

Invited Review

Catenins and their associated proteins in colorectal cancer

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Summary. Colorectal cancer is the second most common cause of cancer mortality in the western world. Colorectal cancer has been well studied, and the genetic steps involved in the adenoma to carcinoma sequence have been well elucidated. The first genetic alteration, found in 85% of adenomas, are mutations in the adenomatous polyposis coli (APC) gene. However, the consequences of this and the exact function of APC in the colon is not fully understood. It has been suggested that APC could function through its regulation of β -catenin, an ubiquitous cytoskeletal protein with multiple binding specificities resulting in diverse functions including cell growth, adhesion, and migration. Any change in these associations may play a role in colorectal cancer development and progression.

Key words: Cadherins, APC, EGFR, Cell adhesion, Cell signalling

Catenins – An introduction

The catenins are a family of cytoplasmic proteins that are involved in a number of cellular processes namely adhesion and signal transduction. This multigene family comprises of α - (102Kda), β - (92Kd), γ - (Plakoglobin) (83Kd) catenin, and p120^(CAS), and are found on chromosomes 5q, 3p and 17q respectively, the chromosomal location of p120 is currently unknown (Peifer et al., 1992; Liu et al., 1997; Reviewed by Smith and Pignatelli, 1997). The catenins have three structurally distinct regions; an amino- and carboxyl-terminal domain, and a hydrophobic region comprising of arm repeats, each consisting of 42 amino acids. The number of these arm repeats vary between the catenins, β - and γ -catenin having 13 arm repeats, whereas p120 has 11 (Reynolds et al., 1994; Ruiz et al., 1996; Troyanovsky et al., 1996; Reviewed by Ilyas and

Thomlinson, 1997). β -catenin has been shown to be homologous to the armadillo protein found in *Drosophila*, suggesting that the catenins have been evolutionarily conserved (Reviewed by Ilyas and Thomlinson, 1997). Of the catenins, γ -catenin and β -catenin share the greatest homology, with the γ -catenin arm repeat region sharing 85% homology with β -catenin (Troyanovsky et al., 1996).

The catenins are found to associate with a large number of proteins (Fig. 1) namely APC, Cadherins (in adhesive junctions), Epidermal growth factor receptor (EGFR), C-erb2, Tyrosine Kinases (v -src) and Phosphatases (Vandate, PTP 1B and PTP μ), vinculin, α -actinin, fascin, paxillin, axin, ezrin, GSK-3 β and the transcription factors TCF (T cell Factor) and LEF (Leukocyte enhancing factor) (Brady-Kalnoy et al., 1995; Reviewed by Jawhari et al., 1997a; Reviewed by Nakamura, 1997; Reviewed by Yamada and Geiger, 1997; Balsamo et al., 1998; Hiscox et al., 1998; Reviewed by Polakis, 1998).

β -catenin is the most widely studied of the catenins and has been shown to have diverse binding specificities (Fig. 1A) and as a consequence functionality, with it being involved in cell adhesion (Via binding to members of the Cadherin family) (Peifer et al., 1992; Nagafuchi et al., 1994; Reviewed by Jawhari et al., 1997a; Lewis et al., 1997), cell signalling (via the wingless-Wnt pathway (Fig. 1B) and receptor and non-receptor tyrosine kinases (Fig. 1C) (Reviewed by Ilyas and Thomlinson, 1997; Reviewed by Nusse et al., 1997; Muller et al., 1998) and cell motility (β -catenin complexed with APC has been found associated with the microtubule system (Barth et al., 1997a,b). Many of the activities of β -catenin are regulated through the control of these functional complexes (Shibamoto et al., 1995; Reviewed by Ilyas and Thomlinson, 1997).

γ -catenin, like β -catenin has also been shown to associate with APC, E-Cadherin and TCF, via its ARM repeat region (Troyanovsky et al., 1996) and is phosphorylated by both receptor tyrosine kinases and non-receptor tyrosine kinases (Ruiz et al., 1996), but unlike β -catenin, γ -catenin is also found associated with the desmosomal proteins desmoglein and desmocollin

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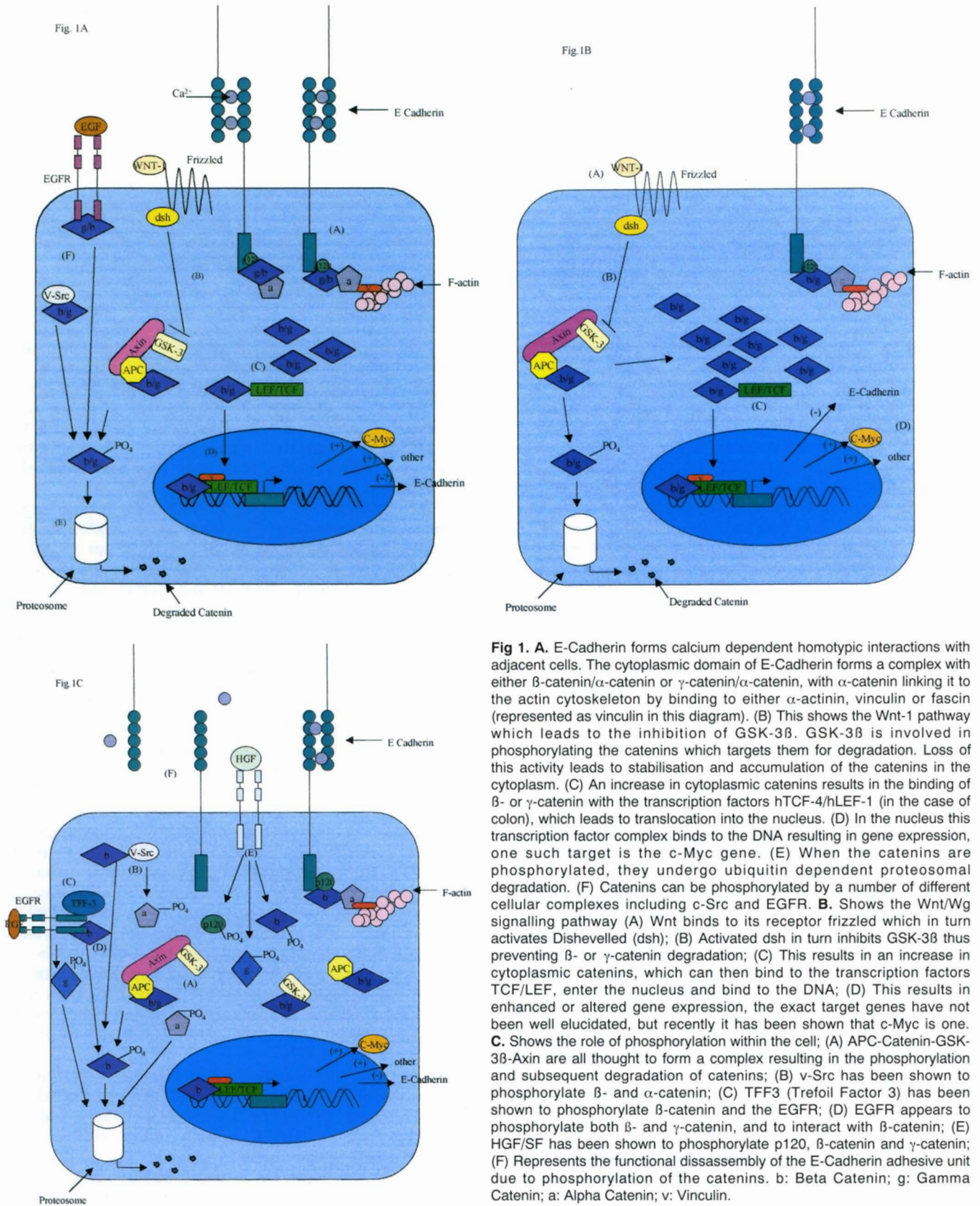


