhibited by phosphorylation, which results in nuclear accumulation (Prasad et al., 2009). When β-catenin accumulates in cell-cell junctions, it has a second function as nuclear transcriptional co-activator for the lymphoid enhancer-binding factor-1 (LEF1)/T-cell specific factor (TCF) family of DNA-binding proteins (Jamora & Fuchs, 2002; Kobielak & Fuchs, 2004).

α-catenin, or cadherin-associated protein (CAP)-102, (Figure 3) is a conserved protein across the eukaryotic kingdom that can bind β-catenin in the adherens junction complex. There are two different subtypes of α-catenin, epithelial αE-catenin and neural αN-catenin. Human and mouse αE-catenin proteins are highly conserved, with 99,2% identity. The gene encoding for α-catenin is called CTNNA1.

It used to be the general idea that αE-catenin linked E-cadherin to the actin cytoskeleton. In 2005, Drees and Yamada indicated that αE-catenin may not able to bind the E-cadherin-β-catenin complex and actin at the same time. This inability to simultaneously bind suggests a role of αE-catenin as a molecular switch. When αE-catenin binds one of those two binding partners, the ability to interact with the other

changes (Drees et al., 2005; Yamada et al., 2005). Purified α-catenin can exist as a monomer and homodimer in solution and as a heterodimer with β-catenin (Drees et al., 2005). Homodimerization and heterodimerization compete with one another (Gates & Peifer, 2005). The domain for β-catenin binding and the domain for homodimerization overlap within amino acids 57-143 (Pokutta & Weis, 2000). The domains for actin and β-catenin binding do not overlap. α-catenin in its monomeric state can bind β-catenin, but not actin. Homodimeric α-catenin cannot bind β-catenin, but does bind actin (Yamada et al., 2005).

α-catenin is a relative of vinculin that shows sequence similarities (Figure 4). Both vinculin and α-catenin have the same binding partners; actin and α-actinin. While vinculins show interaction between their head and tail segments, α-catenin has a less flexible state. α-catenin probably comprises a series of repeating antiparallel α-helical domains (Kobielak & Fuchs, 2004).

α-catenin must be locally concentrated for making dimerization possible by clustering the cadherin-catenin complex during cell-cell adhesion. α-catenin switches between the α-catenin plasma membrane pool that is monomeric and bound to β-catenin, the cytoplasmic pool that is monomeric, and the cytoskeleton pool that is dimeric and can bind actin. This dynamic changing makes it possible to bind either the actin cytoskeleton or the adherens junction complex (Drees et al., 2005).

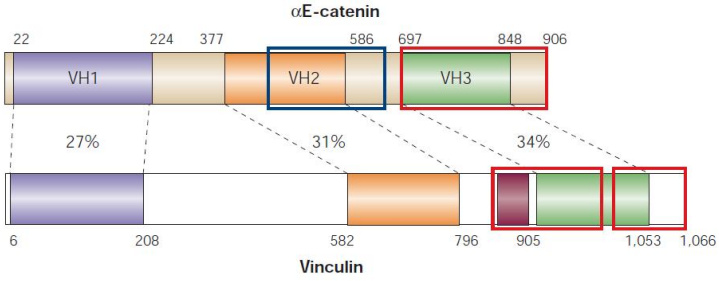


Figure 4: Sequence similarities between αE-catenin and vinculin. The colored areas show similarities between the two proteins, with the degree of similarity shown in percentages. The magenta region in vinculin shows the proline rich hinge segment. The open red boxes indicate actin binding domains. In the blue open box the intercellular adhesion modulation (M) domain is depicted. The numbers in this picture are the amino acids of the protein sequence. VH stands for vinculin-homology domain. (Kobielak & Fuchs, 2004)

α-catenin is required in the formation of adherens junction in epithelial cells. Cells that lack α-catenin show impaired cell-cell adhesion, and when α-catenin is reintroduced, the formation of adherens junctions is restored (Vasioukhin et al., 2001). It is assumed that disturbed cell-cell adhesion is preceded by mutations in genes involved in cell-cycle regulation that lead to uncontrolled growth. Conditional inactivation of αE-catenin in skin epithelium results in epidermal hyperproliferation (Vasioukhin et al., 2001). Additional experiments showed activation of the Ras-ERK/MAPK (extracellular signal-regulated kinase/mitogen-activated protein kinase) pathway, which seemed to involve the insulin-insulin-like-growth-factor signal-

transduction pathway (Vasioukhin et al., 2001). To test whether these perturbations occurred independently of the effects on intercellular adhesion, skin from α-catenin null mice was compared with skin conditionally targeted for loss of desmoplakin, a protein that links the cytoskeleton to desmosomes. Both animals showed severe intercellular adhesion defects. However, the desmoplakin knockout animals showed normal epidermal polarity and proliferation in contrary to the α-catenin null animals. The defects in polarity and cell proliferation are a specific and direct consequence of the absence of α-catenin and these disturbances occurred independently of the effects on intercellular adhesion (Vasioukhin et al., 2001). This indicates that the E-cadherin-β-catenin complex may interact with components of signal transduction pathways involved in cell-cycle regulation, when α-catenin is not present (Kobielak & Fuchs, 2004).

