

Review

Involvement of μ -protocadherin in colorectal carcinogenesis: a promise for clinico-pathological evaluation

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Summary. Cell-cell adhesion is a fundamental activity to allow the maintenance of epithelial integrity, and defects impairing this process promote the formation of tumors such as colorectal cancer (CRC). In this regard, a crucial role is played by adhesion molecules, named cadherins, which exert their function through the inhibition of the β -catenin signaling proliferation pathway, constitutively activated in CRC. A number of reports, published over the last decade, have highlighted the existence of a novel cadherin family member, called μ -protocadherin, to underline the hybrid nature of its extra-cellular region, including both cadherin-like and mucin-like domains. It has been shown that this protein plays an important role in inter-cellular adhesion processes, inhibits β -catenin activity in normal colorectal mucosa, undergoes a down-regulated expression in CRC and is up-regulated upon treatment with chemoprevention agents against this tumor.

Key words: μ -protocadherin, MUCDHL, β -catenin, Colorectal cancer, Mesalazine

Colorectal cancer pathways

Colorectal cancer (CRC) is one of the most common cancers in developed countries. Due to the worldwide scale of the problem, colorectal carcinogenesis is an extensively studied process. CRC is traditionally divided into sporadic and familial (hereditary) cases. In this review, we will focus on sporadic CRC.

It has been established that sporadic CRCs can arise from at least two different carcinogenic pathways. The traditional (“canonical” or “suppressor”) pathway is present in 80-85% of colorectal carcinomas and involves chromosomal instability (CIN) (Worthley et al., 2007) with mutations in oncogenes, such as K-ras and alterations in tumour suppressor genes, such as Adenomatous Polyposis Coli (APC), p53, DCC (Deleted in Colon Cancer) and also a high frequency of allelic imbalance (most commonly involving chromosomal arms 5q, 8q, 17p, and 18q) (Fearon and Vogelstein, 1990; Losi et al., 2005). The second (“mutator”) pathway, observed in nearly 15% of all cases of sporadic colorectal cancer (Imai and Yamamoto, 2008), involves microsatellite instability (MSI), is characterized by the presence of mutations in repetitive sequences, such as those contained in the microsatellite DNA clusters, and is caused by defects in the DNA mismatch repair genes (MMR), mostly hMLH1 or hMSH2. Examples of genes mutated through this modality and implicated in colorectal carcinogenesis are represented by TGFR beta II, IGFR, BAX (Moran et al., 2010). In sporadic tumours, MSI and loss of mismatch repair occur mainly due to methylation of the MLH1 gene promoter with a resultant epigenetic loss of protein expression (Bouzourene et al., 2010).

Wnt pathway and beta-catenin

The wnt/ β -catenin signalling pathway is considered to be the initiating event of tumour transformation in colon epithelial cells. In resting cells, such as the differentiated cells of colon crypt (not exposed to Wnt), β -catenin forms a macromolecular complex containing

the APC protein together with Axin-1 and GSK-3 (β -catenin destruction complex). This complex leads to the destruction of β -catenin and to a consequent reduction of its intra-cellular levels. Under these conditions, a fraction of β -catenin is protected by degradation and is attached to the plasma membrane, where it associates to cadherins in adherens junctions (Dihlmann and von Knebel Doeberitz, 2005). When cells are stimulated by Wnt, as occurs normally in colon crypt stem cells, this molecule binds to the frizzled receptor causing the recruitment of dishevelled protein that blocks the activity of β -catenin destruction complex. This event results in the stabilization and nuclear translocation of β -catenin, where it binds to TCF family proteins, forming a transcription factor dimer that activates the expression of genes involved in cell cycle progression, such as c-myc, cyclin D1 and VEGF (Fuchs et al., 2005). This effect is further supported by the capacity of β -catenin to negatively regulate the transcription of the p21 gene, an important inhibitor of cell cycle. When APC is mutated, destruction of β -catenin cannot occur, and this protein is continuously translocated to the nucleus of transformed cells and proliferation is constitutively activated, leading the acquisition of a persistent stem cell phenotype (Fodde and Brabletz, 2007).