Fig 1. A. E-Cadherin forms calcium dependent homotypic interactions with adjacent cells. The cytoplasmic domain of E-Cadherin forms a complex with either β -catenin/ α -catenin or γ -catenin/ α -catenin, with α -catenin linking it to the actin cytoskeleton by binding to either α -actinin, vinculin or fascin (represented as vinculin in this diagram). (B) This shows the Wnt-1 pathway which leads to the inhibition of GSK-3 β . GSK-3 β is involved in phosphorylating the catenins which targets them for degradation. Loss of this activity leads to stabilisation and accumulation of the catenins in the cytoplasm. (C) An increase in cytoplasmic catenins results in the binding of β - or γ -catenin with the transcription factors hTCF-4/hLEF-1 (in the case of colon), which leads to translocation into the nucleus. (D) In the nucleus this transcription factor complex binds to the DNA resulting in gene expression, one such target is the c-Myc gene. (E) When the catenins are phosphorylated, they undergo ubiquitin dependent proteosomal degradation. (F) Catenins can be phosphorylated by a number of different cellular complexes including c-Src and EGFR. **B.** Shows the Wnt/Wg signalling pathway (A) Wnt binds to its receptor frizzled which in turn activates Dishevelled (dsh); (B) Activated dsh in turn inhibits GSK-3 β thus preventing β - or γ -catenin degradation; (C) This results in an increase in cytoplasmic catenins, which can then bind to the transcription factors TCF/LEF, enter the nucleus and bind to the DNA; (D) This results in enhanced or altered gene expression, the exact target genes have not been well elucidated, but recently it has been shown that c-Myc is one. **C.** Shows the role of phosphorylation within the cell; (A) APC-Catenin-GSK-3 β -Axin are all thought to form a complex resulting in the phosphorylation and subsequent degradation of catenins; (B) v-Src has been shown to phosphorylate β - and α -catenin; (C) TFF3 (Trefoil Factor 3) has been shown to phosphorylate β -catenin and the EGFR; (D) EGFR appears to phosphorylate both β - and γ -catenin, and to interact with β -catenin; (E) HGF/SF has been shown to phosphorylate p120, β -catenin and γ -catenin; (F) Represents the functional disassembly of the E-Cadherin adhesive unit due to phosphorylation of the catenins. b: Beta Catenin; g: Gamma Catenin; a: Alpha Catenin; v: Vinculin.

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(Ruiz et al., 1996; Troyanovsky et al., 1996).

α -catenin has also been shown to bind directly to a number of proteins, including γ -catenin and β -catenin, vinculin, α -actinin and v-src. It has also been shown to bind indirectly to E-cadherin via γ -catenin and β -catenin, having an essential role in adhesion (Oyama et al., 1994; Reviewed by Barth et al., 1997b; Reviewed by Hirohashi, 1998). Sequence analysis of cloned α -catenin has revealed two isoforms of the protein, α -E which is found in epithelial tissue and α -N which is found in neuronal tissues (Hirano et al., 1992; Hoschuetzky et al., 1994).

p120 (Cas, cadherin-associated src substrate) was originally identified as one of several substrates of the tyrosine kinase pp60src (Reynolds et al., 1994; Liu et al., 1997). Subsequently it was shown to associate with β -catenin and E-Cadherin (Liu et al., 1997). Unlike β - and γ -catenins, p120 has been shown to have at least 4 different isoforms, which appear to be expressed differentially in a variety of cell types (Reynolds et al., 1994).

Catenins in cell adhesion

Cell adhesion to neighbouring cells (via cadherin-catenin) and the extracellular matrix (via Integrins) plays an important role in cell motility, growth, differentiation and survival (Watabe et al., 1994; Reviewed by Ben Zev and Geiger, 1998; Reviewed by Efstathiou and Pignatelli, 1998). Perturbation of any of these interactions results in changes in intercellular adhesion and cell transformation (Valizadeh et al., 1997; Guilford et al., 1998).

The cadherins are a family of transmembrane glycoproteins that mediate calcium dependent adhesion and share common amino acid sequences. They have been divided up into more than 10 subclasses dependent on their tissue distribution, including E- (Epithelial), N- (Neuronal) and P-cadherin (Gagliardi et al., 1995; Shiozaki et al., 1996; Reviewed by Smith and Pignatelli, 1997). A novel member of the family, Fat, found in *Drosophila* suggests that cadherins have been evolutionary conserved (Mahoney et al., 1991).

The E-Cadherin gene (CDH 1), found on chromosome 16q22.1, was first isolated by Berx et al. (1995), and was found to span 100Kb consisting of 16 exons (Berx et al., 1995). E-Cadherin (also known as uvomorulin, L-Cam, Arc 1 and cell-CAM 120/80 (Gagliardi et al., 1995) is found at adherens junctions forming homophilic interactions between cells in epithelial tissues (Gagliardi et al., 1995; Shiozaki et al., 1996; Reviewed by Smith and Pignatelli, 1997). It is linked to the actin cytoskeleton via its interactions with the cytoplasmic proteins the catenins (α -, β -, γ -catenins, p120), α -actinin and vinculin (Barth et al., 1997a; Hulsken et al., 1994). Changes in the expression levels of cadherins and catenins have been shown to affect cell-cell adhesion (Reviewed by Adams and Nelson, 1998), thus suggesting that catenins mediate E-cadherin

adhesive functions (Reviewed by Smith and Pignatelli, 1997).

β -catenin has been shown to mediate epithelial cell adhesion by binding to the cytoplasmic domain of E-Cadherin, and then linking E-Cadherin to the actin cytoskeleton by binding to α -catenin via its amino terminal, or by associating directly with the actin cytoskeleton (Reviewed by Smith and Pignatelli, 1997; Reviewed by Polakis, 1998). This E-cadherin/ β -catenin complex has been shown to also interact with the α 2-integrin, a collagen receptor, an interaction influenced by stimulation with TGF- α (Transforming growth factor alpha) and hSP (Human spasmodic polypeptide), which alters cell migration and cell matrix adhesion (Jawhari et al., 1997a,b; Reviewed by Pignatelli, 1998). β -catenin is also found in association with APC, where APC together with GSK-3 β is thought to regulate β -catenin by controlling its free cytoplasmic levels (Reviewed by Gordon, 1998; Reviewed by Polakis, 1998). E-Cadherin is never found in association with these APC/ β -catenin complexes and it has been shown that APC and E-Cadherin both compete for the binding to β -catenin in transient expression assays (Hulsken et al., 1994; Reviewed by Barth et al., 1997b).

