

Review

Cadherins down-regulation: towards a better understanding of their relevance in colorectal cancer

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Summary. The down-regulation of cadherin expression in colorectal cancer (CRC) has been widely studied. However, existing data on cadherin expression are highly variable and its relevance to CRC development has not been completely established. This review examines published studies on cadherins whose down-regulation has been already demonstrated in CRC, trying to establish a relationship with promoter methylation, the capacity to influence the Wnt / CTNNB1 (catenin beta 1, beta-catenin) signaling pathway and the clinical implications for disease outcome. Moreover, it also analyses factors that may explain data variability and highlights the importance of considering the altered subcellular localization of the examined cadherins. The results of this survey reveal that thirty of one hundred existing cadherins appear to be down-regulated in CRC. Among these, ten are cadherins, sixteen are protocadherins, equally divided between clustered and non clustered, and four are cadherin - related. These findings suggest that, to better define the role played by cadherin down-regulation in CRC pathogenesis, the expression of multiple rather than individual cadherins should be taken into account and further functional studies are necessary to clarify the relative ability of individual cadherins to inhibit CTNNB1 therefore acting as tumor suppressors.

Key words: Cadherins, Wnt / CTNNB1 signaling, CDH1, Protocadherin, Cadherin-related protein

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Molecular alterations of CRC carcinogenesis

Colorectal cancer (CRC) is among the most frequent tumors in the general population and the second cause of cancer-related death in western countries. The development of CRC follows a multi-step process in which several genetic and epigenetic alterations lead to the activation of proto-oncogenes and the inactivation of tumor suppressor genes, causing the malignant transformation of an initially normal colorectal mucosa. Interestingly, the vast majority of these alterations affect genes involved in signalling pathways regulating cell proliferation. The first and most important of such modifications determines the constitutive activation of the CTNNB1 (catenin beta 1, beta-catenin) signaling pathway, resulting in an increased proliferation of CRC cells. Although mutations inactivating the Adenomatous Polyposis Coli (APC) tumor suppressor gene represent the main pathogenic mechanism responsible for CTNNB1 activation, a growing body of evidence suggests that loss of cadherin expression, believed to occur as a consequence of epigenetic events, significantly contributes to CTNNB1 activation, also playing an important role in the acquisition of an invasive phenotype.

The CTNNB1 signalling proliferation pathway

CTNNB1 activation via its nuclear localization represents a proliferation-promoting pathway controlled by two distinct mechanisms that are respectively mediated by an extra-cellular signaling molecule, called Wnt, and by a family of intercellular adhesion molecules, called cadherins. In both cases, CTNNB1 is

the main transducer of the pathway acting as a transcription co-activator able to induce the expression of a set of genes which, when transcribed, promote cell proliferation. Although the CTNNB1 pathway is ubiquitous, it assumes a crucial importance in colorectal mucosa.

Wnt-dependent regulation

In the Wnt - dependent regulation modality, the stimulus responsible for the activation of the pathway is represented by the Wnt growth factor. In absence of Wnt, the majority of CTNNB1 is localized in the cytosol where it is bound by Adenomatous Polyposis Coli (APC), a component of a multi-protein complex called the CTNNB1 degradation complex. In addition to APC and CTNNB1, the CTNNB1 degradation complex also contains: Axin-1, a scaffold protein promoting the assembly of all proteins participating in its formation; Glycogen Synthase Kinase 3 β (GSK3 β) and Casein Kinase 1 (CK1), two serine - threonine kinases phosphorylating CTNNB1 on serine residues; and the β -TrCP ubiquitin ligase that promotes the ubiquitination and proteasome-dependent degradation of phosphorylated CTNNB1 (Fodde and Brabletz, 2007; MacDonald et al., 2009).

In the extra-cellular environment, Wnt acts like a ligand inducing the dimerization of a surface receptor formed by two distinct subunits named Lipoprotein receptor - Related Protein (LRP) and Frizzled. Then, the cytoplasmic portion of the dimerized receptor recruits an intra-cellular protein called Dishevelled (Dsh) which contacts the CTNNB1 degradation complex inhibiting the activity of GSK3 β and the destruction of CTNNB1. Stabilized CTNNB1 can consequently translocate to the nucleus where it dimerizes with a transcription factor of the T Cell Factor (TCF) family. Once formed, this dimer activates the transcription of its target genes, the most important of which are Myc and Cyclin D1 which promote cell

cycle progression (Fuchs et al., 2005) (Fig. 1).