A role for α-catenin was shown in the formation of radial actin cables, by looking at keratinocytes in which the αE-catenin gene (CTNNA1) was inactivated. The actin cables seal membranes and assemble epithelial sheets necessary for the stabilization of AJ (Kobielak & Fuchs, 2004).

2.4.3 Binding partners of α-catenin

There are several binding partners of αE-catenin (Figure 5); α-actinin, vinculin, zonula occludens-1 (ZO1) and afadin. Afadin binds both α-catenin and actin filaments and might recruit α-catenin to adherens junctions. Afadin also binds nectin, the protein that forms the transmembrane core of another intercellular adhesion system. Other association partners of αE-catenin are amongst others vasodilator-stimulated phosphoprotein (VASP) and Enabled (Ena). The localization of these proteins to adherens junctions is αE-catenin dependent (Kobielak & Fuchs, 2004).

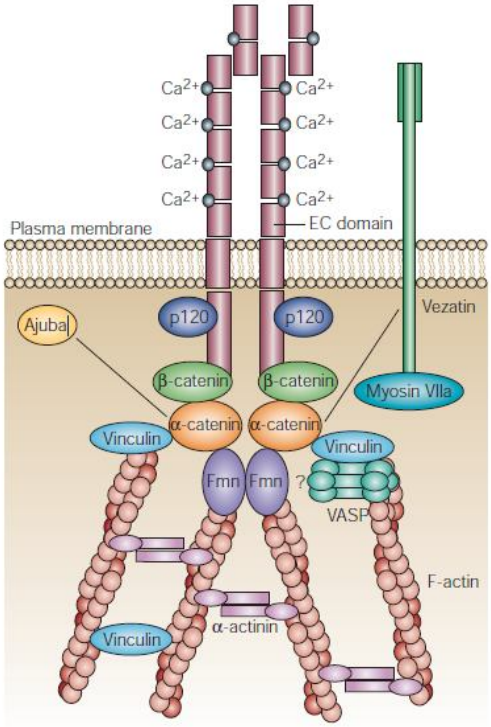


Figure 5: The adherens junction complex. E-cadherin homodimerizes at the membrane. Stabilization of intercellular adhesion requires the cytoplasmic domain of E-cadherin, which binds β-catenin. β-catenin binds α-catenin, which is central in recruiting a number of cytoskeletal proteins, such as filamentous-actin-nucleating formin proteins (Fmn), and actin-binding proteins vinculin, Ajuba, myosin VIIa, vezatin, α-actinin and members of the vasodilator-stimulated phosphoprotein (VASP) family of F-actin-elongating proteins. Zonula occludens-1, afadin and Enabled are not shown in this picture. (Kobielak & Fuchs, 2004)

Further, αE-catenin associates with members of the zyxin family, which are also able to bind actin and to recruit members of the Ena and VASP families. Ajuba is a member of the zyxin family. It has been shown to interact directly with αE-catenin and it might contribute to connecting adherens junctions to actin filaments. The presence of these Ena/VASP proteins can explain addition of actin subunits to existing actin filaments. For *de novo* actin polymerization this might not be sufficient (Kobielak & Fuchs, 2004).

2.4.4 Interactions in the adherens junction complex

Cell-cell adhesion mediated by cadherins is determined by distinct protein interactions where both extracellular and cytoplasmic domains are involved. The extracellular domain forms relatively weak bonds with adjacent cells. The cell-cell adhesion is strengthened by lateral clustering of cadherins mediated by proteins linking the cytoplasmic domain to the actin cytoskeleton. The cytoplasmic domain of E-cadherin forms a complex with β-catenin, which on its turn binds α-catenin (Yamada et al., 2005).

Different domains of αE-catenin can interact with actin-binding proteins or the actin skeleton (Figure 6), showing overlapping binding domains for β-catenin binding and α-catenin dimerization (Kobielak & Fuchs, 2004). This is also the case for the domain for binding actin filaments and vinculin.