γ -catenin has also been shown to bind to E-cadherin at adherens junctions in epithelial cells (Knudsen and Wheelock, 1992; Troyanovsky et al., 1996; Reviewed by Barth et al., 1997b), with evidence pointing to β -catenin and γ -catenin forming mutually exclusive complexes (Nagafuchi et al., 1994; Reviewed by Barth et al., 1997b). The domains of γ -catenin and β -catenin which associate with cadherins and α -catenin have been mapped. It was found that while the central arm repeats of catenins interact with cadherins, the amino terminal domain, as well as the first arm repeat bind to α -catenin (Ruiz et al., 1996). Unlike β -catenin, γ -catenin is also found associated with desmosomal cadherins, mainly desmoglein (Dsg) and desmocollin (Dsc) (Troyanovsky et al., 1996; Reviewed by Barth et al., 1997b). In desmosomes, plakoglobin has been shown to specifically interact with Dsg and Dsc, but no association with α -catenin or p120^(Cas) in these complexes have been observed (Troyanovsky et al., 1996). Ruiz et al. (1996) generated a null mutation of the plakoglobin gene in mice to help elucidate the role of plakoglobin in development and consequently cell adhesion. They found that homozygous null mice died between days 12-16 of embryogenesis due to defects in heart function. These defects revealed loss of desmosome formation, with increased adherens junction formation, thus suggesting that plakoglobin is an essential component of myocardial desmosomes, playing a crucial role in the sorting of desmosomal and adherens junction components, and as a consequence tissue architecture of the heart (Ruiz et al., 1996; Reviewed by Barth et al., 1997b; Lewis et al., 1997). Recent observations of Troyanovsky et al. (1996) suggest that it is the arm repeat region of plakoglobin that mediates these functions. The Arm repeat region was shown to

comprise of two functionally distinct regions, with the first 5 arm repeats involved in specific binding of E-cadherin and desmoglein, with the remaining arm repeats being involved in targeting plakoglobin/cadherin complexes to junctional structures (Trojanovsky et al., 1996).

α -catenin, an actin bundling protein *in vitro*, has been shown to be involved in adhesion by linking cadherin to the actin cytoskeleton (Reviewed by Adams and Nelson, 1998; Weiss et al., 1998). Biochemical evidence suggests that α -catenin does not directly associate with cadherins but binds to the cytoplasmic domain of cadherins via β -catenin or γ -catenin, mediating the connection between the cadherin-catenin complex with the actin cytoskeleton (Hoschuetzky et al., 1994; Oyama et al., 1994; Reviewed by Efstathiou and Pignatelli, 1998; Reviewed by Hirohashi, 1998). Simcha et al (1998) showed that α -catenin, along with cadherins, were able to sequester β -catenin in the cytoplasm, thus inhibiting its transcriptional activity. Amino acid sequencing revealed that α -catenin is homologous to vinculin, a protein found localised at adherens junctions and focal contacts where it functions as a cytoplasmic anchorage for receptors of extracellular matrix proteins (Nagafuchi et al., 1994; Reviewed by Shiozaki et al., 1996; Weiss et al., 1998). Adams and Nelson (1998) have thus suggested that vinculin may bind to the Cadherin/ β -catenin or Cadherin/ γ -catenin complex, thereby compensating for the lack of α -catenin. Furthermore α E-catenin has been shown, in association with vinculin, to play a role in the assembly of apical junctional complex in epithelia (Watabe-Uchida et al., 1998).

p120, like β - and γ -catenin, has also been shown to bind directly to E-Cadherin, and is found associated with both the E-Cadherin/ β -catenin and the E-Cadherin/ γ -Catenin complexes (Reynolds et al., 1994; Jou et al., 1995; Shibamoto et al., 1995; Trojanovsky et al., 1996; Reviewed by Barth et al., 1997b). But unlike β - and γ -catenin p120 does not bind α -catenin (Jou et al., 1995). The binding site of p120 on E-Cadherin, although in close proximity, is distinct from that of its structural homologues β - and γ -catenin (Valizadeh et al., 1997), although other groups have suggested otherwise (Reynolds et al., 1994; Shibamoto et al., 1995). The exact role of p120 catenin in cell to cell adhesion remains a matter of debate (Reviewed by Adams and Nelson, 1998). Skoudy et al. (1996) results suggest that p120 catenin plays a role in regulating E-Cadherin. Cadherin-p120 complexes maybe formed initially to increase the rates of homotypic recognition and binding between cadherin molecules on two adjacent cells (Reviewed by Adams and Nelson, 1998). Subsequently α -catenin, β -catenin and the actin cytoskeleton may associate with and stabilize the cadherin-p120 cluster and, thereby, strengthen the cell-cell adhesion. This interpretation of their results would explain why cells that overexpress cadherin, which have an intact β -catenin binding region, but lacks the p120 binding region, adhere very slowly (Reviewed Adams and

Nelson, 1998). Alternatively as p120 is a major src substrate and is phosphorylated in response to ligand stimulation of receptor tyrosine kinases (Reynolds et al., 1994; Liu et al., 1997; Valizadeh et al., 1997), it may act to mediate the regulation of cadherin adhesion by these signalling pathways (Hoschuetzky et al., 1994; Reynolds et al., 1994; Reviewed by Barth et al., 1997b).

A number of other proteins have been shown to be associated with the E-Cadherin/catenin complex (Liu et al., 1997). Non-receptor and receptor tyrosine kinases, including EGFR, and receptor tyrosine phosphatase, PTP μ and PTP 1B, alter the phosphorylation status of components in the cadherin-catenin complex (Hoschuetzky et al., 1994; Brady-Kalnay et al., 1995; Liu et al., 1997; Balsamo et al., 1998; Soler et al., 1998). The phosphorylation of tyrosine residues in the β -catenin protein was observed in mammalian fibroblasts transduced with v-src, where src was shown to accumulate at adherens junctions and increase tyrosine phosphorylation levels (Tsukita et al., 1991). Each of the different kinases/phosphatases have different specificity for cadherin/catenin complex components (Fig. 1C). For example TFF3 (Trefoil factor 3) has been shown to cause rapid and specific tyrosine phosphorylation of β -catenin and epidermal growth factor receptor, but not E-Cadherin or α -catenin (Liu et al., 1997; Efstathiou et al., 1998). Hepatocyte growth factor/scatter factor (HGF/SF) has been shown to induce the phosphorylation of p120, β - and γ -catenin (Shibamoto et al., 1995). Hoschuetzky et al. (1994) clearly demonstrated that EGF results in the tyrosine phosphorylation of β - and γ -catenin, whereas with v-src the primary targets are α -, β -catenin and p120^(Cas) (Hoschuetzky et al., 1994; Liu et al., 1997; Valizadeh et al., 1997). Hoschuetzky et al. (1994) also demonstrated that this is also cell type specific. The precise biological consequence of this tyrosine phosphorylation of catenins is currently unknown. It has been suggested that it inactivates cadherin-mediated adhesion, this is supported by the fact that phosphorylated β -catenin is exclusively found in detergent soluble fractions, suggesting that tyrosine phosphorylation induces disassembly of the cadherin-catenin complex from the actin filament network, although other explanations are possible (Hoschuetzky et al., 1994; Reviewed by Barth et al., 1997b; Liu et al., 1997). This loss of cadherin-mediated adhesion, through tyrosine phosphorylation, resulting in destabilisation of the adherens junctions, was shown to induce cell dispersion/migration (Liu et al., 1997).

Catenins in cell signalling

Studies in *Drosophila* have revealed the role of the armadillo protein in the wingless-wnt pathway that mediates cell fate determination (Reviewed by Barth et al., 1997b). As β -catenin is highly homologous to the armadillo protein it was proposed that β -catenin would also play a part in the vertebrate wingless-wnt pathway (Reviewed by Ilyas and Thomlinson, 1997; Reviewed by

Nusse et al., 1997), which subsequently revealed the importance of β -catenin in embryogenesis (Reviewed by Barth et al., 1997b).