Cadherin - dependent regulation

Under resting conditions, a fraction of cytoplasmic CTNNB1 is associated to the cell membrane inner layer, where it is bound to cadherins. Cadherins are calcium-dependent adhesion molecules and are involved in homotypic cell - cell interaction, which is the interaction between cells belonging to the same tissue (Takeichi, 1990). This activity is exerted by specific cell junctions that are called adherence junctions. Like many other integral membrane proteins, cadherins are composed of three parts: an extra-cellular portion, containing the so called cadherin domain responsible for the adhesion function; a trans-membrane portion, mediating the interaction with the cell membrane phospholipid bilayer; and an intra-cellular portion, sequestering CTNNB1 through a particular amino acidic sequence named PDZ domain (Nollet et al., 2000). Together with other similar proteins, named α -catenin and γ -catenin, CTNNB1 forms, at this site, a multi-protein complex endowed with the function to anchor the cell membrane to the actin filaments of the cytoskeleton. It is worth noticing that the extra- and intra-cellular portions of a cadherin, probably through a conformational change, reciprocally influence their functions, implying that the activation or inhibition of one of the two determines a similar effect on the other (Halbleib and Nelson, 2006; Sotomayor et al., 2014).

In the presence of a proper stimulus, such as the activation of the receptor tyrosine kinase (RTK) signaling pathway, the phosphorylated receptor, perhaps acting indirectly through the cytosolic and membrane - anchored c-src tyrosine kinase, phosphorylates CTNNB1 on tyrosine residues. This phosphorylation event, in contrast to that involving serine residues, promotes the dissociation of CTNNB1 from the intra-cellular portion of cadherins, followed by its translocation to the nucleus where it activates cell proliferation, as described above.

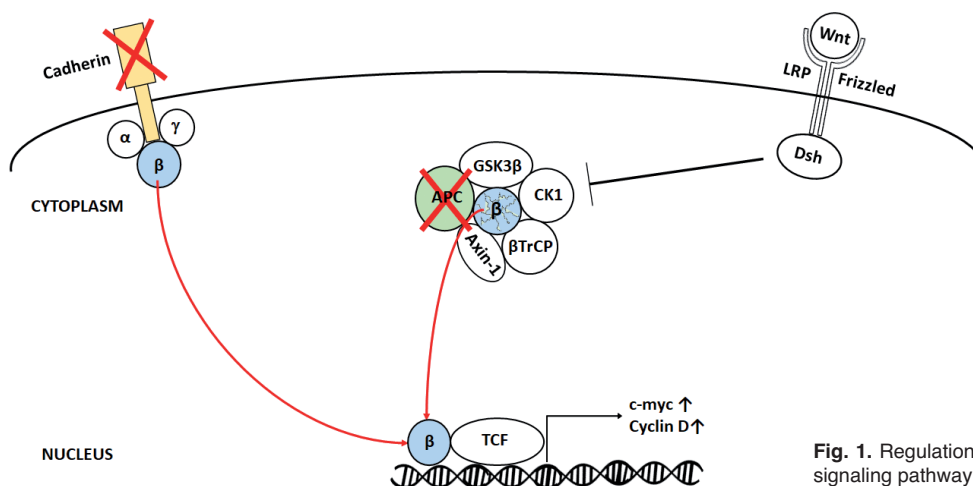


Fig. 1. Regulation modalities controlling the Wnt / CTNNB1 signaling pathway.

At the same time, the release of CTNNB1 from the cytoplasmic tail of cadherins promotes the detachment of cell membrane from the cytoskeleton, leading to the formation of pseudopods, and the inhibition of adhesion to neighbouring cells. Both phenomena contribute to the activation of cell migration (McCrea et al., 2015). Therefore, the cadherin - dependent regulation of CTNNB1 is involved in quiescence/adhesion (also known as contact inhibition) or, alternatively, proliferation/migration indicating its peculiar function in coordinating cell proliferation and cell motility.

In colorectal cancer (CRC), both regulatory mechanisms controlling the CTNNB1 signaling pathway are altered (Fig. 1). In particular, the first mechanism involves gene mutation inactivating APC function, whereas the second involves an epigenetic (hypermethylation) event inactivating Cadherin expression. The former is an early and almost constant alteration of CRC carcinogenesis whereas the second is believed to occur later and less frequently. These two situations share the inactivation of an onco-suppressor gene (APC or Cadherin) physiologically inhibiting CTNNB1 activity, leading to the final and common effect of a constitutive activation of the CTNNB1 signaling pathway which is in turn responsible for an increased proliferation activity. The loss of Cadherin expression determines an even more dramatic effect on CRC cells characterized by the fact that they lose their anchorage to neighbour cells and begin to migrate, giving rise to a process known as Epithelial Mesenchymal Transition (EMT) that is responsible for the appearance of tumor metastasis (Thiery et al., 2009).

