

• REVIEW •

CDH1 germline mutation in hereditary gastric carcinoma

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Abstract

This paper provides a bird's-eye view both in preclinical and clinical aspects of E-cadherin germline gene (CDH1) in gastric cancer patients and their families. E-cadherin, a product of CDH1 gene, belonging to the functionally related trans-membrane glycoprotein family, is responsible for the Ca^{2+} -dependent cell-cell adhesion mechanism and contributes to dissociation followed by acquisition of cell motility, which usually occurs in the first step of cancer invasion and metastasis. CDH1 gene germline mutation is common in many types of carcinoma, and occurs very frequent in hereditary gastric carcinoma (HGC) patients and their families. Recently, more and more researches support that E-cadherin plays an important role in the differentiation, growth and invasion of HGC. So it is of great value to clarify its mechanisms both for understanding HGC pathogenesis and for clinical therapy, especially in China, where there are a high risk population of gastric cancer and a high HGC incidence rate. In this paper, recent researches on CDH1 gene mutation, especially its role in tumor genesis and progress of HGC, are reviewed, and advances in evaluation of its mutation status for HGC diagnosis, therapy and prognosis, are also discussed briefly.

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INTRODUCTION

CDH1 mutation in HGC

Gastric carcinoma is one of the leading causes of cancer mortality in many countries, especially in most Asian countries. It is also one of the leading malignant diseases in China. According to data, approximately one Chinese person died of gastric cancer every 2 to 3 min. In the past decades, the incidence tended to increase gradually. Some population based surveys revealed that about 10% gastric cancer patients demonstrated familial clustering. The identification of genes predisposing to HGC is an essential step towards understanding the molecular events underlying tumorigenesis and critical for the clinical

management. In 1998, a germline mutation in the CDH1 gene, which located on chromosome locus 16q22.1, was first recognized in Maori kindred from New Zealand with diffuse gastric carcinoma. The product of CDH1 gene, E-cadherin protein, a 120 ku transmembranous glycoprotein, is involved in Ca^{2+} -dependent intercellular adhesion. More and more researches confirm that impaired expression of E-cadherin contributes to histogenesis, tumor growth, metastasis and poor survival of gastric cancer patients, especially HGC patients. So further analyses are needed to clarify the mechanism of gastric cancer and provide better strategies for treatment of HGC patients and prophylaxis of their families.

E-CADHERIN: STRUCTURE AND FUNCTION

The newly mutated gene discovered in HGC, CDH1, is composed of 16 exons and 15 introns encoding the E-cadherin protein. E-cadherin, also called epithelial cadherin or uvomorulin, is a member of the subclass of cadherins, which are Ca^{2+} -dependent cell-cell adhesion receptors identified in vertebrates. The 120 ku E-cadherin molecule is made up of a 27 amino acid signal peptide (exons 1-2), a 154 amino acid precursor peptide (exons 2-4), and a 728 amino acid mature peptide. The mature protein consists of three major domains, five extracellular repeats (CAD repeats) (exons 4-13), a single membrane-spanning region (exons 13-14) and a cytoplasmic region (exons 14-16). The N-terminal CAD1 repeat is essential for the homophilic binding specificity that directs "like" E-cadherins with each other. The cytoplasmic domain is bound to the actin cytoskeleton via intracellular attachment proteins, the catenins, forming the E-cadherin/catenin complex (Figure 1).

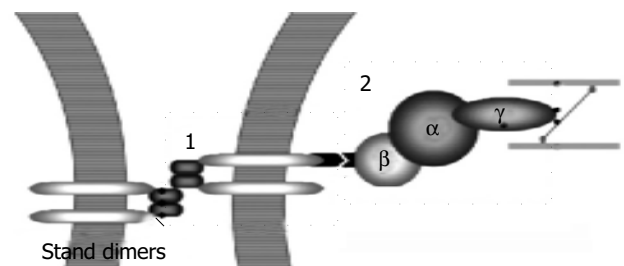


Figure 1 Formation of E-cadherin/catenin complex. Step1. E-cadherin stand dimers on the two adjacent cells' surface combine together and transfer the signals to catenin complex which is composed of β + α + γ subunits. Step2. The complex promotes actins to contract and enhance the intra-cellular adhesion.