Catenins have already been shown to be involved in the tyrosine kinases and phosphatase signalling pathways that regulate cell adhesion (Barth et al., 1997a), with evidence pointing to cellular adhesion itself mediating signalling pathways (Benze'Ve, 1997). β -catenin was also found to be a major player in the Wnt/wingless signalling pathway. Hinck et al. (1994a,b) showed that Wnt-1 expression resulted in the accumulation of β -catenin and γ -catenin into the cytoplasm, coupled with an increase in cellular adhesion due to the increased stability between the cadherins and catenins (Bradley et al., 1993; Hinck et al., 1994a). An increase in the stability between APC and the catenins has also been observed (Papkoff et al., 1996). The Wnt-1 pathway has been fairly well elucidated and is shown in fig. 1B, with Cook et al. (1996) using 10T1/2 fibroblasts showing that Wnt acted through the Wingless pathway to inhibit GSK-3 β . Fig. 1B shows that Wnt-1 binds to its receptor frizzled which in turn activates the vertebrate homologue of dishevelled. Dishevelled in turn inhibits the vertebrate homologue of ZW-3 known as GSK-3 β . GSK-3 β is a serine/threonine kinase that negatively regulates β -catenin, when β -catenin levels increase to excess. In *Xenopus* and *Drosophila* GSK-3 β serves to downregulate both β -catenin and γ -catenin, through phosphorylation (Rubinfeld et al., 1996; Reviewed by Jankowski et al., 1997; Morin et al., 1997a). Subsequent studies revealed the involvement of GSK-3 β in the phosphorylation of both APC (increasing APCs affinity for γ -catenin) and β -catenin (on the N-terminal region) resulting in the increased degradation of β -catenin (Rubinfeld et al., 1996; Reviewed by Moon and Miller, 1997) via the ubiquitin dependent proteosomal pathway (Aberle et al., 1997; Müller et al., 1998). GSK-3 β and APC may also regulate β -catenin independently of each other (Reviewed by Moon and Miller, 1997; Sakanaka et al., 1998), or work in a complex which also consists of the Axin (Conductin) molecule (Reviewed by Gordon, 1998; Ikeda et al., 1998). Axin was shown to bind both β -catenin and GSK-3 β in colorectal cancer cells that contained mutant APC, blocking β -catenin transcription (Sakanaka et al., 1998). Thus inhibition of GSK-3 β results in increased stability of β -catenin. This increase in cytoplasmic β -catenin leads to translocation of β -catenin into the nucleus where it associates with the transcription factors LEF and TCF (Behrens et al., 1996; Reviewed by Barth et al., 1997b; Reviewed by Jankowski et al., 1997; Korinek et al., 1997; Morin et al., 1997a; Reviewed by Nusse, 1997; Müller et al., 1998). There is some discrepancy over whether β -catenin enters the nucleus of its own accord (but it is a large molecule with no signal peptide), or more likely whether it associates with Lef or Tcf in the cytoplasm and these molecules carry β -catenin into the nucleus (Behrens et al., 1996).

LEF and TCF are members of the high mobility

group sex determining (*sry*) family of transcription factors, that induce bends in the DNA helix on binding. These transcription factors, LEF and TCF, by themselves are poor promoters of transcription. However when β -catenin is complexed with these transcription factors the DNA bend produced by these transcription factors (Behrens et al., 1996) is altered, which is thought to enhance and alter gene transcription (Reviewed by Moon and Miller, 1997; Reviewed by Nusse et al., 1997). The target genes of these transcription factors have long been sort after, but are thought to be involved in inhibiting apoptosis or promoting cellular proliferation. Recently, He et al. (1998) have shown that one of the target genes of Tcf-4 is *c-myc*, which is a well known oncogene over expressed in colon cancer. γ -catenin has also been shown to enter the nucleus and interact with these transcription factors, thus suggesting some functional homology between the two proteins, β -catenin and γ -catenin. Simcha et al. (1998) have recently shown that γ -catenin and β -catenin differ in their nuclear translocation and their association with the transcription factor Lef-1. They found that Lef-1 dependent transcription is preferentially driven by β -catenin, with β -catenin binding to Lef-1 in association with vinculin. The β -catenin-Lef complex has also been shown to bind to the 5' end of E-cadherin gene (Ben-Ze've, 1997). Whether tyrosine phosphorylation of β -catenin is involved in the wingless-Wnt signalling pathway has not been clarified (Reviewed by Nakamura, 1997).

Catenins in cancer

Colorectal cancer is the second most common cause of cancer death, causing 17000 deaths in England and Wales alone each year (Dept. of Health, 1998). The development of colorectal cancer has been well studied, and the genetic steps involved in the adenoma to carcinoma sequence have been well elucidated (Fearon and Vogelstein, 1990; White, 1998). The first genetic alteration in the multistep process, in inherited and sporadic tumours, is the loss of APC (Wasan et al., 1998) with 80-85% of adenomas carrying an APC mutation (Reviewed by Nakamura, 1997). The APC gene is located on chromosome 5q21-22 and codes for a 310 Kd protein (Mulken et al., 1998). APC has been described as a 'gatekeeper' gene in that it directly regulates the growth of tumours by inhibiting growth and/or promoting death (Reviewed by Kinzler and Vogelstein, 1996; Reviewed by Barth et al., 1997b; Reviewed by Morin et al., 1997b; Reviewed by Kinzler and Vogelstein, 1998; Mulken et al., 1998). APC is a cytoplasmic protein with multiple functional domains (Reviewed by Moon and Miller, 1997). Both γ -catenin and β -catenin have been found to directly associate with APC (Rubinfeld et al., 1993; Su et al., 1993; Reviewed by Jawhari et al., 1997a), whereas α -catenin indirectly associates with APC via β -catenin (Su et al., 1993). APC is thought to function by controlling the cytoplasmic levels of β -catenin, with it having two β -catenin binding

sites, and in association with GSK-3 β and axin, results in β -catenin degradation (Reviewed by Moon and Miller, 1997; Reviewed by Ben Ze-ev and Geiger, 1998; Reviewed by Gordon, 1998; Reviewed by Ikeda et al., 1998).

Familial Adenomatous Polyposis (FAP) is an inherited autosomal dominant disease, resulting in the inheritance of a mutant APC gene leading to the early onset of colorectal cancer. FAP has provided a useful model for studying CRC and is characterised by the development of thousands of adenomatous polyps at a young age (Reviewed by Kinzler and Vogelstein, 1996). Stabilization and accumulation of cytoplasmic β -catenin, which result from mutations in either the APC or β -catenin genes are causitively associated with colon carcinogenesis (Takahashi et al., 1998; Rubinfeld et al., 1996). APC mutations usually involve at least one of the β -catenin binding sites, thus resulting in increased stability of the β -catenin protein (Reviewed by Moon and Miller, 1997). This is shown in cells expressing mutant APC, as they possess an abnormally large pool of monomeric β -catenin (Reviewed by Moon and Miller, 1997). Using immunohistochemical techniques. Hao et al. (1997) showed that in normal tissue β -catenin was found associated with the cell membranes, but in the adenoma and carcinoma tissues there was reduced β -catenin membranous staining which was accompanied with an increase in cytoplasmic and nuclear staining (Hao et al., 1997; Sheng et al., 1998; Takahashi et al., 1998), this was also found to correlate with the progression from adenomas to carcinomas (Takayama et al., 1996; Hao et al., 1997). However, where there are no APC mutations observed, there are a number of other possible candidates which could be involved in colorectal carcinogenesis, namely the catenins.