Cadherin down-regulation in colorectal cancer

Adhesion molecules of the cadherin superfamily can be classified into three distinct families: 1) cadherins in the strictest sense, also called major cadherins (CDH); 2) protocadherins (PCDH); 3) cadherin-related proteins (CDHR) (Gul et al., 2017). All these proteins are structurally different one from another (Nollet et al., 2000; Hulpiau and van Roy, 2009; Sotomayor et al., 2014), due to the fact that their extracellular (EC) domain harbors a number of extracellular repeats (repeated motifs of about 110 amino acid residues) varying significantly among the three families. Among the major cadherins, classical and desmosomal cadherins exhibit a cleavable domain, five EC repeats and a variable cytoplasmic domain able to bind catenins. Protocadherins contain six to seven EC repeats lacking the conserved sequence elements represented in major cadherins and contain a cytoplasmic domain that is distinct from that of major cadherins. In general, protocadherins exhibit weaker adhesive properties in cell aggregation assays, and it is unclear whether they mediate homophilic or heterophilic adhesion (Chen and Gumbiner, 2006). In addition, their different cytoplasmic domain could mediate novel unknown interactions and their precise functions have not been completely

elucidated (Halbleib and Nelson, 2006). The cadherin-related family contains the most diverse members and shares only a few similarities with major cadherins. The presence of EC repeats varies from 2 to 34. It is currently unclear whether their primary role is cell-to-cell adhesion and it seems that they are actually involved in maintaining planar cell polarity (Halbleib and Nelson, 2006).

In the next sections, we will focus on cadherins exhibiting down-regulated expression in CRC, following the classification mentioned above.

Major cadherins (CDH)

CDH1 (cadherin 1, E-cadherin)

The prototype of cadherin superfamily is CDH1, the most studied so far, due to its role in the epithelial-mesenchymal transition (EMT) (Thiery et al., 2009; Bezdekova et al., 2012). The function of CDH1 is quite complex and involves the binding of its intracellular domain to the cellular cytoskeleton via proteins of the catenin family (in particular CTNNB1) (Nollet et al., 2000). Not surprisingly, a decline of CDH1 expression leads to the release of CTNNB1, which translocates to the nucleus and activates the transcription of genes promoting cell proliferation, thus mimicking the state of activation of the Wnt / CTNNB1 signaling pathway (Strumane et al., 2004; van Roy and Berx, 2008; Mitselou et al., 2016). Down-regulation of CDH1 expression has been frequently observed in CRC and is believed to give rise to cell growth and invasion (Chen et al., 2012; Lu et al., 2012). Several papers have examined the expression of CDH1 in histological samples using an immunohistochemical approach. These studies reported controversial results concerning both the expression levels of CDH1 observed in CRC and the possible association with a clinically aggressive tumor behavior. As far as expression is concerned, membranous presence of CDH1 varied from lower (El-Bahrawy et al., 2001; Losi et al., 2011; Bezdekova et al., 2012; Yun et al., 2014; Jurčić et al., 2019; Losi et al., 2019) to higher levels (Kim et al., 2016; Bendardaf et al., 2019). Regarding the association with prognosis, several studies reported that loss of CDH1 expression is associated with lymph node metastasis (Kwak et al., 2007; Lugli et al., 2007; Karamitoupoulou et al., 2011; Kim et al., 2016), distant metastasis (Filiz et al., 2010; Jie et al., 2013) and higher mortality (Nanashima et al., 1999; Ikeguchi et al., 2000; Aoki et al., 2003; Roca et al., 2006; Shioiri et al., 2006; Shiono et al., 2006; Ngan et al., 2007; Filiz et al., 2010; Kang et al., 2011; Karamitoupoulou et al., 2011; Elzagheid et al., 2012) whereas other studies failed to confirm such findings (Bondi et al., 2006; Kwak et al., 2007; Zlobec et al., 2007; Chen et al., 2008; Andras et al., 2012; Kim et al., 2016; Jurčić et al., 2019; Losi et al., 2019).