Linkage between transmembranous cadherins and actin filament of the cytoskeleton is localized at the cell-cell adherent junction(AJ) and mediated by many associated undercoat proteins of the junction including catenins, vinculins, and actin^[1]. Catenins are a series of cadherin-associated cytoplasmic proteins that are co-immunoprecipitated with cadherin by nonionic detergent, and classified by their molecular mass as α -(102 ku), β -(88 ku), γ -(80 ku)^[2]. Furthermore, catenins connect cadherin to other integral membrane proteins, such as Na^+ - K^+ -ATPase, or to cytoplasmic structures, such as fodrin or ankyrin^[3]. All these form a transcellular network, mediating structural integrity, cellular polarity and epithelial morphogenesis^[4,5].

The CAD1 domain conformation of E-cadherin contains seven β strands (β A - β G) and two short α helices (α A, α B). The homophilic binding specificity appears to be governed by CAD1 domains through a surface including a HAV motif, which is found in or following β F. CAD1 also possesses the PEN (10-13), LDRE (66-69), DXND (100-104) motifs, while CAD2 possesses a DAD motif. These four Ca^{2+} -binding motifs can form a shared Ca^{2+} -binding pocket. The ligation of Ca^{2+} between tandem CAD domains explains the vulnerability of cadherins to proteolytic degradation when Ca^{2+} is depleted. Removal of Ca^{2+} would alter the junction between tandem CAD domains and likely expose the linkers to proteases. The binding of Ca^{2+} could provide the rigidity suggested by Ca^{2+} -induced changes of the entire extracellular region of E-cadherin from a globular to a rod-like structure observed by electronic microscopy^[2,6].

Full length E-cadherin has a cleavage site near the transmembrane domain and soluble E-cadherin (s-E-cadherin), a soluble 80-84 ku amino-terminal fragment, can be produced artificially in the culture medium digested by trypsin, so E-cadherin is also named as cell CAM120/80^[7]. s-E-cadherin can be detected in the protein extract of tissues, and in serum of peripheral blood or urine by means of immunoenzymometric assay (IEMA) and the volume did not depend on either age or sex^[8]. Research also found it could be released from some human carcinoma cell lines (for instance MCF-7) into the culture medium^[9].

E-CADHERIN ABNORMAL EXPRESSION IN CARCINOMA

E-cadherin is a crucial adhesion molecule which hampers tumor invasion and metastasis. Down-regulation of expression or impaired function would lead cells to poor intercellular adhesion through inhibiting homophilic binding. To normal epithelial tissues, it means poor differentiation, while to tumor, it means invasive growth pattern and easy to spread from the primary place. Disturbance of epithelial cell adhesion has been shown to be due to several mechanisms as shown in Figure 1. The first is inefficiency of E-cadherin homophilic binding, the second is abnormality or deletion of catenins, the last is biochemical modification of catenins, such as tyrosine phosphorylation of β -catenin^[10,11]. Among the factors that lead to inefficient homophilic binding of E-cadherin, the most important and widely recognized is E-cadherin down regulation or dysfunction due to CDH1 mutations.

Down-regulation of E-cadherin expression or CDH1 mutations could cause a change towards a less epithelial morphology and also interfered with initial adhesion of cells. Moreover, changed E-cadherins could sharply increase cell motility and change the organization of actin cytoskeleton. Nagafuchi *et al.*^[12] showed that the enhanced expression of E-cadherin cDNA in fibroblastic cells could generate epithelial structures in their recent research. McNeill *et al.*^[13] found that E-cadherin could cause polarized distribution of Na^+ - K^+ -ATPase, an important factor in the establishment of cell polarities. Mutations have been also found at very early non-invasive stages, thus associating E-cadherin mutations with loss of growth control and defining CDH1 as a real tumor suppressor^[14,15]. All of these may contribute to the significant correlation between abnormal E-cadherin expression and degree of differentiation ($P = 0.0001$) or local tumor size ($P = 0.002$)^[16].