Cadherins function in colorectal carcinogenesis

Cell-cell adhesion plays a fundamental role in the development, and maintenance of epithelial structure integrity and defects in cell adhesion molecules are critical for both initiation and progression of CRC (Takeichi, 1991). In particular, the cadherin family has an important role in colon cancer progression.

Cadherins are single pass trans-membrane glycoproteins which serve as calcium-dependent adhesion molecules and present 1-34 extracellular cadherin repeated domains (Nollet et al., 2000). The cadherin family includes classical cadherins, protocadherins, desmosomal cadherin (desmogleins and desmocollins). The classical cadherins are expressed in various tissues. E-cadherin is expressed in epithelial cells and mediates homophilic cell adhesion (Yagi and Takeichi, 2000; van Roy and Berx, 2004).

It is well recognized that the adhesion properties of E-cadherin are positively influenced by the association of its intra-cytoplasmic domain with catenins, among which the most important is represented by β -catenin (Gumbiner and McCrea, 1993).

β -catenin exerts a stabilizing function on epithelial structure that is realized through connection of E-cadherin to the cytoskeleton's actins, and at the same time it is an important transcription factor that regulates biological functions, such as organogenesis, tissue morphogenesis, cell proliferation and apoptosis (Olmeda et al., 2003; van de Wetering et al., 2002; Fevr et al., 2007).

The crucial role that the E-cadherin / β -catenin complex plays in tumor development resides in its

capacity to suppress either the proliferation or the invasion capacity of epithelial cells in normal conditions (Birchmeier, 2005). Recent findings have indicated that down-regulation of E-cadherin expression facilitates proliferation and invasion activities in epithelial cancers (Cristofori, 2006). This phenomenon is only rarely attributed to gene mutations (Efsthathiou et al., 1999) and is instead more frequently ascribed to epigenetic changes such as those deriving from promoter methylation of the considered gene (Wheeler et al., 2001; Garinis et al., 2002).

Other classical cadherins tend to be expressed at high levels in various types of tissue during development: N-cadherin in neural tissue and muscle, R-cadherin in forebrain and bone, P-cadherin in the basal layer of epidermis (Halbleib and Nelson, 2006).

Several cadherins, such as N-cadherin, VE-cadherin, P-cadherin and cadherin LI have been implicated in cancer disease progression (Wheeler et al., 2008).

Numerous preclinical and clinical studies have shown that the loss of E-cadherin occurs concurrently with the up-regulation of N-cadherin (Hazan et al., 2004) or other cadherin family members implicated in invasive growth (Wheeler et al., 2008). This process, known as cadherin switching, has been reported to promote epithelial-mesenchymal transition and leads to tumour cell invasion and metastasis (Christofori, 2006).

N-cadherin over-expression via cadherin switching was observed in various invasive cancer cell lines and tumours and, therefore, is emerging as a potential therapeutic target (Mariotti et al., 2007; Augustine et al., 2008).

P-cadherin was often reported to correlate with increased tumor cell mobility and invasiveness when over-expressed (Taniuchi et al., 2005; Paredes et al., 2007). The up-regulation of P-cadherin was frequently observed in various malignant tumours, including breast, colon, and pancreatic tumours and correlated with poor survival of breast cancer patients (Hardy et al., 2002; Paredes et al., 2005; Taniuchi et al., 2005). Recent findings suggest that disrupting the N or P-cadherin signaling pathway by a therapeutic agent such as monoclonal antibody or peptide antagonist resulted in a anti-metastatic and anti-proliferative activity and induction of apoptosis, representing a novel approach for anti-cancer therapy targeting tumours (Mariotti et al., 2007; Augustine et al., 2008; Zhang et al., 2011).

Protocadherins

Protocadherins (PCDHs), which are predominantly expressed in the nervous system and implicated in synaptic junction adhesive interactions (Sano et al., 1993; Kim et al., 2007) constitute the largest subgroup (about 80 members) in the cadherin superfamily (Nollet et al., 2000; Kim et al., 2001).