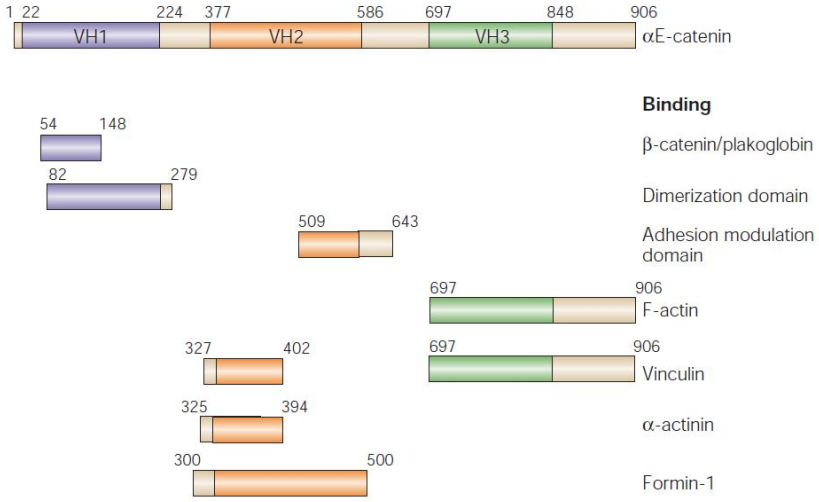


Figure 6: Functionally important regions of αE-catenin. Three vinculin homology (VH) domains are shown (VH1-VH3). The direct binding partners of αE-catenin are listed on the right, next to the corresponding domain diagrams where the regions and encompassing amino-acid residues of αE-catenin are depicted. αE-catenin interacts with the E-cadherin-β-catenin complex through its amino-terminal β-catenin-binding domain. In solution αE-catenin from a homodimer through its dimerization domain. The adhesion modulation domain is the part of αE-catenin necessary for mediating intercellular adhesion. The vinculin-, α-actinin- and formin-1 binding domains on αE-catenin facilitate organization of the actin cytoskeleton and regulation of actin dynamics during formation of cell-cell adhesion.(Kobielak & Fuchs, 2004)

Newly synthesized E-cadherin associates with β-catenin in the endoplasmic reticulum (ER). At the cell surface the E-cadherin-β-catenin complex is joined by α-catenin (Hinck et al., 1994). This was shown by using pulse chase experiments to track protein movement in a cell. In *Drosophila*, during disassembly of the adherens junction, α-catenin and actin detach from the complex (Oda et al., 1998).

α-catenin seems to link the E-cadherin-β-catenin complex and the actin cytoskeleton. How this is exactly regulated is not clear. Since it was shown that α-catenin binds to the cadherin-catenin complex and actin filaments independently (Huber et al., 2001; Pokutta & Weis, 2000; Pokutta et al., 2002), it was assumed that E-cadherin-β-catenin complexes are directly linked to actin filaments through α-catenin forming a quaternary complex (Rimm et al., 1995). However, this simultaneous binding has not been formally demonstrated. Yamada and colleagues found that there is no simultaneous interaction of α-catenin with actin filaments and the E-cadherin-β-catenin complex. Based on *in vitro* evidence, they suggest that α-catenin shuttles between E-cadherin-β-catenin complexes and actin filaments. In the cytosol α-catenin exist as a homodimer and when it has the form of a monomer it can bind to β-catenin (Drees et al., 2005; Yamada et al., 2005).

Moreover, α-catenin may also mediate interaction between E-cadherin-β-catenin complexes and the actin cytoskeleton through other proteins like vinculin and EPLIN (epithelial protein lost in neoplasm) (Abe & Takeichi, 2008; Watabe-Uchida et al., 1998; Weiss et al., 1998). EPLIN links α-catenin and actin filaments at adherens junction. The recruitment of vinculin at adherens junctions by α-catenin requires actomyosin tension (Miyake et al., 2006). Another possibility is direct α-catenin-mediated linkage through additional (unknown) proteins. This would be with such a low affinity that it is not visible *in vitro*, but it would be there *in vivo* in clusters of a few thousand molecules (Lecuit, 2010).

Knudsen and colleagues (Knudsen et al., 1995) are one of the research groups that proposed that the cadherin-catenin complexes are linked to the actin cytoskeleton via a direct association between α-catenin and α-actinin. They based this idea on the results from their experiments in which they showed that α-actinin coimmunoprecipitates with the N-cadherin-catenin complex in an actin–independent manner. When α-catenin was not present, α-actinin did not associate with the N-cadherin-catenin complex, hence concluding that the cadherin-catenin complex is linked to actin via a direct interaction between α-catenin and α-actinin (Knudsen et al., 1995). Recent work of le Duc and colleagues shows that upon cell-cell adhesion, the actin binding protein vinculin is localized at cell-cell junctions where the junctions contact actin filament bundles (le Duc et al., 2010). This was shown in MDCK cells coated with E-cadherin-cartilage oligomerizing protein (COMP), which induces formation of actin-anchored E-cadherin adhesions. They showed that the E-cadherin complex is a mechanosensor that leads to a change in the mechanics of adherens junctions. This requires a direct mechanical link to an organized and contractile actin

cytoskeleton. From the results of le Duc and colleagues can be concluded that there is a role for vinculin in the modulation of the E-cadherin-cytoskeleton mechanics and remodeling of cell-cell junctions (le Duc et al., 2010).