Takahashi et al. (1998) showed that in AMO (azoxymethane) treated rats there was an increase in both cytoplasmic and nuclear β -catenin. Further analysis of β -catenin revealed that in this model a mutation in the GSK-3 β consense motif for β -catenin resulted in its increased stability. There are a number of other possible mutation sites in β -catenin, it has been shown that in epithelial cells β -catenin may undergo tyrosine phosphorylation due to a mutation in the coding sequences of the serine residues (Reviewed by Jankowski et al., 1997). Amino terminal deletion in β -catenin also results in the stabilisation of the protein along with hyperphosphorylation of APC (Munemitsu et al., 1996). Alterations in cell signalling, as in inappropriate Wnt-1 signalling, can also result in increased free cytoplasmic β -catenin (Hinck et al., 1994). As mentioned previously an increase of free β -catenin results in increased and inappropriate transcription of hLEF-1 and hTCF-4 (Reviewed by Moon and Miller, 1997), which has recently been shown by He et al. (1998), to result in c-Myc expression, thus leading to a possible understanding of why c-Myc is overexpressed in colorectal tumours. Tcf-4 has also recently been shown to maintain the crypt stem cells of

the small intestine, suggesting that when APC or β -catenin are mutated in human epithelial cells, these cells may retain stem cell characteristics thus resulting in malignant transformation (Korinek et al., 1998). γ -catenin has also been shown to associate with APC, Tcf and Lef and also to enter the nucleus, suggesting there may be further functional homology. Mutations in APC and β -catenin are thought not to be the only reasons for loss of the regulation of β -catenin levels. Recent data have also revealed a number of other complexes involved in β -catenin degradation, namely Axin which is thought to form a complex with APC and GSK-3 β leading to phosphorylation and subsequent β -catenin degradation (Reviewed by Gordon, 1998; Ikeda et al., 1998), thus potentially mutations in Axin could also play a role in the fundamental disruption of this multiprotein complex (Reviewed by Gordon, 1998; Reviewed by Pennisi, 1998). Mutations in GSK-3 β could also be a significant factor, suggested by the fact that mutations in the GSK-3 β consensus sequence of β -catenin has been shown to result in its increased stability and increased activity (Reviewed by Moon and Miller, 1997; Takahashi et al., 1998). A further possible candidate is the ubiquitin dependent proteosomal degradation (Aberle et al., 1997), any abnormality in the proteins involved in this pathway could also have a significant effect on β -catenin degradation, and function.

As early as 1989, Behrens et al. suggested that the loss of adhesive function of uvomorulin (E-Cadherin) was a critical step in the promotion of the malignant phenotype of epithelial cells. This is coupled with the emerging concept that this loss of adhesion leads to an increase in genomic instability, thus increasing the risk of further mutations (Tlsty, 1998). E-Cadherin is essential for the formation and maintenance of epithelia, and any perturbation of the adhesive complex, i.e. any mutation in any of the components results in a disruption in E-Cadherin function leading to increased invasiveness and decreased differentiation (Reviewed by Barth et al., 1997a,b; Reviewed by Jankowski et al., 1997; Guilford et al., 1998). Loss of E-cadherin mediated adhesion appears to be a fundamental aspect of the neoplastic process (Jawhari et al., 1997b). Mutations in E-cadherin itself, β -catenin, α -catenin and γ -catenin have all been shown to result in the perturbation of cellular adhesion (Gagliardi et al., 1995). In vitro non invasive, differentiated cell lines tend to express E-cadherin, whereas the invasive, undifferentiated cell lines had reduced E-cadherin expression, suggesting that E-cadherin is an invasion suppressor molecule (Frixen et al., 1991). Gagliardi et al. (1995) used immunohistochemical techniques to evaluate the expression of E-cadherin in normal and cancerous tissues. They found that in normal colorectal epithelial cells there was typical membranous staining at the adherens junctions. But in the adenoma and carcinoma tissues there were changes in the immuno-reactivity and cellular localisation, and these changes correlated with tumour size, tumor type, growth patterns and degree of dysplasia (Dorudi et al.,

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Table 1. Examples of genetic and epigenetic changes in catenin associated proteins in colorectal cancer.

MOLECULE	MUTATION TYPE	MUTATION	CODON	PHENOTYPE
APC	Deletion	AA	MCR 1310	Truncated protein, Moderate dysplasia, Tubular adenoma (Mulken et al., 1998)
APC	Missense	AAA>TAA	MCR 1370	Truncated protein, Moderate dysplasia, Tubular adenoma (Mulken et al., 1998)
APC	Insertion	A	MCR 1384	Truncated protein, Moderate dysplasia, Tubulovillous adenoma (Mulken et al., 1998)
APC	Deletion	G	1399	Found in 2/84 ACF (Otori et al., 1998)
APC	Missense	Gln--Stop	1338	Most common mutation found resulting in a truncated protein (Smith and Pignatelli, 1997)
APC	Deletion	5bp	1309	Germline mutation found (Müller et al., 1998)
β-catenin	G:C to A:T	GGA>GAA		Found in GSK-3β consensus motif (Takahashi et al., 1998)
β-catenin	Deletion		234-760	Stabilisation of β-catenin protein, found in 7/222 patients with CRC (Iwao et al., 1998)
β-catenin	Deletion	3bp	codon 45	Stabilised protein, found in the RER+ cell line HCT116 (Ilyas et al., 1997; Müller et al., 1998)
β-catenin	Missense			Stabilised protein, found in RER+ cell line SW48 (Ilyas et al., 1997; Müller et al., 1998)
β-catenin	Point Mutation or Intestinal deletion			Loss of phosphorylation sites (Polakis, 1998)
E-cadherin	Methylation	Promoter region	Intron 1?	Only been found in breast and gynaecological cancers (Yoshiura et al., 1995)
E-cadherin	Phosphorylation			Post translational modification, resulting in non-functional E-cadherin (Jawahari et al., 1997a,b)
E-cadherin	Missense	G--T	Exon 7	Found as a germline mutation in gastric carcinomas (Guilford et al., 1998)
Catenins β and γ	Tyrosine Phosphorylation			Reduced cellular adhesion, resulting in increased invasion, with decreased differentiation.

MCR: Mutational Cluster Region; ACF: Aberrant Crypt Foci; RER+: Replication Error Positive.

1993; Gagliardi et al., 1995). It has been suggested that loss of E-cadherin mediated cell-cell adhesion could allow cells to escape normal growth control signals resulting in cellular proliferation. Alternatively Guilford suggests that the cytoplasmic domain of E-cadherin may modulate the Wnt signalling pathway by inhibiting the availability of free β-catenin, similarly to APC (Guilford et al., 1998).

It has also been shown that there is an increase in the activation of several receptor tyrosine kinases, i.e. EGFR and c-MET in cancer cells, resulting in tyrosine phosphorylation of the catenin molecules, thus disrupting cellular adhesion (Behrens et al., 1993; Reviewed by Jankowski et al., 1997). The activation of the tyrosine kinase Src has also been shown to be an early event in colorectal cancer, resulting in enhanced anchorage independence, although this is thought not to be cadherin mediated (Pories et al., 1998). Other mechanisms of E-Cadherin inactivation have also been described by Yoshiura et al. (1995) who showed that methylation on CpG islands near the promoter region also inactivated E-Cadherin at least in ovarian, breast and prostate carcinomas.

Shiozaki et al. (1994) looked at whether the possible down regulation of α-catenin expression played a role in

tumour invasion and metastasis through E-Cadherin dysfunction. They found that normal epithelium expressed α-catenin strongly, without exception. However, α-catenin was reduced or absent in a number of primary tumours of the esophagus, stomach and colon, thus suggesting that E-Cadherin mediated adhesion may be abrogated by downregulation of α-catenin (Shiozaki et al., 1994). Skoudy et al. (1996) also reported altered α-catenin expression in colorectal cancer.

Skoudy et al. (1996) demonstrated that in the normal colon, p120-catenin was present in the crypt and surface epithelium, the cells showed reactivity in both the membrane and cytosol, with Valizadeh et al. (1997) showing that the staining intensity was greatest in the proliferating crypt cells. Reduced expression of p120 was observed in 20% of adenomatous polyps, with loss of membranous p120 expression correlating with reduced E-Cadherin expression. Decreased expression of p120 was also found to correlate with the larger size tumour (Skoudy et al., 1996). These findings are difficult to explain as the physiological role of p120 is unknown, but Skoudy et al. (1996) results suggest that changes in p120 catenin levels are a common event in colorectal tumours, and suggest that

the distribution of this protein and E-cadherin is coordinately regulated.