The protocadherin subgroup has only been identified and characterized in studies from the past decade, which have revealed a divergent cytoplasmic domain, as well

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as six or seven extra-cellular cadherin (EC) domains with low sequence similarities to the EC domains of the classical cadherin subgroup (Nollet et al., 2000).

The protocadherin family can be largely divided into two subgroups based on their genomic structure: clustered genes (alfa, beta and gamma), which are organized as a large cluster in the genome and often localized at three chromosome loci: 4p28-31; 5q31-33; 13q21, and non clustered genes (delta), which are scattered throughout the genome (Morishita and Yagi, 2007). A striking difference in the genomic organization of classical cadherin genes and PCDH genes is represented by the presence of unusually large exons in PCDH genes (Wu and Maniatis, 2000). The ectodomain of each member of the PCDH gene is encoded by a single large exon, while the classical cadherin extracellular domain is encoded by multiple exons, with few exceptions (Redies et al., 2005).

Non-clustered PCDHs are expressed predominantly in the nervous system and exhibit spatio-temporally diverse expression patterns suggesting that they play roles in circuit formation and maintenance (Kim et al., 2001). The non clustered PCDHs appear to have homophilic / eterophilic cell-cell adhesion properties and could act as a regulator via interaction with a variety of intracellular binding partners. Furthermore, each PCDH has several isoforms with differential cytoplasmic sequences, suggesting that each PCDH isoform could activate a specific intracellular signalling. Although research on the protocadherins family is still in its infancy, recent findings show that some protocadherins (9, 11, 17, 19, and 15) have been found to be involved in neuropsychiatric disorders and visual dysfunction (Dibbens et al., 2008; Depienne et al., 2009; El-Amraouri and Petit, 2010) suggesting that they play multiple, tightly regulated roles in normal brain function. In addition, recently, some PCDHs have been reported as candidate tumor suppressor genes. The expression of PCDH8 in breast and hematologic tumors, PCDH9 in glioblastoma, PCDH10 in gastric, colorectal, nasopharyngeal, esophageal, breast, cervical, lung, hepatocellular, testicular and haematological neoplasias, PCDH17 in esophageal squamous cell carcinoma and PCDH20 in non-small cell carcinoma, are reduced or silenced through gene inactivation, such as promoter hyper-methylation and / or somatic mutations (Kim et al., 2001; Morishita and Yagi, 2007). Furthermore re-expression of PCDH8 (Yu et al., 2008) or PCDH10 (Yu et al., 2009) suppresses tumor cell proliferation and inhibits cell migration. Notably, PCDH8, 9, 17 and 20 genes are located around 13q21.1 and are closely positioned within 16 megabases. These results suggest that PCDHs on chromosome 13q21 might be broadly involved in tumor suppression in a variety of tumors.

Elevated expression of PCDH24 or LKC (Protocadherin liver, kidney, and colon) in HCT116 colon carcinoma cells have been shown to induce contact inhibition, thereby completely abolishing tumour formation in vivo (Okazaki et al., 2002). PCDH24

appears to be a distinct negative regulator for nuclear translocation of β -catenin and subsequent transcriptional activation. In PCDH24-expressing cells β -catenin is retained in a submembranous location where it interacts with actin cytoskeleton and vimentin intermediate filaments (Ose et al., 2009), as already observed for classical cadherins.