Immunohistochemical studies have shown that expression of E-cadherin is reduced or lost in more than 15 different human carcinomas^[17]. The frequency of reduced E-cadherin expression is higher in tumors with infiltrative growth than in those with expansive growth. These observations are supported by *in vitro* studies, which evaluated the influence of E-cadherin on cell behavior. Behrens *et al.*^[18] found MDCK cells could invade into collagen gels when their E-cadherins were inactivated with antibodies. Frixen *et al.*^[19] also observed the similar phenomenon

that the invasiveness of several human carcinoma lines was suppressed by mouse E-cadherin cDNA transfection. Down regulation of E-cadherin or its functional defects could change the construction of actin cytoskeleton, which can weaken intracellular adhesion, increase the activity of epithelial cells and lead to more invasions.

Extensive immunohistopathologic studies, including those of squamous cell carcinomas of the head, neck and prostate also support the possibility that loss of E-cadherin expression may promote tumor metastasis to lymph nodes. Reduced E-cadherin expression of breast cancer was also found to have a higher frequency of blood-borne distant metastasis (*e.g.* bone or lung) than preserved E-cadherin expression of other tumors. There was a significant correlation between the degree of E-cadherin expression and the degree of tumor differentiation, as well as histological type according to the Lauren or the WHO classifications. Similarly, the correlation between E-cadherin expression and prognostic parameters (depth of invasion, lymph node involvement and vascular invasion) could be demonstrated according to some researches. These findings are consistent with the previous hypothesis that inhibition of E-cadherin function could enhance the release of cancer cells from their primary sites.

CDH1 MUTATION IN HGC PATIENTS AND FAMILIES

Lauren's classification of gastric cancer includes the intestinal (glandular) and diffuse type, as well as the mixed type. It has been reported that two histologic types have differences in epidemiology, clinicopathologic profile and molecular genetics, while both originate from *Helicobacter pylori* positive gastritis. The intestinal (glandular) type is especially predominant in high-risk population and elderly patients, and related with environmental factors, and their incident rates are decreasing in developed countries, suggesting that the pathogenesis of intestinal-type gastric carcinoma has an environmental component. Diffuse gastric cancer is determined significantly by heredity and the criteria for familial gastric cancer worked out by some investigators^[20,21] are as follows. There should be at least 3 relatives with gastric cancer. One should be a first-degree relative of the other 2. At least 2 successive generations should be affected. At least 1 should be diagnosed before age 50. Other familial tumors should be excluded.

Several investigations outlined the role of lost or mutated junctional molecules (E-cadherin, catenins, integrins, *etc.*) in the pathogenesis of diffuse gastric cancer (DGC). In particular, more than 50% of advanced diffuse gastric cancer patients showed mutations of CDH1 gene^[22-25]. Germline mutations of the CDH1 gene have been recently reported in gastric cancer families with diffuse-type tumors but not in those with intestinal-type tumors^[26]. These families are characterized by a highly penetrant susceptibility to DGC with an autosomal dominant pattern of inheritance, predominantly in young persons. Familial aggregation of GC occurs in about 1% of gastric cancer patients. Although the genetic factors resulting in this aggregation have been unclear, the study indicates that germline mutations of CDH1 genes, do contribute to such a clustering. Mutations of the CDH1 gene has a high penetrance and confers a lifetime risk of gastric cancer of 75-80% for carriers^[27-30].