Conclusion

The catenins have been shown to associate with a large number of different proteins which direct their function within the cell. Any perturbation in these associations could potentially lead to cancer development. The importance of catenins in cellular function has been shown to be at the level of cell growth, adhesion and signalling, with APC playing a fundamental role in regulating catenin levels. Changes in cellular expression of the catenins, largely a reduction in membranous staining associated with increased cytoplasmic and nuclear staining, suggests that they play a fundamental role in cancer development. The abrogation of catenin function has been shown to be through a number of genetic and epigenetic factors, with changes also occurring in the catenin associated proteins. For example it is well established that most mutations involving APC result in a truncated protein which can no longer degrade β -catenin, thus resulting in increased cytoplasmic β -catenin which is free to bind the transcription factors altering gene expression. Table 1 gives examples of those mutations and epigenetic factors that contribute to the development of colorectal cancer. This table shows that the characterisation of mutations (outside of APC) are not well defined and are relatively rare. These factors all suggest that loss of catenin function either directly or indirectly can result in cancer development. Thus it will be interesting to elucidate the exact role of catenins and associated proteins in cancer, especially whether an excess of β -catenin protein in association with Tcf may result in the regulation not only of c-Myc, but of other oncoproteins overexpressed in colorectal cancer.

References

- Aberle H., Bauer A., Stappert J., Kispert A. and Kemler R. (1997). β -Catenin is a target for the ubiquitin-proteasome pathway. *EMBO J.* 16, 3797-3804.
- Adams C.L. and Nelson W.J. (1998). Cytomechanics of Cadherin-mediated cell-cell adhesion. *Curr. Opin. Cell Biol.* 10, 572-577.
- Balsamo J., Arregui C., Leung T. and Lilien J. (1998). The nonreceptor protein tyrosine phosphatase PTP1B binds to the cytoplasmic domain of N-cadherin and regulates the cadherin-actin linkage. *J. Cell Biol.* 143, 523-532.
- Barth A.I.M., Pollack A.L., Altschuler Y., Mostov K.E. and Nelson W.J. (1997a). NH2-terminal deletion of β -catenin with adenomatous polyposis coli protein and altered MDCK cell adhesion. *J. Cell Biol.* 136, 693-706.
- Barth A.I.M., Nathke I.S. and Nelson W.J. (1997b). Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways. *Curr. Opin. Cell Biol.* 9, 683-690.
- Behrens J., Mareel M.M., Van Roy F.M. and Birchmeier W. (1989). Dissecting tumour cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. *J. Cell Biol.* 108, 2435-2447.
- Behrens J., Vakaet L., Friis R., Winterhager E., Van Roy F., Mareel M.M. and Birchmeier W. (1993). Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-Cadherin/ β -Catenin complex in cells transformed with a temperature-sensitive v-SRC Gene. *J. Cell Biol.* 120, 757-766.
- Behrens J., von Kries J.P., Kühl M., Bruhn L., Wedlich D., Grosschedl R. and Birchmeier W. (1996). Functional interaction of β -catenin with the transcription factor LEF-1. *Nature* 382, 638-642.
- Ben-Ze'Ve A. (1997). Cytoskeletal and adhesion proteins as tumour suppressors. *Curr. Opin. Cell Biol.* 9, 99-108.
- Ben-Ze'Ve A. and Geiger B. (1998). Differential molecular interactions of β -catenin and plakoglobin in adhesion, signaling and cancers. *Curr. Opin. Cell Biol.* 10, 629-639.
- Berx G., Staes K., von Hengel J., Molemans F., Bussemakers M.J.G., van Bokhoven A. and van Roy F. (1995). Cloning and characterisation of the human invasion suppressor gene E-cadherin (CDH1). *Genomics* 26, 281-289.
- Bradley R.S., Cowin P. and Brown A.M.C. (1993). Expression of Wnt-1 in PC12 cells results in modulation of plakoglobin and E-cadherin and increased cellular adhesion. *J. Cell Biol.* 123, 1857-1865.
- Brady-Kalnay S.M., Rimm D.L. and Tonks N.K. (1995). Receptor protein tyrosine phosphatase PTP μ associates with cadherins and catenins in vivo. *J. Cell Biol.* 130, 977-986.
- Cook D., Fry M.J., Hughes K., Sumathipala R., Woodgett J.R. and Dale T.C. (1996). Wingless inactivates glycogen synthase Kinase-3 via an intracellular signalling pathway which involves a protein kinase C. *EMBO J.* 15, 4526-4536.
- Department of Health (1998). www.open.gov.uk/doh/dhhome.htm
- Dorudi S., Sheffield J.P., Poulosom R., Northover J.M.A. and Hart I.R. (1993). E-Cadherin expression in colorectal cancer. *Am. J. Pathol.* 142, 981-986.
- Efstathiou J.A. and Pignatelli M. (1998). Modulation of epithelial cell adhesion in gastrointestinal homeostasis. *Am. J. Pathol.* 153, 341-347.
- Efstathiou J.A., Noda M., Rowan A., Dixon C., Chinery R., Jawhari A., Hattori T., Wright N.A., Bodmer W.F. and Pignatelli M. (1998). Intestinal trefoil factor controls the expression of the adenomatous polyposis coli-catenin and the E-cadherin-catenin complexes in human colon carcinoma cells. *Proc. Natl. Acad. Sci. USA.* 95, 3122-3127.
- Fearon E.R. and Vogelstein B. (1990). A genetic model for colorectal tumorigenesis. *Cell* 61, 759-767.
- Frixen U.H., Behrens J., Sachs M., Eberle G., Voss B., Warda A., Lochner D. and Birchmeier W. (1991). E-Cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J. Cell Biol.* 113, 173-185.
- Gagliardi G., Kandemir O., Liu D., Guida M., Benvenuto S., Ruers T.G.M., Benjamin I.S., Northover J.M.A., Stamp G.W.H., Talbot I.C. and Pignatelli M. (1995). Changes in E-cadherin immunoreactivity in the adenoma-carcinoma sequence in the large bowel. *Virchows Arch.* 426, 149-154.
- Gordon D. (1998). β -Catenin links the APC gene to MYC in colon cancer. *Gastroenterology* 115, 1041-1042.
- Guilford P., Hopkins J., Harraway J., McLeod M., McLeod N., Harawira P., Taite H., Scoular R., Miller A. and Reeve A.E. (1998). E-cadherin germline mutations in familial gastric cancer. *Nature* 392, 402-405.
- Hao X., Tomlinson I., Ilyas M., Palazzo J.P. and Talbot I.C. (1997). Reciprocity between membranous and nuclear expression of β -