μ -protocadherin: gene, protein and function

Working on a rat cDNA library, in 2000, Goldberg et al. were able to isolate the nucleotide sequence coding for a novel member of the cadherin protein family that was named μ -protocadherin, to underline the hybrid nature of its extra-cellular region, explained below in more detail, and additionally called MUCDHL, MU-PCDH or CDHR5. Examination of the deduced aminoacid sequence of this cDNA allowed authors to disclose the presence of four cadherin-like domains (unlike other cadherins, harbouring more than six) and three mucin-like domains (reflected in its denomination), in the extra-cellular portion of the protein, and four prolin-rich domains and a PDZ-binding domain (previously shown to bind β -catenin and cytoskeleton proteins), in its intra-cellular tail (Goldberg et al., 2000). Interestingly, sixty-nine percent of the aminoacids, contained inside the mucin-like repeats, were represented by serine, threonine or prolines, thus providing a considerable number of potential sites available for O-linked glycosylation. Five putative N-glycosylation sites were also identified in the extra-cellular domain of the polypeptide chain. Northern blot analysis evidenced a preferential expression of the investigated gene in adult rat kidney, where it revealed the existence of two mRNA isoforms, the longer of which was represented by the full length version of the coding sequence, while the shorter appeared to be devoid of the mucin-like repeats. Western blot analysis, performed on cell extracts of the same cells upon treatment with O- and N-glycosidases, confirmed the glycosylation pattern of μ -protocadherin that had been previously predicted on the base of its aminoacid sequence. Immunofluorescence assay, carried out on a similar tissue sample, showed some colocalization spots of μ -protocadherin and β -catenin that resulted especially evident at the junctions between basal and lateral membranes of tubule epithelial cells (Goldberg et al., 2000). Transfection experiments conducted on L929 fibroblast cells using an expression vector containing μ -protocadherin induced a remarkable cell aggregation effect, confirming the role that the investigated protein could play in inter-cellular adhesion processes.

Two years later, by using a fluorescent in situ hybridization (FISH) approach, the same authors demonstrated that the μ -protocadherin gene was localized in the distal end of mouse chromosome 7, syntenic to the human 11p15.5, an imprinted region often subject to loss of heterozygosity in Wilms tumour (Goldberg et al., 2002). Analysis of Basic Local

Alignment Search Tool (BLAST) revealed that human μ -protocadherin maps to 11p15.5, matching a previously identified gene called MUCDHL (or MUPCDH). This bioinformatic tool also allowed authors to demonstrate the existence of three alternatively spliced mRNA isoforms of human μ -protocadherin, two of which corresponded to those that had been previously characterized, whereas the third isoform exhibited a shortened 13th exon, a specific cytoplasmic region and appeared devoid of the mucin-like and transmembrane domains. This isoform could potentially encode for a secreted form or, alternatively, could be linked to the membrane by a glycosyl phosphatidylinositol (GPI) linkage, although the consensus sequence for GPI linkage was not detected. Although μ -protocadherin contains many similarities to the protocadherin superfamily, the analysis reported above is in contrast to the clusterized genomic organization of other protocadherin genes (Wu and Maniatis, 2000). A subsequent Northern blot analysis experiment confirmed the existence of all the mRNA isoforms, demonstrating their expression in the gastroenteric tract where, more precisely, it was detected in liver and in small and large intestine. Immunocytochemistry analysis, performed in human adult kidney, revealed that μ -protocadherin is expressed in the apical surface of epithelial cells in proximal convoluted tubules. Transfection experiments performed in Madin-Darby canine kidney cells demonstrated that both the longer mRNA isoforms of μ -protocadherin were targeted to the apical surface of polarized cells and a truncated form lacking the COOH terminus was still capable of targeting to the same cellular site. Deletion of NH2 terminus, conversely, disrupted this targeting and μ -protocadherin also localized to the lateral membrane. These experiments consequently demonstrated that the extra-cellular domain is required for targeting μ -protocadherin to the apical surface.

In a subsequent paper, an extended gene expression study performed by an independent research group confirmed the data described so far and demonstrated that μ -protocadherin is expressed in virtually all organs of the gastroenteric tract (Moulton et al., 2004).

Taken together these observations suggested an involvement of μ -protocadherin in the biological functions that are normally regulated by other cadherins, such as proliferation, apoptosis and development. In spite of the biological relevance of these findings, several years were later necessary before other authors could again address their interest in the functional role of μ -protocadherin.