Examining these studies, it emerges that there is a great heterogeneity concerning source and dilutions of

primary antibodies, clinicopathological parameters, study location, number of patients and especially quality score used to define the loss of expression by immunohistochemistry. Therefore, there may be several explanations for the different results. A meta-analysis that considered 27 eligible studies published from 1997 to 2012, all using immunohistochemistry to assess CDH1 expression (He et al., 2013), revealed that the most important factor that may explain the different results is represented by the threshold of CDH1 expression adopted to define its “down-regulation”: in this regard, some authors suggested that this parameter should be fixed at a 50% reduction as compared to normal controls. On the same topic, a further study (Yun et al., 2014) which considered the down-regulation of CDH1 expression as the complete lack of protein, reported positive expression in 98% of stage III CRC samples and underlined an association with poor prognosis and a correlation with poor differentiation for the 7 negative cases on the total of 409 patients examined. Similarly, lower frequency of complete loss of CDH1 expression was observed in other studies (Losi et al., 2011, 2019) in which no correlation with stage of tumors or prognosis was reported. In the study of Palaghia et al. (2016) two scores, previously used for gastric cancer, were considered and only for one of them was it possible to find a significant correlation with survival. This score considered the abnormal cytoplasmic expression pattern of CDH1 present in CRC and not observed in normal mucosa (Jawari et al., 1997) suggesting a cytoplasmic redistribution of CDH1 in CRC. Also, other articles evaluate CDH1 localization (El-Bahrawy et al., 2001; Elzagheid et al., 2012; Bendardarf et al., 2019) and some of them (Elzagheid et al., 2012; Bendardarf et al., 2019) report for CRC a lower disease-free survival rate in the case of cytoplasmic expression. These papers propose that the change in CDH1 expression is qualitative rather than strictly quantitative, underlining that the tumors continue to express CDH1 but aberrantly. Nevertheless, most primary CRCs conserve membranous expression, suggesting that CDH1 is an adhesion molecule necessary to maintain cell adhesion also in primary tumor and that loss of membranous expression represents a dynamic event occurring together with epithelial-mesenchymal transition (EMT). Gagliardi et al. (1995) compared E-cadherin expression in primary CRC and liver metastases finding membranous expression in more than 50% of the metastases. Loss of membranous expression could therefore represent a transient process by which cells detach from the tumor, but the formation of metastasis requires the rescue of membranous expression. Similar results were found by other studies (Kanazawa et al., 2002; Batistatou et al., 2006; Truant et al., 2008; Palaghia et al., 2016), suggesting that neoplastic cells regain membranous expression upon arrival to lymph nodes in the process known as epithelial-mesenchymal transition (EMT). In this regard, studies concerning EMT-related proteins such as Snail

and Vimentin, underline that these proteins are more expressed at the invasive front with respect to tumor center and, in combination with loss of CDH1 expression, they are significantly associated with aggressive clinicopathological factors and shorter disease-free survival (Choi et al., 2017). This finding was not observed when the expression was evaluated in the tumor center. Considering all these studies, it follows that to establish the role of CDH1 down-regulation several variables should be considered, such as the percentage of positive cells, the localization of staining (membranous or cytoplasmic), the location in the tumor (center or invasive front). To clear up the contrasting data obtained by immunohistochemistry, perhaps the expression at the molecular level should be evaluated. In this regard, a study that reported results of microarray databases on CDH1 expression showed that only a little decrease was observed in adenoma and carcinoma compared to normal colorectal mucosa (Losi et al., 2011). Another study (Losi et al., 2014) based on microarray data meta-analysis showed that the expression of the CDH1 gene exhibited a slight decrease in CRC. Nevertheless, a correlation with clinical and pathological parameters such as grade, stage and survival was displayed.

CDH4 (cadherin-4)

Another cadherin involved in CRC is CDH4, which was for the first time connected to human cancer and in particular to CRC in a paper of Miotto et al. which reported a correlation between loss of CDH4 expression in CRC cell lines and samples and methylation (Miotto et al., 2004) and suggested that this cadherin might represent a tumor suppressor gene. The same authors proposed that detection of CDH4 methylation also in peripheral blood of patients with CRC (Miotto et al., 2004) and in stool (Nishioka et al., 2015) might be a marker for early detection of CRC.

CDH13 (cadherin-13, H-cadherin, heart cadherin, T-cadherin, truncated cadherin)

CDH13 is the only known cadherin that is membrane anchored via a GPI (glycosylphosphatidylinositol) anchor instead of a transmembrane domain and it is expressed at the apical membrane of polarized cells (Berx and Van Roy, 2009). Down-regulation of CDH13 expression, observed in numerous cancer types including CRC, increases their proliferation, migration and invasion (Andreeva and Kutuzov, 2010). A recent meta-analysis highlighted that promoter methylation of CDH13 is very frequent in CRC where it plays an important role in disease initiation and progression and might be correlated with patient overall survival (Ye et al., 2017).