The International Gastric Cancer Linkage Consortium (IGCLC) predicted that up to 25% of families fulfilling the criteria for hereditary DGC would harbour CDH1 germline mutations^[29]. CDH1 is well known as a strong invasion suppressor in experimental tumor cell systems. Inactivated mutations have been identified for the CDH1 gene in HGC. To date, 69 somatic mutations have been reported comprising, in addition to few missense mutations, mainly splice site mutations and truncation mutations caused by insertions, deletions and nonsense mutations. Up to now,

27 germline mutations previously reported are distributed along with CDH1 gene in Table 1. While in sporadic gastric cancers, truncation mutations are uncommon and sequence changes usually result in either missense mutations (most commonly in exons 8 and 9) or exon skipping, especially in exons 6-9^[30]. From the 27 CDH1 mutations described to date, 24 are inactivated ones (splice-site, frame shift and nonsense) and the remaining 4 are missense^[31-37].

Table 1 CDH1 gene germline mutation patterns in HGC families

Ethnic background	Age at diagnosis	Mutation type	Mutation site
White	30-67	45insT	Exon 1
White	34-69	49-2A→G	Exon 2
-	-	53delC	Exon 2
White	27-50	59G→A	Exon 2
White	37-46	70G→T	Exon 2
Japanese	46-72	185G→T	Exon 3 ¹
White	33-69	187C→T	Exon 3
Maori	22-28	190C→T	Exon 3
White	41-47	283C→T	Exon 3
White	15-58	372-377del1	Exon 3
White	31-55	586G→T	Exon 5
Korean	30-63	731A→G	Exon 6 ¹
White	23-70	832 G→A	Exon 6
White	14-67	1008G→T	Exon 7
White	35-47	1018 A→G	Exon 8 ¹
African-American	29-58	1137+1G→A	Intron 8
Korean	42-49	1460T→C	Exon 10 ¹
White	32-40	1472insA	Exon 10
White	31	1487del7	Exon 10
-	-	1565+1 G→T	Intron 10
White	40-63	1588insC	Exon 11
-	-	1710delT	Exon 11
White	30-68	1711insG	Exon 11
White	23-43	1792C→T	Exon 12
Maori	30	2095C→T	Exon 13
-	-	2295+5 G→A	Intron 14
White	16-35	2381insC	Exon 15

¹Missence mutation; -No information obtained from the published paper.

Mutant E-cadherins are not localized in the lateral regions of cell-to-cell contact sites of adherin junctions (AJs) and show apical and perinuclear localization. It is explained by an inability of the cells to complete polarization^[38]. These may result in disassembly of AJs and consequently conversion to a spindled and more scattered cell morphology^[39]. Impaired processing of mutant E-cadherin, loss of the putative calcium binding sites or essential amino acid motives, may cause a transition from a rigid to a more globular molecular structure of the extracellular domain. In response to extracellular signals, cells react by dissolving their cell-to-cell contacts and by changing their shape and the strength of their attachment to the substrate, thus resulting in significant reduction or loss of its ability to bind in a homophilic fashion to neighboring cells^[40,41]. Cell motility is thought to be the result of the concerted action of all these individual events.

Mutant E-cadherin lacking the cytoplasmic domain or the β -catenin-binding site may tie up essential catenins, which influences its function to suppress cell motility. Actin filaments are drastically reduced^[42]. The actin belt characteristics of epithelial cells are lost^[43]. Because proper anchorage to the cytoskeleton is necessary for E-cadherin function, suppression of E-cadherin expression or of its functional activity, or a defect in any of the molecules involved in this system, may account for the ability of cancer cells to detach from the primary tumor and invade locally. Cells with initial calcium-dependent cell aggregation exhibits decreased aggregation and remarkably increase cell motility when E-cadherin is not expressed (Figure 2).

We can conclude that these E-cadherin mutations may not only affect cell adhesion but also play an important role in a trans-dominant-active manner, thus leading to increased cell motility. They are the cause of multiple morphological and functional disorders and could induce scattered morphology and invasive behavior of diffuse gastric cancer.