Chemoprevention of CRC and involvement of μ -protocadherin

In the last years, a number of authors have focused their attention on drugs characterized by a chemoprevention activity against CRC. Among them, NSAIDs exhibited a clear (Janne and Mayer, 2000;

Sandler et al., 2003) efficacy in a general population as well as in patients with an increased risk of developing the considered disease. Unfortunately the systemic and gastrointestinal toxicity of NSAIDs drastically limits their administration in the context of clinical protocols requiring a long term treatment of interested patients. Both therapeutic and toxic effects elicited by these compounds are largely dependent on the inhibition of Cyclooxygenase (COX)-1 and COX-2, enzymes that, in turn, are responsible for a reduced synthesis of prostaglandins normally sustaining a number of physiological functions.

Several reports indicate that mesalazine (5-Aminosalicylic Acid or 5-ASA) could be a promising alternative to achieve a comparable anti-CRC chemoprevention effect, avoiding, at the same time, the severe side effects usually provoked by NSAIDs (Allgayer, 2003; Cheng and Desreumeaux, 2005; Stolfi et al., 2008). In fact, despite the chemical similarity with a typical NSAID such as aspirin, 5-ASA is characterized by a weak COX inhibitory activity and a poor systemic absorption. Like some classical FANS, 5-ASA also exhibits COX independent effects that largely reside in its capacity to modulate the activity of transcription factors regulating the immune and / or inflammatory response, such as Peroxisome Proliferator Activated Receptors (PPARs) and Nuclear Factor (NF) κ B (Allgayer, 2003; Cheng and Desreumeaux, 2005; Gasche et al., 2005; Rousseaux et al., 2005; Stolfi et al., 2008). Due to its anti-inflammatory properties 5-ASA therapy is the treatment of choice in Ulcerative Colitis where this compound leads to a parallel up to 49% reduction of the risk of developing CRC or CRC / dysplasia (van Staa et al., 2005; Velayos et al., 2005). Although these chemoprevention effects are quite encouraging, it has to be pointed out that they remain to be confirmed in other risk categories, such as patients undergoing colon polypectomy, tumour genetic syndromes and healthy people, through specifically designed clinical trials.

Several papers have, over the last decade, tried to characterize the biological actions determined by 5-ASA on colon cancer cells, which could reasonably underlie its chemoprevention properties. These reports evidenced a number of effects, spanning from inhibition of proliferation and induction of apoptosis and DNA repair processes, which collectively can be interpreted as a capacity of 5-ASA to potentiate cell cycle checkpoints (Reinacher-Schick et al., 2003; Gasche et al., 2005; Luciani et al., 2007; Campregher et al., 2010).

At the molecular level, 5-ASA has been demonstrated to inhibit the Wnt/ β -catenin proliferation signaling pathway, by blocking the activity of the protein phosphatase 2A (PP2A) enzyme, normally required to allow the nuclear translocation of β -catenin protein (Bos et al., 2006). Other authors ascribed this activity of 5-ASA to its capacity to activate the PPAR γ nuclear receptor (Rousseaux et al., 2005), a transcription factor that has been previously demonstrated to inhibit the same signaling pathway by promoting β -catenin

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degradation (Jansson et al., 2005; Rubin et al., 2008). Regardless of the precise molecular mechanisms that are activated to inhibit the β -catenin signaling pathway, it is worth considering that this activity of 5-ASA explains many of the cellular effects that this compound is able to determine on colorectal cancer cells.