Vinculin and α-actinin are considered to be good candidates for linking the cadherin-catenin complex via α-catenin with the actin cytoskeleton. However, Yamada and colleagues showed that vinculin and α-actinin are both not sufficient to mediate binding of the cadherin-catenin complex to the actin cytoskeleton (Yamada et al., 2005). If the E-cadherin-β-catenin complex would bind directly and stably to actin at cell-cell contacts, a significant immobile fraction of membrane-associated actin is expected. At the membrane high mobility of actin was seen by Yamada and colleagues. This is due to rapid exchange the pool of cytoplasmic actin. The state of actin organization or dynamics has no influence on the mobility of the cadherin-catenin complex at cell-cell contacts. (Yamada et al., 2005)

If there is no direct linkage of the E-cadherin-β-catenin complex to the actin cytoskeleton via proteins, α-catenin could play a role as molecular switch. In solution, purified α-catenin was shown to be a homodimer (Koslov et al., 1997). There is an overlap in the domain for homodimerization and the β-catenin binding domain (Pokutta & Weis, 2000), so before α-catenin can bind β-catenin the α-catenin homodimer has to dissociate. Data showed that 60-75% of the cytosolic α-catenin in MDCK cells was monomeric. The monomeric α-catenin preferentially binds to β-catenin and the homodimer to actin (Drees et al., 2005). α-catenin exchanges between the E-cadherin-β-catenin complex and actin filaments. So, whether interaction is possible depends on the molecular state of α-catenin.

Actin polymerization is mediated by Arp (actin related protein) 2/3, which can be suppressed in a concentration-dependent manner by α-catenin. The α-catenin monomer concentration in the cytoplasm is low and needs to be concentrated for α-catenin to be able to dimerize and to bind actin. There is a significant increase in the local concentration when the cadherin-catenin complex accumulates. The pool of cadherin bound α-catenin can exchange with the cytoplasmic pool. (Drees et al., 2005)

The Arp2/3 complex plays a role in the regulation of the actin cytoskeleton. The complex alone has little effect on actin polymerization but in the presence of a certain protein expressed in cells of the hematopoietic system, WASp-VCA (Wiskott–Aldrich Syndrome Protein -Verprolin, cofilin, acidic), the polymerization is enhanced (Brieher et al., 1997). Monomeric α-catenin has no influence, but the α-catenin homodimer completely suppressed actin polymerization in the presence of the Arp2/3 complex and WASp-VCA. A possibility is that actin filaments bundling by α-catenin homodimers sterically inhibits the Arp2/3 complex from binding to the actin filaments, which suppresses actin polymerization. The Arp2/3 complex does not bind α-catenin neither as monomer nor homodimer. This implies that α-catenin does not

cap the growing barbed end of the actin filaments or sequester G-actin or the Arp2/3 complex. When β-catenin is also present, the inhibitory effects of α-catenin are reduced. This reduces the effective concentration of α-catenin that can bind actin. It is likely that the inhibitory effect of α-catenin on actin polymerization mediated by Arp2/3 is because of direct competition with the Arp2/3 complex for binding actin filaments (Drees et al., 2005).

α-catenin plays different roles in the adherens junction complex. Not only is it a link between E-cadherin-β-catenin and the actin cytoskeleton, it also contributes to the binding strength of the catenin p120ctn to the cadherin-catenin complex. A large loop in the middle of the p120ctn arm-repeat domain is close-by the amino-terminal VH1 domain of α-catenin. The p120ctn binding to cadherin is weakened by the amino acid extension of this loop, caused by an alternative splicing (Troyanovsky et al., 2011).

Lack of α-catenin expression results in reduced cell-cell adhesion and loss of the epithelial phenotype. Bajpai and colleagues (Bajpai et al., 2009) used single-molecule force spectroscopy to probe the tensile strength, unstressed bond lifetime, and interaction energy between E-cadherins expressed on the surface of live human parental breast cancer cells lacking α-catenin and these cells where α-catenin is re-expressed. The results showed that the intracellular protein α-catenin stabilized extracellular E-cadherin-E-cadherin bonds against tensile forces. Bajpai states that the bond strength changes are the result of a change in configuration of E-cadherin-E-cadherin bonds, mediated by α-catenin. The dimerization of α-catenin is disrupted by binding of E-cadherin. This makes it seem improbable that dimerization of α-catenin enhances that bond strength of E-cadherin-E-cadherin bonds in cell expressing α-catenin (Bajpai et al., 2009).