Tissue specific gene expression has been explained by the interaction of promoter sequences with their specific transcription factors. *In vivo*, foot printing analysis revealed that positive regulatory elements of the E-cadherin promoter were specifically bound to transcription factors in E-cadherin-expressing cells. Mapping of DNase type I hypersensitive sites showed that the chromatin structure in the promoter region was loosened in expressing cells but condensed in non-expressing cells. Furthermore, endogenous E-cadherin promoter was methylated at CpG island sites in undifferentiated cells. All these suggested that silencing of the promoter during epithelial mesenchymal transition and tumor progression was due to a loss of factor-binding *in vivo* and chromatin rearrangement in

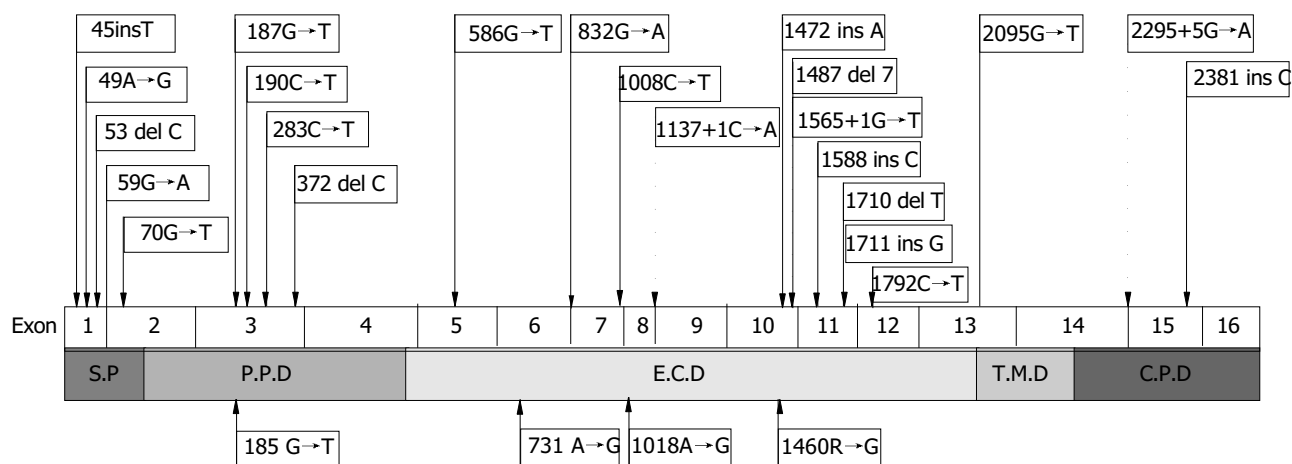


Figure 2 Schematic of CDH1 germline mutations in HGC. Truncating mutations are shown above the gene and missense mutations below. Arrowhead indicates the status in the dot frame refers to the alteration of intron pointed. S.P.: signal peptide, P.P.D.: protein precursor domain, E.C.D.: extracellular domain, T.M.D.: transmembrane domain and C.P.D.: cytoplasmic domain.

the regulatory region^[10,44]. Comijn *et al.*^[11] reported that multi-zinc finger protein, SIP1 (ZEB-2) and snail showing specific DNA binding activity could bind to partly overlapping promotor sequences and had similar silencing effects.