To better characterize the molecular mechanisms underlying the chemoprevention capacity of 5-ASA, Parenti et al., 2010 through a study that was mainly conducted in the Caco2 CRC cell line, has recently demonstrated that treatment of colorectal cancer cells with 5-ASA determines a remarkable up-regulation of μ -protocadherin. This result was obtained using a number of molecular analysis procedures, such as microarray mRNA expression profiling performed with the Affymetric methodology, QRT-PCR, and Western blot analysis. Interestingly, under the same experimental conditions, the authors of this report did not observe any significant variation of E-cadherin expression, usually indicated as the preferential interactor of β -catenin on the plasmatic membrane of epithelial cells. Furthermore, by using a combined co-immunoprecipitation and co-localization approach, they were able to demonstrate that μ -protocadherin sequesters β -catenin on the plasmatic membrane of 5-ASA treated CaCo2 cells, inhibiting its nuclear translocation and transcriptional activity. The conclusion of this report was, therefore, that 5-ASA interferes with the β -catenin proliferation signaling pathway through the induction of μ -protocadherin expression (Parenti et al., 2010). The relevance of this finding is based on the observation that the β -catenin pathway is constitutively activated in CRC, raising the possibility that μ -protocadherin is the product of a tumor suppressor gene undergoing a down-regulated expression during the transformation process accompanying such a neoplastic condition.

μ -protocadherin is a common event in colorectal carcinogenesis

To address this issue, in a recent paper the same authors (Losi et al., 2011) assessed the mRNA and protein expression of μ -protocadherin in normal and tumor colorectal cell samples by using microarray expression profiling, QRT-PCR and immunohistochemical analysis. The results obtained evidenced that the expression of the investigated gene was down-regulated in all colorectal cancer cell lines as well as in the majority of tumors (Losi et al., 2011). More in detail, immunohistochemical evaluation of μ -protocadherin revealed localization at the lateral borders and the apical extrusion zone of normal colon enterocytes. Intensity of staining remarkably increased from the former to the latter site of cells and from the bottom to the top of the crypt, indicating that this protein was more expressed in differentiated cells. A complete loss of μ -protocadherin protein expression was observed in most examined CRC tumor samples (71%) together with a normal E-cadherin expression and higher proliferation levels as estimated

by Ki-67 labeling index, different from the remaining set of CRC in which a normal expression of μ -protocadherin was accompanied by retention of β -catenin on cell membrane of cancer cells and low proliferative activity. Co-localization immunofluorescence analysis clearly demonstrated that μ -protocadherin is associated to β -catenin on such a cellular site, both in normal and positive μ -protocadherin tumours cells, implying that β -catenin is released from that site and translocates to the nucleus in CRC cells at least partly as a direct consequence of the down-regulation of μ -protocadherin itself. All these data were globally consistent with an onco-suppressive activity exerted by μ -protocadherin on colon epithelial cells.

To explain these data, the authors postulated that promoter methylation could be the mechanism explaining the down-regulated expression of μ -protocadherin gene observed in CRC. This hypothesis was supported by a previous report demonstrating that the hypermethylation of β -catenin related genes is a common event in neoplastic colon disorders (Dhir et al., 2008). Future studies, aimed at assessing the DNA methylation pattern of the μ -protocadherin gene in the above mentioned colorectal disease, will help to verify this hypothesis. Taken together, the data indicate the down-regulation of μ -protocadherin as a common event in colorectal carcinogenesis, evidencing the important role played by this protein in biological functions, such as proliferation and differentiation, which are normally regulated by other cadherins in colon enterocytes.

Further and even more relevant information regarding the biological role played by μ -protocadherin in colorectal carcinogenesis has been recently obtained by a different group of researchers (Hinkel et al., 2012), working on a homeodomain transcription factor called Cdx2 and using, as experimental models, a considerable panel of CRC cell lines such as CaCo2, HT29, Hct116 and SW480. The biological rationale underlying this report is based on the observation that, in mice, the mentioned protein is involved in the embryonic development of gut and its expression becomes gradually restricted to the intestinal epithelium in adult animals (Guo et al., 2004), whereas in human it is characterized by a dysregulated expression in CRC (Brabletz et al., 2004). Additional properties of Cdx2 are also represented by its capacity to inhibit the proliferation activity of enterocytes and to promote, at the same time, the differentiation, adhesion and polarization processes of these cells (Gross et al., 2008).