3. αE-catenin in cancer

Perturbations in the adherens junction complex can lead to metastasis and invasion of tumors.

3.1 Cancer

90% of all human malignancies develop in epithelial tissues and are therefore termed carcinomas. They are usually treated with surgery, radiation and chemotherapy. The majority of deaths from cancer is caused by metastases that are resistant to therapy and cause organ failure. Metastases are often difficult to treat because of the nonuniformity of cells in both primary and secondary neoplasms. Malignant neoplasms contain different cell populations with a wide range of heterogeneity, such as growth rate, immunogenicity, sensitivity to various drugs and the ability to invade metastasize (Fidler & Balch, 1987).

The most common malignancy in women of the Western World is breast cancer, a disease that develops from mammary gland epithelium. 30-40% of the patients develop metastatic disease, which is the primary cause of death. There are different types of breast cancer, of which most frequently observed types are invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC). Of all cases is 10-15% is ILC, characterized by non-cohesive invasive cells that are arranged in trabecules without mass formation and calcification. This makes it difficult to diagnose using physical examination or mammography (Derksen et al., 2006; Derksen et al., 2011). Ductal tumors tend to form more glandular structures (Korkola et al., 2003). Breast cancer cells show disturbances in the adherens junctions. Most cases of ILC (85%) are completely E-cadherin negative, but in a minority of ILC patients (15%) expression of E-cadherin and α-catenin is maintained. However, this E-cadherin expression is atypical as it is nonpolarized (tumor cells are stained all over their surface). E-cadherin is already absent in LCIS (lobular carcinoma in situ), seen as a risk factor but not a precursor for ILC (Moll et al., 1993). While IDC shows mostly no or heterogeneous loss of E-cadherin, associated with epigenetic transcriptional downregulation, ILCs are in most cases completely E-cadherin-negative (Berx & Van Roy, 2001).

It is thought by several researchers that the majority of carcinomas loses E-cadherin mediated cell-cell adhesion and undergoes epithelial-mesenchymal transformation (EMT) (Figure 7). This is only an assumption as it is impossible to recognize EMT in human tumors, because of the great diversity of cellular organization (Thiery, 2002). In most types of human breast cancer, metastases are most frequent to lung, liver and bone marrow. In ILC metastases are mostly gastrointestinal and peritoneal (Arpino et al., 2004). The majority of carcinomas lose E-cadherin, however, the timing of loss is different between ILC and IDC. While ILC loses E-cadherin expression already in early stages, IDC shows no or more heterogeneous loss. This leads to a different

tumor etiology. Lobular tumors often grow slower than ductal tumors and are less cohesive (Coradini et al., 2002).

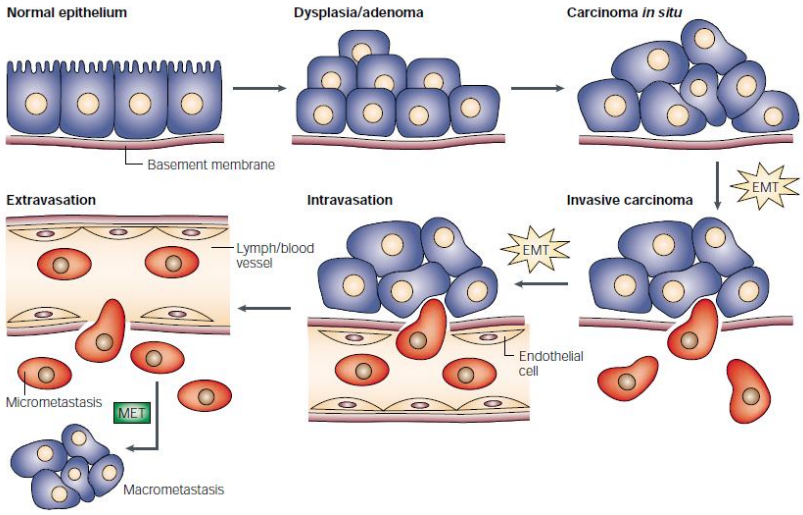


Figure 7: EMT and MET in emergence and progression of carcinoma. Epithelial cells proliferate locally and give rise to an adenoma. Transformation by epigenetic changes and genetic alteration leads to a carcinoma *in situ*. Local dissemination of carcinoma cells, possibly through an EMT, can be induced by further alterations. The basement membrane becomes fragmented and the cells intravasate into lymph or blood vessels. Carcinoma cells extravasate at secondary site and either remain solitary (micrometastasis) or form a new carcinoma through a mesenchymal-epithelial transition (MET). (Thiery, 2002)

In metastasis, cancer cells lose contact with the primary tumor, either as individual cells using amoeboid- or mesenchymal type movement, or as cell sheets, strands and clusters using collective migration (Friedl & Wolf, 2003). They invade the lymph or blood circulatory system and leave the circulatory system for surrounding tissue. The last step in metastasis is the colonization and proliferation in their new environment.