Several Cadherins such as CDH9, 15, 16, 17, 19 and 22 were found to be down-regulated in CRC in a microarray meta-analysis but with little variation of their

expression level (Log₂ Fold Change from 0 to -1) (Losi et al., 2014). These results were partially confirmed by another genome-wide expression study (Bujko et al., 2015a) in which down-regulation of CDH19 and DSC2 (desmosomal cadherin, desmocollin 2) in CRC compared to normal mucosa was observed. The role of these cadherins in colorectal carcinogenesis is so far unknown even if some studies are trying to elucidate it. Indeed, desmosomal cadherins DSC 1,2,3, which do not bind catenins but various plakophilins and plakoglobin (paralogues of catenins) to assemble highly tension-resistant desmosomes (Van Roy, 2014) were found to be down-regulated in CRC. Their reduced expression was statistically linked to higher grading and, for DSC2, to shorter survival (Knösel et al., 2012). Recently, a paper demonstrated that loss of DSC2 promotes cell proliferation and tumor growth through the activation of AKT/CTNNB1 signaling (Kolegraff et al., 2011). Similarly, a study of Cui et al demonstrated that DSC3 has a tumor suppressive activity through inhibition of AKT pathway in CRC (Cui et al., 2019).

Protocadherins (PCDHs)

Protocadherins (PCDH) are divided into two families: clustered and non-clustered, reflecting the localization of their genes in vertebrate genomes. They play a role predominantly in the nervous system, where they act in circuit formation and maintenance (Hulpiau and Van Roy, 2009; Losi and Grande, 2014).

Clustered protocadherins

Evidence for the involvement of clustered protocadherins in cancer is limited. PCDHA5, PCDHA6, PCDHA10, PCDHGA8, PCDHGA10, PCDHGA11, PCDHB14 were found down-regulated in CRC in a microarray meta-analysis but with little variation of their expression level (Log₂ Fold Change from 0 to -1). A statistically significant association between low expression of PCDHGA8 and the probability to develop a relapse within 5 years after diagnosis was reported in CRC (Losi et al., 2014). Similar observations were obtained for some non-clustered protocadherins, such as PCDH1, PCDH8, PCDH9 and PCDH21 (Losi et al., 2014). A subsequent work confirmed that PCDHB14 together with PCDH7 exhibits decreased expression in CRC (Bujko et al., 2015a). The expression of PCDHGC3, a clustered protocadherin, is suppressed in hypermethylated CRC cell lines and tumor tissues recovered from patients (Dallosso et al., 2012).

Non clustered protocadherins

Among the non-clustered protocadherins, down-regulation of PCDH10 was observed in CRC cell lines and genetic aberrations (loss of heterozygosity) were reported to be an independent predictor of poor survival

for CRC patients. Subcutaneous injection of PCDH10-expressing CRC cells into SCID mice revealed a decreased tumor growth. Furthermore, re-expression of PCDH10 in silenced cells suppressed intrahepatic metastasis of CRC, suggesting that loss of PCDH10 is involved in tumorigenesis and metastasis (Jao et al., 2014).

Loss of expression of PCDH17 has been frequently found in CRC and it appears to be linked to genetic (deletions) or epigenetic (methylation) alterations. PCDH17 acts as a tumor suppressor, exerting its anti-proliferative activity by inducing apoptosis and autophagy (Hu et al., 2013).

A recent study, performed through bioinformatic analysis (Hong Kong colorectal dataset and The Cancer Genome Atlas expression array), demonstrated for the first time that PCDH18 is down-regulated in CRC samples and CRC cell lines due to promoter methylation (Zhou et al., 2017). Furthermore, the authors validated this finding with a clinical study performed on patient tissues and plasma samples. They clarified the role played by PCDH18 in the suppression of cell viability, colony formation and migration, showing that PCDH18 neutralizes the Wnt / CTNNB1 signaling pathway in colonic NCM460 cells (immortalized human colonic epithelial cell line) by inactivation of GSK-3 β . Moreover, the detection of PCDH18 methylation in DNA circulating in the plasma of patients affected by CRC suggests the possibility to use it as a plasma biomarker for early detection of the disease (Zhou et al., 2017).