Preliminary data obtained by the authors allowed them to demonstrate that vector mediated over-expression of Cdx2 and its inactivation by transfection of oligonucleotide siRNA, respectively, gave rise to an up- and down-regulation of μ -protocadherin expression in the analyzed colorectal cancer cells. This finding clearly suggested that μ -protocadherin could be a direct target gene of Cdx2 in the investigated cell context. Luciferase assay and Chip experiments, performed to validate a putative binding site for Cdx2 that had been

previously identified inside μ -protocadherin proximal promoter region, subsequently confirmed the hypothesis. By using a doxycycline inducible plasmid expression vector, the authors were then able to demonstrate that the μ -protocadherin mRNA isoform, lacking the mucin-like domains, is able to sequester β -catenin on plasmatic membrane, inhibiting its transcriptional activity and the proliferation capacity of colorectal cancer cells. This effect became especially evident using an experimental system based on colony formation in soft agar, also adopted by the authors to mimic the tri-dimensional microenvironment of colon mucosa, and was only minimally detected with the full length mRNA isoform of μ -protocadherin, indicating that the presence of mucin-like domains probably exerts an inhibitory activity on the anti-proliferation properties of this cadherin. More strikingly, data obtained by Hinkel et al., 2012, showed that with similar differences between the two mRNA isoforms, over-expression of μ -protocadherin was able to determine an onco-suppressive effect in a classical tumorigenic assay based on inoculation of colorectal cancer cells in nude mice, providing, for the first time, the proof of principle demonstration that the gene under consideration actually is an anti-oncogene.

Altogether, these observations allowed the involved researchers to clarify relevant aspects on the biological role played by μ -protocadherin in normal and neoplastic colon epithelium, although the precise role of the third and shorter mRNA isoform, coding for a putative secretive variant of the protein, remains to be elucidated.

Future challenges

Taken together all the data presented so far help to better understand the biological function of μ -protocadherin, highlighting the critical role that it plays in colorectal carcinogenesis. Additional efforts are required to study the molecular interactions among the different components of cell-cell adhesion complexes and the modulation they undergo during the CRC progression. In this regard particular attention should also be dedicated to cadherin independent molecular mechanisms regulating the process under consideration, such as those represented by β -catenin phosphorylation, stability or interactions with specific partners.

As already reported, the up-regulated expression of μ -protocadherin, elicited by drugs such as 5-ASA, might represent one of the molecular mechanisms underlying CRC chemoprevention. To date, this effect has been exclusively observed in IBD and it remains to be confirmed, through specifically designed clinical trials, on different and large categories of patients such as those affected by multiple polyps and hereditary / familial tumour syndromes or even healthy people. In this context, μ -protocadherin might be used as a biological marker to monitor the chemoprevention efficacy of 5-ASA. In addition, it should be interesting to evaluate, on large series of patients, the possible synergism existing

between this chemoprevention agent and other drugs characterized by a similar pharmacological activity.

The relevance of such clinical trials also resides in the possibility to use them as an opportunity to perform a number of pharmaco-genomic pilot studies, carried out by molecular techniques such as DNA microarrays and QRT-PCR, aimed at characterizing other genes involved in the μ -protocadherin pathway, which could themselves represent additional biomarkers of the response to chemoprevention treatment.

Another attractive challenge would be to assess whether μ -protocadherin could play a role in the clinico-pathological evaluation of CRC. Knowledge on this issue is, to date, still limited. It is therefore of great interest to examine, in a large series of CRC tissues, the potential relationship existing between μ -protocadherin expression and peculiar histo-pathological parameters, such as grade and stage, which are related to prognosis.

Moreover, it has been observed that alterations of some genes are particularly related to the development of specific types of metastases (Losi et al., 2004, 2007; Zhang et al., 2010; Mannan and Hahn-Stromberg, 2012). Due to the intercellular adhesion role that is normally exerted by μ -protocadherin, an involvement of this cadherin-like protein in the above mentioned processes deserves a proper investigation.

Data arising from all these activities could contribute to a detailed molecular classification of CRC helping to design therapeutic strategies based on patient clinical stratification.

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