Catenins and associated proteins in colorectal cancer

- catenin in colorectal tumours. *Virchows Arch.* 431, 167-172.
- He T.-C., Sparks A.B., Rago C., Hermeking H., Zawel L., da Costa L.T., Morin P.J., Vogelstein B. and Kinzler K.W. (1998). Identification of c-MYC as a target of the APC pathway. *Science* 281, 1509-1512.
- Hinck L., Nelson W.J. and Papkoff J. (1994). Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing β -catenin binding to the cell adhesion protein cadherin. *J. Cell Biol.* 124, 729-741.
- Hirano S., Kimoto N., Shimoyama Y., Hirohashi S. and Takeichi M. (1992). Identification of a neural α -catenin as a key regulator of cadherin function and multicellular organization. *Cell* 70, 293-301.
- Hirohashi S. (1998). Inactivation of the E-Cadherin-mediated cell adhesion system in human cancers. *Am. J. Pathol.* 153, 333-339.
- Hiscox S., Mansel R.E. and Jiang W.G. (1998). Ezrin Regulates cell-cell adhesion and motility and associates with intercellular junctional proteins in cancer cells. *BACR Conference proceedings.*
- Hoschuetzky H., Aberle H. and Kemler R. (1994). β -catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. *J. Cell Biol.* 127, 1375-1380.
- Hulsken J., Birchmeier W. and Behrens J. (1994). E-Cadherin and APC compete for the interaction with β -catenin and the cytoskeleton. *J. Cell Biol.* 127, 2061-2069.
- Ikeda S., Kishida S., Yamamoto H., Murai H., Koyama S. and Kikuchi A. (1998). Axin, a negative regulator of the Wnt signalling pathway, forms a complex with GSK-3 β and β -catenin and promotes GSK-3 β -dependent phosphorylation of β -catenin. *EMBO J.* 17, 1371-1384.
- Ilyas M. and Tomlinson I.P.M. (1997). The interactions of APC, E-cadherin and β -catenin in tumour development and progression. *J. Pathol.* 182, 128-137.
- Ilyas M., Tomlinson I.P.M., Rowan A., Pignatelli M. and Bodmer W.F. (1997). β -catenin mutations in cell lines established from human colorectal cancers. *Proc. Natl. Acad. Sci. USA* 94, 10330-10334.
- Iwao K., Nakamori S., Kameyama M., Imaoka S., Kinoshita M., Fukui T., Ishiguro S., Nakamura Y. and Miyoshi Y. (1998). Activation of the β -catenin gene by interstitial deletion involving exon 3 in primary colorectal carcinomas without adenomatous polyposis coli mutations. *Cancer Res.* 58, 11021-11026.
- Jankowski J.A., Bruton R., Shepherd N., Scott D. and Sanders A. (1997). Cadherin and catenin biology represents a global mechanism for epithelial cancer progression. *J. Clin. Pathol. Mol. Pathol.* 50, 289-290.
- Jawhari A., Farthing M. and Pignatelli M. (1997a). The importance of the E-Cadherin-catenin complex in the maintenance of intestinal epithelial homeostasis: more than intercellular glue? *Gut* 41, 581-584.
- Jawhari A., Noda M., Farthing M. and Pignatelli M. (1997b). New evidence of cross talk between the E-cadherin-catenin complex and α 2 integrin: An interaction influenced by transforming growth factor- α and the trefoil peptide hSP. *Gut* 41, A27.
- Jou T.-S., Stewart D.B., Stappert J., Nelson W.J. and Marris J.A. (1995). Genetic and biochemical dissection of protein linkages in the cadherin-catenin complex. *Proc. Natl. Acad. Sci. USA* 92, 5067-5071.
- Kinzler K.W. and Vogelstein B. (1996). Lessons from hereditary colorectal cancer. *Cell* 87, 159-170.
- Kinzler K.W. and Vogelstein B. (1998). Landscaping the cancer terrain. *Science* 280, 1036-1037.
- Knudsen K. and Wheelock M.J. (1992). Plakoglobin, or a 83-kD homologue distinct from β -Catenin, interacts with E-cadherin and N-cadherin. *J. Cell Biol.* 118, 671-679.
- Korinek V., Barker N., Morin P.J., van Wichen R., de Weger R., Kinzler K.W., Vogelstein B. and Clevers H. (1997). Constitutive transcriptional activation by a β -catenin-Tcf complex in APC^{-/-} colon carcinoma. *Science* 275, 1784-1787.
- Korinek V., Barker N., Moerer P., van Donselaar E., Huls G., Peters P.J. and Clevers H. (1998). Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nature Genet.* 19, 379-383.
- Lewis J.E., Wahl III J.K., Sass K.M., Jensen P.J., Johnson K.R. and Wheelock M.J. (1997). Cross talk between adherens junctions and desmosomes depends on plakoglobin. *J. Cell Biol.* 136, 919-934.
- Liu D., El-Hariry I., Karayiannakis A.J., Wilding J., Chinery R., Kmiot W., McCrea P.D., Gullick W.J. and Pignatelli M. (1997). Phosphorylation of β -Catenin and epidermal growth factor receptor by intestinal trefoil factor. *Lab. Invest.* 77, 557-563.
- Mahoney P.A., Weber U., Onofrechuk P., Biessmann H., Bryant P.J. and Goodman C.S. (1991). The fat tumour suppressor gene in *Drosophila* encodes a novel member of the cadherin gene superfamily. *Cell* 67, 853-868.
- Moon R.T. and Miller J.R. (1997). The APC tumour suppressor protein in development and cancer. *Trends Genet.* 13, 256-258.
- Morin P.J., Sparks A.B., Korinek V., Barker N., Clevers H., Vogelstein B. and Kinzler K.W. (1997a). Activation of β -catenin-Tcf signalling in colon cancer by mutations in β -catenin or APC. *Science* 275, 1787-1790.
- Morin P.J., Sparks A.B., Korinek V., Barker N., Clevers H., Vogelstein B. and Kinzler K.W. (1997b). The Gatekeeper has many keys: dissecting the function of the APC gene. *Gastroenterology* 113, 2009-2012.
- Mulkens J., Poncin J., Arends J.W. and De Goeij A.F.P.M. (1998). APC mutations in human colorectal adenomas: analysis of the mutation cluster region with temperature gradient gel electrophoresis and clinicopathological features. *J. Pathol.* 185, 360-365.
- Müller O., Nimmrich I., Finke U., Friedl W. and Hoffmann I. (1998). A β -catenin mutation in a sporadic colorectal tumour of the RER phenotype and absence of β -catenin germline mutations in FAP patients. *Gene Chromosome. Canc.* 22, 37-41.
- Munemitsu S., Albert I., Rubinfeld B. and Polakis P. (1996). Deletion of an amino-terminal sequence stabilizes β -Catenin in vivo and promotes hyperphosphorylation of the adenomatous polyposis Coli tumour suppressor protein. *Mol. Cell Biol.* 16, 4088-4094.
- Nagafuchi A., Ishihara S. and Tsukita S. (1994). The roles of catenins in the Cadherin-mediated cell adhesion: functional analysis of E-cadherin- α catenin fusion molecules. *J. Cell Biol.* 127, 235-245.
- Nakamura Y. (1997). Cleaning up on β -Catenin. *Nature Med.* 3, 499-500.
- Nusse R. (1997). A versatile transcriptional effector of wingless signalling. *Cell* 89, 321-323.
- Otori K., Konishi M., Sugiyama K., Hasebe T., Shimoda T., Kikuchi-Yanoshita R., Mukai K., Fukushima S., Miyaki M. and Esumi H. (1998). Infrequent somatic mutation of the adenomatous polyposis Coli gene in aberrant crypt foci of human colon tissue. *Cancer Res.* 58, 896-900.
- Oyama T., Kanai Y., Ochiai A., Akimoto S., Oda T., Yanagihara K., Nagafuchi A., Tsukita S., Shibamoto S., Ito F., Takeichi M., Matsuda H. and Hirohashi S. (1994). A truncated β -catenin disrupts the interaction between E-cadherin and α -catenin: A cause of loss of intercellular A adhesiveness in human cancer cell lines. *Cancer Res.* 54, 6282-6287.