3.2 Adherens junction complex and cancer

A disturbance in interaction between proteins in the E-cadherin-catenin complex is one of the main events in both early and late steps of cancer development. In many different types of cancers, changes in levels of proteins from the adherens junction complex have been observed. Vleminckx and colleagues indicated in 1991 that E-cadherin could acts as an invasion suppressor by showing that inhibition of E-cadherin function induced invasiveness of epithelial tumor cells in kidney and mammary gland of dogs and mice (Vleminckx et al., 1991).

In 1995, E-cadherin was found somatically mutated in lobular breast cancer (Berx et al., 1995) and Mayer *et al* showed inactivating germ line mutation in E-cadherin in metastatic gastric carcinoma (Mayer et al., 1993). COSMIC (Catalogue of Somatic Mutations in Cancer) of the Trust Sanger Institute displays somatic mutation information and related details relating to human cancers. It shows a list of mutations in the E-cadherin gene (CDH1) found in many types of cancer, such as breast, stomach, endometrium, lung, oesophagus, ovary, thyroid and cervix carcinoma.

Some years later Perl and colleagues showed in a transgenic mouse model of pancreatic β-cell carcinogenesis that the inhibition of E-cadherin function was linked with the transition from a benign tumor to a invasive carcinoma. However, whether the loss of cell-cell adhesion mediated by E-cadherin is a cause or consequence of tumor progression, was not clear (Perl et al., 1998).

Derksen and colleagues showed for mammary tumors that a combined loss of E-cadherin and p53 in the mammary epithelium induces metastatic carcinomas resembling human ILC (Derksen et al., 2006; Derksen et al., 2011). Mammary-specific loss of E-cadherin led to metastatic dissemination through the acquisition of anoikis resistance (Derksen et al., 2006). Moreover, dual inactivation of conditional E-cadherin and p53 results in impaired mammary gland function during pregnancy through loss of epithelial organization and a dysfunctional mammary gland (Derksen et al., 2011). The results establish that loss of E-cadherin is causal to mammary tumor initiation and metastasis.

Thiery suggested that loss of E-cadherin leads to EMT in cancer (Thiery, 2002). Therefore E-cadherin is very important for the epithelial phenotype. In most differentiated tumors E-cadherin is maintained (Thiery, 2002). Derksen and colleagues showed that loss of E-cadherin alone is not enough to induce EMT (Derksen et al., 2006). However, the cells of ILC may need mesenchymal properties during tumor invasion, which can be seen as a partial EMT. EMT correlates with β-catenin presence in the nucleus. Overexpression of β-catenin does not lead to EMT, but leads to apoptosis (Kim et al., 2000).

A lot of research was done to investigate the effects of E-cadherin on cancer. It was shown that loss or down regulation of E-cadherin was correlated with aggressiveness and invasiveness of cancer, in ductal and lobular breast cancer (Bukholm et al., 1998; Bukholm et al., 2000; Moll et al., 1993), renal cancer (Shimazui et al., 1996), liver (Kozyraki et al., 1996), esophagus, stomach, colon (Shiozaki et al., 1994), and high E-cadherin was associated with better survival in endometrial cancer (Singh et al., 2011).

3.2.1 α-catenin in cancer

The role of α-catenin is less clear. Since α-cadherin is crucial for tissue integrity, knocking out the gene results in embryonic or neonatal lethality (Silvis et al., 2011). COSMIC shows 28 mutations found in CTNNA1, the gene encoding for α-catenin, in breast, ovary, kidney, lung, pancreas, pleura, large intestine, biliary tract, skin, urinary tract, upper aerodigestive tract and central nervous system cancer.

Hollestelle and colleagues used 55 human breast cancer cell lines and looked for mutations in E-cadherin and α-catenin coding sequences. They found four α-catenin mutant breast cancer cell lines. All four lines showed biallelic mutation predicting

premature termination of the encoded proteins, which is a hallmark of a tumor suppressor gene. None of the cell lines expressed α-catenin proteins and three of the mutant cell lines showed rounded cell morphology and had mutually exclusive mutations of either E-cadherin or α-catenin. The rounded cells are a feature of lobular breast cancers and diffuse-type gastric cancers. The results suggest that E-cadherin and α-catenin are in the same suppressor pathway (Hollestelle et al., 2010).

In human gastric tumors expressing E-cadherin, but no α-catenin, the frequency of lymph node metastasis is higher than in tumors where both E-cadherin and α-catenin are present. Those tumors without α-catenin had almost the same frequency of metastasis as the tumors where both E-cadherin and α-catenin lacked (Matsui et al., 1994).