Cadherin-related family (CDHR)

The cadherin-related family (CDHR) is the smallest in the cadherin superfamily and gathers the most diverse members. They are phylogenetically distinct from genuine cadherins and protocadherins. Some authors also suggest that genes that have been so far included in the protocadherins group should instead be part of the CDHR group (Gul et al., 2017). This is especially true for CDHR2 (cadherin related family member 2, PCDH24 or protocadherin-24) and CDHR5 (cadherin related family member 5, MUCDHL, Mucin and Cadherin-like protein, or MUPCDH, μ -protocadherin), which can also heterophilically trans-interact to form intermicrovillar adhesion links between adjacent microvilli in the intestinal brush border (Crawley et al., 2014).

CDHR5 (cadherin related family member 5, MUCDHL, Mucin and Cadherin-like protein, or MUPCDH, μ -protocadherin)

CDHR5 was first described by Goldberg et al, 2000 (Godlberg et al., 2000) and also named μ -prothocadherin to underline the hybrid nature of its extra-cellular region containing four cadherin-like

domains and three mucin-like domains (Crawley et al., 2014). This cadherin is down-regulated during colorectal carcinogenesis (Parenti et al., 2010; Losi et al., 2011) and is up-regulated upon treatment with a chemoprevention agent called 5-ASA (5-Aminosalicylic Acid or Mesalazine) causing the sequestration of CTNNB1 to the plasma membrane and a consequent inhibition of its signaling pathway. Down-regulation of CDHR5 expression exhibited a correlation with CTNNB1 nuclear localization and higher levels of the Ki-67 proliferation marker in CRC samples (Losi et al., 2011). To confirm this finding, obtained in a limited set of samples, the expression of CDHR5 was assessed in a CRC database of microarray expression profiles which considered the expression of cadherins in a large number of samples (Losi et al., 2014). Among assessed cadherins, μ -protocadherin showed the most appreciable variation (Log2 Fold Change <-1) and a statistically significant association between its lower expression and the probability to develop a 5-year relapse. In the same paper, CDHR5 down-regulation was investigated in a series of sporadic colorectal adenoma and carcinoma samples screened by immunohistochemical analysis and subsequently subjected to methylation analysis using bisulfite pyrosequencing. A concordance between high methylation and absence of expression was observed in CRC, whereas colorectal adenomas exhibited a methylation status that appeared intermediate between those of normal colorectal mucosa and CRC. A similar finding was obtained from several CRC cell lines previously tested by RT-PCR indicating that hypermethylation of the promoter region of the gene represents the molecular mechanism explaining the down-regulation of CDHR5 (Losi et al., 2014). Similar findings on the down-regulation of CDHR5 obtained by microarray and methylation analysis were later reported by the studies of Bujko et al. (2015b).

Further experiments, performed to elucidate the role of CDHR5, indicated that its expression is negatively regulated by CTNNB1 and suggested that the activity of these two proteins is regulated by a mechanism of reciprocal inhibition (Montorsi et al., 2016). Another study, performed to better characterize the chemoprevention activity of 5-ASA, demonstrated that this drug activates the expression of KLF4 (Kruppel-like factor 4), a transcription factor expressed in the adult intestine and important in the differentiation process. This activation leads to the expression of CDHR5 which then sequesters CTNNB1 to the plasma membrane and, with a direct mechanism, inhibits the transcriptional activity of CTNNB1 in the nucleus (Parenti et al., 2018). CDHR5 also seems to play a role in hereditary CRC, where lower frequency of CDHR5 down-regulation is observed in a set of hereditary CRC compared to the sporadic forms (Losi et al., 2019). This observation is in agreement with the best clinical outcome and with the carcinogenic mechanisms of hereditary CRC, based more on the impairment of MMR genes, rather than on the activation of the Wnt / CTNNB1 pathway, typical of

sporadic CRCs.

CDHR2 (cadherin related family member 2, PCDH24 or protocadherin-24)