Catenins and associated proteins in colorectal cancer

- Papkoff J., Rubinfeld B., Schryver B. and Polakis P. (1996). Wnt-1 regulates free pools of catenins and stabilizes APC-Catenin complexes. *Mol. Cell. Biol.* 16, 2128-2134.
- Peifer M., McCreas P.D., Green K.J., Wieschaus E. and Gumbiner B.M. (1992). The vertebrate adhesive junction proteins β -Catenin and plakoglobin and the *Drosophila* segment polarity gene armadillo form a multigene family with similar properties. *J. Cell Biol.* 118, 681-691.
- Pennisi E. (1998). How growth control Path takes a wrong turn to cancer. *Science* 281, 1438-1441.
- Pignatelli M. (1998). Integrins, cadherins, and catenins: molecular cross-talk in cancer cells. *J. Pathol.* 186, 1-2.
- Polakis P. (1998). The many partners of β -Catenin. *Insights-Transduction Laboratories* 4, 1-3.
- Pories S.E., Hess D.T., Swenson K., Lotz M., Moussa R., Steele G., Shibata G., Riegerchrist K.M. and Summerhayes I.C. (1998). Overexpression of pp60(c-Src) elicits invasive behaviour in rat colon epithelial cells. *Gastroenterology* 114, 1287-1295.
- Reynolds A.B., Daniel J., McCreas P.D., Wheelock M.J., Wu J. and Zhang Z. (1994). Identification of a new catenin: the tyrosine kinase substrate p120cas associates with E-cadherin complexes. *Mol. Cell. Biol.* 14, 8333-8342.
- Rubinfeld B., Souza B., Albert I., Müller O., Chamberlain S.H., Masiarz F.R., Munemitsu S. and Polakis P. (1993). Association of the APC gene product with β -catenin. *Science* 262, 1731-1734.
- Rubinfeld B., Albert I., Porfiri E., Fiol C., Munemitsu S. and Polakis P. (1996). Binding of GSK-3 β to the APC- β -catenin complex and regulation of complex assembly. *Science* 272, 1023-1025.
- Ruiz P., Brinkmann V., Ledermann B., Behrend M., Grund C., Thalhammer C., Vogel F., Birchmeier C., Gunthert U., Franke W.W. and Birchmeier W. (1996). Targeted mutation of plakoglobin in mice reveals essential functions of desmosomes in the embryonic heart. *J. Cell Biol.* 135, 215-225.
- Sakanaka C., Weiss J.B. and Williams L.T. (1998). Bridging of β -catenin and glycogen synthase kinase-3 β by axin and inhibition of the β -catenin-mediated transcription. *Proc. Natl. Acad. Sci. USA* 95, 3020-3023.
- Sheng H., Shao J., Williams C.S., Pereira M.A., Taketo M.M., Oshima M., Reynolds A.B., Washington M.K., DuBois R.N. and Beauchamp R.D. (1998). Nuclear translocation of β -catenin in hereditary and carcinogen-induced intestinal adenomas. *Carcinogenesis* 19, 543-549.
- Shibamoto S., Hayakawa M., Takeuchi K., Hori T., Miyazawa K., Kitamura N., Johnson K.R., Wheelock M.J., Matsuyoshi N., Takeichi M. and Ito F. (1995). Association of p120, a tyrosine kinase substrate, with E-cadherin/catenin complexes. *J. Cell Biol.* 128, 949-957.
- Shiozaki H., Lihara K., Oka H., Kadowaki T., Matsui S., Gofuku J., Inoue M., Nagafuchi A., Tsukita S. and Mori T. (1994). Immunohistochemical detection of α -catenin expression in human cancers. *Am. J. Pathol.* 144, 667-674.
- Shiozaki H., Oka H., Inoue M., Tamura S. and Monden M. (1996). E-Cadherin mediated adhesion system in cancer cells. *Cancer* 77, 1605-1613.
- Simcha I., Shtutman M., Salomon D., Zhurinsky J., Sadot E., Geiger B. and Ben-Ze'ev A. (1998). Differential nuclear translocation and transactivation potential of β -Catenin and plakoglobin. *J. Cell Biol.* 141, 1433-1448.
- Skoudy A., Gomez S., Fabre M. and Garcia De Herreros A. (1996). p120-Catenin expression in human colorectal cancer. *Int. J. Cancer* 68, 14-20.
- Smith M.E.F. and Pignatelli M. (1997). The molecular histology of neoplasia: the role of the cadherin/catenin complex. *Histopathology* 31, 107-111.
- Soler C., Rousselle P. and Damour O. (1998). Cadherin mediated cell-cell adhesion is regulated by tyrosine phosphatases in human keratinocytes. *Cell Adh. Comm.* 5, 13-25.
- Su L.-K., Vogelstein B. and Kinzler K.W. (1993). Association of the APC tumour suppressor protein with catenins. *Science* 262, 1734-1737.
- Takahashi M., Fukuda K., Sugimura T. and Wakabayashi K. (1998). β -Catenin is frequently mutated and demonstrates altered cellular location in azoxymethane-induced rat colon tumours. *Cancer Res.* 58, 42-46.
- Takayama T., Shiozaki H., Shibamoto S., Oka H., Kimura Y., Tamura S., Inoue M., Monden T., Ito F. and Monden M. (1996). β -Catenin expression in human cancers. *Am. J. Pathol.* 148, 39-46.
- Tlsty T.D. (1998). Cell-adhesion-dependent influences on genomic instability and carcinogenesis. *Curr. Opin. Cell Biol.* 10, 647-653.
- Troyanovsky R.B., Chitaev N.A. and Troyanovsky S.M. (1996). Cadherin binding sites of plakoglobin: localization, specificity and role in targeting to adhering junctions. *J. Cell Sci.* 109, 3069-3078.
- Tsukita S., Oishi K., Akiyama T., Yamanashi Y., Yamamoto T. and Tsukita S. (1991). Specific proto-oncogenic tyrosine kinases of src family are enriched in cell-to-cell adherens junctions where the level of tyrosine phosphorylation is elevated. *J. Cell Biol.* 113, 867-879.
- Valizadeh A., Karayiannakis A.J., El-Hariry I., Kmiet W. and Pignatelli M. (1997). Expression of E-cadherin-associated molecules (α -, β -, γ -catenins and p120) in colorectal polyps. *Am. J. Pathol.* 150, 1977-1983.
- Wasan H.S., Park H.-S., Liu K.C., Mandir N.K., Winnett A., Sasieni P., Bodmer W.F., Goodlad R.A. and Wright N.A. (1998). APC in the regulation of intestinal crypt fission. *J. Pathol.* 185, 246-255.
- Watabe M., Nagafuchi A., Tsukita S. and Takeichi M. (1994). Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin-catenin adhesion system in a dispersed carcinoma line. *J. Cell Biol.* 127, 247-256.
- Watabe-Uchida M., Uchida N., Imamura Y., Nagafuchi A., Fujimoto K., Uemura T., Vermeulen S., van Roy F., Adamson E.D. and Takeichi M. (1998). α -Catenin-vinculin interaction functions to organize the apical junctional complex in epithelial cells. *J. Cell Biol.* 142, 847-857.
- Weiss E.E., Kroemker M., Rüdiger A.-H., Jockusch B.M. and Rüdiger M. (1998). Vinculin is part of the Cadherin-Catenin junctional complex: complex formation between α -Catenin and vinculin. *J. Cell Biol.* 141, 755-764.
- White R. (1998). Tumour suppressing pathways. *Cell* 92, 591-592.
- Yamada K.M. and Geiger B. (1997). Molecular interactions in cell adhesion complexes. *Curr. Opin. Cell Biol.* 9, 76-85.
- Yoshiura K., Kanai Y., Ochiai A., Shimoyama Y., Sugimura T. and Hirohashi S. (1995). Silencing of the E-Cadherin invasion-suppressor gene by CpG methylation in human carcinomas. *Proc. Natl. Acad. Sci. USA* 92, 7416-7419.