In prostate cancer, the interaction of β-catenin with E-cadherin was enhanced in presence of α-catenin. This led to decreased cyclin D1 expression (involved in cell proliferation) and decreased cell proliferation. Lack of α-catenin expression resulted in reduced cell-cell adhesion and in loss of the epithelial phenotype. When α-catenin was reintroduced the effects are reversed. Phosphorylation of β-catenin by Src reduces its ability to associate with E-cadherin. However, this Src induced phosphorylation is overridden by α-catenin (Inge et al., 2008).

In contrary to other studies, Elzagheid and colleagues (Elzagheid et al., 2008) state that in colorectal cancer high α-catenin expression was correlated with increased depth of tumor invasion and to increased lymph node involvement.

To study the effect of α-catenin in the adherens junction, complex knockout studies were performed. Some of the consequences of loss of αE-catenin are impairment of hair development and abnormalities in the epidermis. Already shortly after gene ablation in an αE-catenin-mutant mouse developed by Vasioukhin and colleagues, epidermal hyperproliferation of the skin epithelium was seen (Vasioukhin et al., 2001). The morphology, consisting of hyperproliferation, defects in cell polarity, abnormally large and multinucleated keratinocytes, and mitoses in multiple cell layers and the dermal epithelial masses, had a resemblance to a precancerous condition, squamous cell carcinoma in situ (Vasioukhin et al., 2001). Squamous cell carcinoma is developed when there is a genetic deletion of αE-catenin in the hair follicle stem cell compartment. The deletion also leads to inflammatory skin lesions. Further, Silvis and colleagues proved that there was an interaction between αE-catenin and Yap1, where αE-catenin negatively regulated Yap1 nuclear localization by inhibiting the transcriptional activity. αE-catenin was decreased or lost in human keratoacanthoma, which correlated with nuclear Yap1 localization (Silvis et al., 2011). These observations lead to the idea that αE-catenin is a tumor suppressor protein.

Besides α-catenin, other catenins from the adherens junction complex may also have a role in cancer. For example, p120-catenin (p120) is often co-expressed with E-

cadherin and β-catenin in adherens junctions in invasive ductal carcinoma. In invasive lobular carcinoma β-catenin is degraded, but p120-cat is not. It accumulates in the cytoplasm and nucleus where it regulates processes that influence invasiveness. It depends on the type of tumor if p120 has a role as tumor suppressor or oncogene. Localization of p120 can be used as determining if the diagnosis is a ductal or lobular tumor type (Schackmann et al., 2011).

Downregulation of p120ctn leads to destruction of the cadherin complex, as it is an inhibitor of cadherin endocytosis. Often, p120ctn is lost in cancer, which suggests that the downregulation of p120ctn might be an initiating event in a subset of tumors deficient of E-cadherin (Reynolds & Carnahan, 2004).

4. Discussion

Adherens junctions have a role in the initiation and stabilization of cell-cell adhesion, regulation of the actin cytoskeleton, intracellular signaling and transcriptional regulation (Hartsock & Nelson, 2008). It has been thought for a long time that there is a direct binding between the E-cadherin-β-catenin complex and the actin cytoskeleton through α-catenin. A problem with this approach is that the assembly of a quaternary complex of E-cadherin, β-catenin, α-catenin and actin was never shown. A different theory is that α-catenin works as a molecular switch in the complex. When α-catenin binds either the E-cadherin-β-catenin complex or actin, the conformation changes and makes it impossible to bind the other partner (Drees et al., 2005; Yamada et al., 2005). α-catenin exists as monomer, homodimer and heterodimer with β-catenin (Drees et al., 2005). The domain for homodimerization and the domain for β-catenin binding overlap, which makes it impossible for homodimers to bind β-catenin. In its homodimeric state it can bind actin (Yamada et al., 2005). Another option for linkage of the E-cadherin-β-catenin complex with actin filaments is through binding of α-catenin to actin binding proteins such as α-actinin or afadin. However, there is no direct evidence of simultaneous binding to α-catenin and actin.

Besides its function as a linker between the E-cadherin-β-catenin complex and the actin cytoskeleton, α-catenin seems to have different other functions. Based on the study of le Duc and colleagues, who pointed out the cadherin-catenin complex acts as a mechanosensor, it may be the case that α-catenin has a role in this process too. They didn't describe the role of α-catenin directly, however, the results indicate that there may be a role as tension sensor (le Duc et al., 2010).