In the same study (Losi et al., 2019), CDHR2, another cadherin-related protein was examined. This cadherin was first described some years ago (Okazaki et al., 2002) and due to its high expression in liver, kidney and colon is also named LKC. Its role has been investigated in CRC cell lines where it is capable of inhibiting CTNNB1 (Ose et al., 2009). In CRC samples, CDHR2 behaves similarly to μ -protocadherin with a loss of expression that appears higher in sporadic as compared to hereditary CRCs (Losi et al., 2019). Notably, double negative cases lacking both CDHR5 and CDHR2 were 4-fold higher in percentage in sporadic cancer as compared to hereditary CRCs, whereas double positive cases were observed only in hereditary CRCs. Furthermore, CRCs co-expressing or failing to express both proteins (double positive or double negative) largely predominated over those characterized by a single expression. These data suggested that a coordinated regulation in the control of CDHR5 and CDHR2 expression might exist. This was also supported by preliminary results obtained in the CaCo2 cell line where treatment with 5-ASA induces CDHR2 expression similarly to CDHR5 (Losi et al., 2019). CRCs losing the expression of both cadherin-related proteins showed higher proliferative rate as compared to those with single protein loss, suggesting that the evaluation of panels of cadherins / protocadherins rather than single cadherins / protocadherins could be more relevant for a detailed molecular classification of CRC and a more accurate diagnosis.

FAT proteins (CDHR8 to 11)

Among the other cadherin-related proteins, giant cadherin-related proteins such as DCHS (dachshous cadherin-related), and FAT (FAT1, FAT atypical cadherin1; FAT2, FAT atypical cadherin2; FAT3, FAT atypical cadherin3 and FAT4, FAT atypical cadherin4) are involved in planar cell polarity (Zhang et al., 2016) and, in particular, heterophilic interactions between DCHS1 and FAT4 seem to regulate planar cell polarity and cell proliferation (Tsukasaki et al., 2014). FAT1, a susceptibility gene associated to neurological bipolar disorder (Blair et al., 2006; Abou Jamra et al., 2008), shows a de-regulated expression in several types of tumors (Sadeqzadeh et al., 2014; Zhang et al., 2016). A recent paper reported that FAT1 is expressed at higher frequency in CRC at early stages than at more advanced stages (Pileri et al., 2016). Loss of FAT1 might affect interaction with CTNNB1, promoting its translocation to the nucleus (Morris et al., 2013). Little is known about dachshous cadherin-related proteins, with the exception of DCHS2 (dachshous cadherin-related 2) exhibiting some involvement in CRC (An et al., 2015).

RET (ret proto-oncogene, CDHR16)

RET is a tyrosine kinase receptor with cadherin-like domains in its extra-cellular domain. Therefore, based on its structure, it is a complex protein exhibiting both protooncogene and tumor suppressor gene properties exerting its actions depending on the considered cell context. Its role in CRC has been widely studied, showing that expression of RET in CRC cell lines and CRC samples is significantly decreased, which leads to reduced apoptosis levels determined by the activation of MAPK signaling (Luo et al., 2013). RET's down-regulation is associated to hyper-methylation of its gene promoter (Chan et al., 2008; Mokarram et al., 2009; Yi et al., 2011), which correlates to a poor prognosis (Chan et al., 2008; Draht et al., 2014).

Concluding remarks and future perspectives

This review analyzes the role played by cadherin down-regulation in CRC. Among approximately one

hundred existing cadherins, around thirty are known to be down-regulated in colon cancer (Table 1). Among them, ten are cadherins, sixteen are protocadherins (eight clustered and eight non-clustered) and four are cadherin-related. A complete characterization, represented by data on down-regulation, promoter hypermethylation, biological mechanism and clinical implications, is available only for a few of them (CDH1, DSC, CDHR5, PCDH10, PCDH17, PCDH18 and RET). It appears that there are inconsistencies in the literature regarding how different authors evaluate down-regulation of these proteins; it would be advisable to define a common standard in order to avoid misinterpretation of existing data. Moreover, this review highlights the fact that down-regulation is not the only event underlying cadherin involvement in transformation, but altered subcellular localization seems to play an important role as well. In addition, given that cadherin down-regulation seems to be variable in CRC and that its relevance is still unclear, since several cadherins are capable of sequestering CTNNB1 to the plasma membrane, it would probably be more relevant in future to consider the expression of panels of cadherins rather than single cadherins. Moreover, further functional comparative studies of cadherins are required to assess the relative capacity of cadherins to inhibit CTNNB1 and to act as tumor suppressors. Such an approach will allow to better clarify whether partial down-regulation of several cadherins rather than complete down-regulation of a single cadherin play different or comparable roles in relation to Wnt / CTNNB1 pathway activation. Although apparently less related to cadherin expression, an important aspect concerning this pathway is represented by its relationship with the immune response against CRC. Indeed, a recent study has highlighted the association between an activated Wnt signaling, demonstrated by the nuclear expression of CTNNB1 or by the bi-allelic mutation of APC, and a decreased T-cell infiltration, indicating a capacity of tumor to evade immune surveillance (Grasso et al., 2018). Other reports also suggested that diet, alcohol, medications and other lifestyle factors influence the intestinal microbiota which in turn can modulate the immune system, modifying the evolution of tumor (Chen et al., 2017; Zitvogel et al., 2017). Another study has demonstrated the association of a pro-inflammatory diet, such as that based on red and processed meat, with a higher risk of developing CRC subtypes characterized by an absent / low lymphocytic reaction in the tumor microenvironment (Liu et al., 2017). Microbiota have been involved in the initiation and progression of CRC by affecting intestinal inflammation and modulating the tumor-related signaling pathways (Lucas et al., 2017). These aspects have been included in the context of the so-called molecular pathological epidemiology (MPE), a relatively new and evolving field of epidemiology that investigates the correlation existing between exogenous factors such as environment, lifestyle, host factors (i.e. intestinal