CDH1 MUTATION FOR HGC DIAGNOSIS AND THERAPY

Indicator for prophylactic total gastrectomy

HGC was associated with E-cadherin. Penetrance of the gene ranged from 70% to 80%, and the average age of gastric cancer patients was 37 years^[20,45,46]. These characteristics have led to the consideration of prophylactic total gastrectomy (PTG) in family members with CDH1 mutations. Lewis *et al.*^[45] performed this operation on 6 asymptomatic members in 2 families based on family pedigree and genetic analysis. The gastric specimens appeared normal and the results of routine pathologic examination were negative for cancer, but all the patients had microscopic foci of cancer, often at multiple sites, with overlying normal gastric mucosa. These results indicate that CDH1 gene mutations in association with familial gastric cancer are a new disease for PTG. Huntsman *et al.*^[28] also recommended genetic counseling and prophylactic gastrectomy in young people. The morbidity of this operation was much higher than that of other genetic diseases. The morbidity of prophylactic gastrectomy in the young, healthy population has been estimated as following: 1-2% mortality, 10-20% major acute morbidity, principally related to esophageal anastomotic procedure, and 100% long term morbidity related to weight loss, rapid intestinal transit, dumping syndrome, and diarrhoea. The high surgical risk of the procedure should be minimised by operations at centers with experience rich in gastric surgery. The decision to perform prophylactic gastrectomy should be balanced with age based risk and based on age specific penetrance data. Other factors, for example, the decision to operate on children, may affect the decision regarding the timing of prophylactic gastrectomy, and it is essential that patients carrying the gene have the opportunity extensive counseling and discussion with clinicians, geneticists, and counsellors before making the decision. The risk of other cancers (breast, colon) should also be targets of screening, as they may also be increased in this population, and patients should be counselled regarding these. However, germline mutations of E-cadherin could be used to identify individuals with a high risk^[47].

Target for radioimmunotherapy

Radiolabeled mAb used in radioimmunotherapy is limited because of lack of tumor specific antigens. In most cases reported thus far, tumor antigens serving as targets are not tumor-specific, being overexpressed in tumor cells and also at a lower level in normal cells. Only one tumor-specific mAb that recognizes a mutant form of the epidermal growth factor receptor (EGFRv III) has been found in different tumor types^[48]. Inframe deletions of exons 8 or 9 in the mRNA coding for E-cadherin are characteristic of DGC. So a rat mAb (d9mAb) was generated that specifically reacts with this mutation, and d9mAb was found to react with 13% of E-cadherin-expressing DGCs^[49]. Senekowits-Schmidtke *et al.*^[50] conjugated d9mAb with the α -emitting radionuclide ²¹³Bi, and tested for its binding specificity in sc and ip tumors expressing mutant E-cadherin. Seventy-eight percent of the total activity in ascites fluid was bound to free tumor cells, whereas in control cells expressing wild-type E-cadherin, the binding was only 18%. The selective binding of the ²¹³Bi-labeled, mutation-specific monoclonal antibody d9mAb raised its significant potential for the local radio-immunotherapy of disseminated, diffuse-type gastric carcinoma.

Markers for prognosis and evaluation

Gastric cancer remains a major cause of cancer mortality globally

but no good prognostic tumor markers are available. The most frequently used tumor markers in gastric cancer are carcinoembryonic antigen (CEA) and CA19-9^[51]. Gastric cancer antigen MG-7 may be a good marker for GC^[52], or even the detection of circulating DNA in serum reported recently^[53], but only a modest proportion of patients had elevated levels of these markers. Under-expression of E-cadherin molecules has been found in various malignancies and has a potential value as a prognostic marker^[54]. Serum soluble E-cadherin was found in the circulation of normal individuals but was particularly elevated in patients with malignancies. s-E-cadherin might derive from cancer tissues, though the expression of E-cadherin was decreased. With increased protease, the degradation products of tissue E-cadherin could accumulate and release into peripheral blood. However, its value in gastric cancer is controversial. Velikova *et al.*^[55] were unable to show a significant difference in serum soluble E-cadherin between patients with gastric cancer and normal subjects, while Gofuku *et al.*^[8] showed that concentrations were significantly elevated in 67% patients. Chan *et al.*^[56] confirmed its potential value as a prognostic marker. A high concentration could predict palliative/conservative treatment and T4 invasion. Gabbert *et al.*^[57] studied 413 gastric cancer patients, including all histological tumor types and stages. As shown by univariate and multivariate Cox regression analyses, patients with E-cadherin-positive tumors had significantly better 3 and 5 year survival rates than patients with E-cadherin-negative tumors. This prognostic effect remained present in a multivariate Cox regression analysis, including the prognostic parameters pT category, pN category and vascular invasion, suggesting that it is an independent prognostic marker.

In short, hereditary gastric cancer is still a challenge, especially its diagnosis and treatment^[58], although some progress has been achieved in recent years with the development of molecular biology and new methods.

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