Also Kobielak and Fuchs say that α-catenin is not only important in connecting the E-cadherin-β-catenin complex to the actin cytoskeleton, it also coordinates actin dynamics and is inversely correlating cell-cell adhesion with proliferation (Kobielak & Fuchs, 2004). Ozono and colleagues showed in a mouse teratocarcinoma cell line that cytoplasmic α-catenin is not required for cell-cell adhesion and the organization of the actin cytoskeleton. They were unable to determine whether cytoplasmic α-catenin was involved in actin-bundle formation at cell-cell junctions (Ozono et al., 2011). The roles of α-catenin have not been investigated under the condition of complete loss of cytoplasmic α-catenin. Drees *et al* stated that cytoplasmic, and not cadherin-associated, α-catenin, forms dimers and plays a critical role in junctional actin bundle formation (Drees et al., 2005). This can complicate the interpretations of the results. Actin dynamics are regulated by cytoplasmic α-catenin independently of cadherin-mediated cell-cell adhesion (Benjamin et al., 2010).

Another role for α-catenin is affecting the Wnt signaling pathway. It is thought that α-catenin, when overexpressed in cells, affects the Wnt signaling pathway by binding β-catenin (Sehgal et al., 1997). Loss of α-catenin from the E-cadherin-β-catenin

complex can occur through different mechanisms, namely a decrease in binding affinity to β-catenin or direct competition from other β-catenin binding proteins (Benjamin & Nelson, 2008). The decrease in binding affinity can be caused by changes in protein phosphorylation. For example, phosphorylation of Y142 in β-catenin causes decreased binding to α-catenin (Piedra et al., 2003).

The role of α-catenin in the adherens junction complex is essential. As was shown by Setoyama and colleagues, α-catenin has an influence on cancer survival. Loss of α-catenin has a similar effect on cancer survival as E-cadherin, namely a decrease in the 5-year survival rate. This was shown in patients with esophageal squamous cell carcinoma (Setoyama et al., 2007). As is displayed in the COSMIC database of the Trust Sanger Institute, many cancers such as breast, kidney and skin cancer, show mutations in the α-catenin gene, CTNNA1. Knockout studies showed that loss of α-catenin leads to compromised adherens junctions and impairment of cell-cell adhesion, but also to increased proliferation and migration (Vasioukhin et al., 2001). When α-catenin is reintroduced, the adherens junction formation is restored (Vasioukhin et al., 2001). It has been suggested that E-cadherin-β-catenin complexes may find new ways to interact with components of signal transduction pathways involved in cell-cycle regulation when α-catenin is not present (Kobielak & Fuchs, 2004).

The role of α-catenin in cancer can become more clear by looking at its influence on different processes of (cancer) cells, such as proliferation, migration and invasion. It influences proliferation by enhancing the interaction of β-catenin with E-cadherin which leads to decreased cyclin D1 expression and decreased cell proliferation. In absence of α-catenin in prostate cancer, cell proliferation increases (Inge et al., 2008). More effects of α-catenin on cell proliferation were shown by Vasioukhin and colleagues (Vasioukhin et al., 2001). They showed epidermal hyperproliferation of the skin epithelium already shortly after gene ablation in the α-catenin-mutant mouse. As it is assumed that perturbations in cell-cell adhesion are late steps in carcinogenesis, this leads to the thought that there must be other molecular explanations.

The actin cytoskeleton is dynamically remodeled during cell migration. This reorganization produces the force that is necessary for cell migration. When these processes are inhibited the cell motility will decrease, which makes these mechanisms important for cancer therapeutics. Especially Arp2/3 complex dependent actin polymerization and its regulation may play an important role in future cancer intervention. α-catenin homodimers can suppress Arp2/3 in a concentration-dependent manner when the Arp2/3 complex and WASp-VCA are present (Drees et al., 2005).

The dimerization of α-catenin is disrupted by binding of E-cadherin. This makes it seem improbable that dimerization of α-catenin enhances the bond strength of E-cadherin-E-cadherin bonds in cells expressing α-catenin (Bajpai et al., 2009). These results suggest that besides the loss of global cell adhesion caused by down regulation

of E-cadherin, also the reduction of intercellular cadherin bond strength induced by the loss of α-catenin plays a role. When α-catenin is re-expressed in human breast cancer cells, the intercellular bond strength of E-cadherin can be restored. So there is probably an alternative strategy to stop or slow down the metastatic phenotype (Bajpai et al., 2009).

Most studies described in this report show correlation between increase in invasiveness and decrease in α-catenin expression (Bukholm et al., 1998; Bukholm et al., 2000; Inge et al., 2008; Matsui et al., 1994; Moll et al., 1993; Vasioukhin et al., 2001). However, contradictory findings were published by Elzagheid and colleagues, showing correlation between increased depth of tumor invasion with high α-catenin expression (Elzagheid et al., 2008). These inconsistent results might be the result of intrinsic tumor heterogeneity, lack of standardization of positive and negative results etcetera.

More research is needed to clarify the underlying molecular mechanisms regulating α-catenin protein expression, as there is still much unknown. This way, for example by looking further into the role of α-catenin in cell migration, it will be possible to develop new ways for cancer intervention in the future.

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