Table 1. Cadherins exhibiting a down-regulated expression in colorectal carcinogenesis.

Name	Down-regulated expression	Promoter hyper-methylation	Biological characterization	Clinical implication
CDH				
CDH1	✓	✓	✓	✓
CDH4	✓	✓	x	✓
CDH9	✓	x	x	x
CDH13	✓	✓	x	✓
CDH15	✓	x	x	x
CDH16	✓	x	x	x
CDH17	✓	x	x	x
CDH19	✓	x	x	x
CDH22	✓	x	x	x
DSC	✓	x	✓	✓
PCDH				
PCDHA5	✓	x	x	x
PCDHA6	✓	x	x	x
PCDHA10	✓	x	x	x
PCDHB14	✓	x	x	x
PCDHGA8	✓	x	x	✓
PCDHGA10	✓	x	x	x
PCDHGA11	✓	x	x	x
PCDHGC3	✓	✓	x	x
PCDH1	✓	x	x	x
PCDH7	✓	x	x	x
PCDH8	✓	x	x	x
PCDH9	✓	x	x	x
PCDH10	✓	x	✓	✓
PCDH17	✓	✓	✓	x
PCDH18	✓	✓	✓	✓
PCDH21	✓	x	x	x
CDHR				
CDHR2	✓	x	✓	x
CDHR5	✓	✓	✓	✓
FAT	✓	x	x	✓
RET	✓	✓	✓	✓

microbiota), and endogenous factors such as molecular pathology and immunity (Ogino et al., 2011, 2019). In this regard, CRC represents a practical model to provide novel insights into these interactions and cadherins could play a role opening a new research frontier.

A novel entity always related to MPE is represented by the colorectum continuum model (Yamauchi et al., 2012a). The dichotomous description of CRC, accepted until today, is based on the distinction between proximal tumors, often associated to high-level microsatellite instability (MSI) (about 15% of cases) (Vilar and Gruber, 2010), and distal tumors, characterized by microsatellite stability (MSS), chromosome instability (CIN) and mutations affecting critical genes such as p53 and APC (about 85% of cases) (Fodde et al., 2001; Pino and Chung, 2010). Recent data regarding the comprehensive molecular characterizations of CRCs, including The Cancer Genome Atlas (TCGA), have increased the understanding of the genomic and epigenomic landscape of CRCs and have enabled their classification into various subtypes according to their distinct molecular alterations and clinical features (Cancer Genome Atlas Network, 2012; Inamura, 2018). A recent study showed that some subtypes of CRC, i.e. the fraction of high - MSI carcinomas, gradually increased from the rectum to the ascending colon (Yamauchi et al., 2012b) and together with subsequent papers argued against the dichotomous model supporting on the contrary the continuum model of CRC (Phipps et al., 2012, 2013; Jess et al., 2013; Rosty et al., 2013; Loree et al., 2018). How the down-regulated expression of cadherin could be topographically distributed along the various colorectal sites would be, on these basis, an interesting issue to be addressed.

In the scientific literature discussed by this review, the different locations of CRC were indeed considered but the relationship between cadherin down-regulation and anatomical sites of tumors was, in general, not analyzed and / or not reported by the various authors. The study of Martinez et al. found a strong association between a lower CDH1 expression and a worse differentiation degree, although this report exclusively analyzed left colon CRCs (Martinez et al., 2011). In a recent article (Losi et al., 2019), CDHR5 and CDHR2 were found to be less frequently down-regulated in hereditary CRCs exhibiting germline mutations of MMR (Mismatch Repair) genes and a usual localization in the proximal colon. Therefore, the possible link existing between different molecular alterations and distinct tumor sites of CRC represents a future challenge and additional studies are required to achieve a thorough comprehension of this issue.

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