

E-cadherin: A potential biomarker of colorectal cancer prognosis (Review)

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Abstract. Colorectal cancer (CRC) is a common and lethal disease. It is the third most common type of cancer in the world, behind lung and breast cancer, with almost 1.4 million new cases diagnosed in 2012. The risk of developing CRC is influenced by environmental and genetic factors. Adenocarcinomas comprise the vast majority (98%) of CRCs. A patient's likelihood of survival is associated with the tumor stage at the time of diagnosis. With regular screening, CRC can be identified early, when treatment is the most effective. However, CRC is typically asymptomatic until the advanced stages. The combination of the absence of symptoms and current screening methodology results in a significant number of patients being diagnosed in advanced stages. The purpose of the present review is to discuss and summarize the biomarkers linked to CRC progression, particularly the controversial E-cadherin, which is a calcium-dependent cell-cell adhesion molecule involved in the mesenchymal-epithelial transition.

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1. Introduction

Colorectal cancer (CRC) is a prominent cause of mortality worldwide (1). It is surgically curable in early stages, when it is localized or limited to loco-regional invasion; however, the

metastatic stages are associated with a high mortality rate (2). According to GLOBOCAN estimates (1), there were 1,360,602 new cases of CRC worldwide in 2012, making it the third most common type of cancer worldwide and accounting for 9.7% of all cases of cancer that were not non-melanoma skin cancer (2). The majority of CRCs are adenocarcinomas. When these are diagnosed early, the prognosis is good: The 5-year relative survival rate is 91% for localized cancer and 70% for cancer with loco-regional invasion. However, the 5-year survival rate is ~11% in metastatic cases, which includes ~25% of patients at the point of diagnosis (3). The rate of CRC mortality has decreased over the last 20 years due to advances in disease management, including earlier diagnosis and improved treatment modalities. However, a lack of reliable methods for the early detection of CRC is impacting patient prognoses (4).

At present, carcinoembryonic antigen (CEA), a <200-kilo-dalton (kDa) glycoprotein that acts to mediate cell adhesion in cancer, is used in the initial assessment and monitoring of CRC (5). It was first identified in the blood serum of patients with CRC (6). However, 30% of cases of CRC recurrence do not produce CEA, irrespective of the amount associated with the original disease. Furthermore, it is estimated that 44% of patients with normal CEA levels prior to surgery have an increased CEA level in disease recurrence (7). The diagnostic value of CEA also varies according to the site. The sensitivity of CEA for the detection of hepatic and retroperitoneal metastases is greater than for the detection of lung metastases; sensitivity is also improved in cases of multiple recurrences compared with a single recurrence. A number of potential diagnostic and prognostic biomarkers for CRC have been evaluated thus far (Table I), including cytokines, chemokines and enzymes (8-14).

2. CRC and biomarkers

Blood serum biomarkers are of interest for the screening and monitoring of disease as they do not require invasive procedures and may facilitate rapid detection (8). CRC biomarkers in the blood include circulating tumor cells, DNA, RNA and proteins (8). In Table I, a number of serum biomarkers that may be used for the early detection or monitoring of CRC are summarized.

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Circulating tumor cells in CRC have been validated as prognostic markers for advanced disease and are associated with a poor overall survival time (15). The US Food and Drug Administration has validated them as monitoring markers, as they may predict response to treatment (16). Circulating DNA is also considered as a promising type of prognostic marker. For example, the detection of a Kirsten rat sarcoma mutation preoperatively, or its persistence subsequent to surgery, is associated with a poor prognosis (17).

These biomarkers are accessible with simple and rapid methods throughout the fluctuating course of the disease. A number of studies (15,17) have demonstrated the utility of these biomarkers in prognosis and monitoring, and as theranostic markers, and recommend their routine use in the near future. However, according to consensus-based recommendations from the American Society of Clinical Oncology (18), only CEA levels should be monitored preoperatively to assist in staging and surgical planning. There is currently no biomarker for the early detection of CRC, with negative consequences for patient prognosis (10).

3. E-cadherin and cancer

Similar to CEA, one of the putative protein biomarkers for the prediction of tumor progression, E-cadherin, is an adhesion molecule. The loss of cell adhesion is one of the mechanisms underlying cancer invasion and progression (19).

E cadherin belongs to the 'classical' or type-I cadherin subfamily. A total of 16 molecules of ~120 kDa each have been identified in this subfamily. Four subclasses exist: Non-neuronal epithelial (E-), placental (P-), neuronal (N-) and retinal (R-) cadherins. E-cadherin is encoded by the *CDH1* gene; it is a calcium-dependent transmembrane glycoprotein that is localized to adherens junctions at the basolateral surface of epithelial cells, and is involved in cell-cell interactions, including in cancer (20).

The E-cadherin molecule is composed of a cytoplasmic domain, a single-pass transmembrane domain and an extracellular domain that consists of five cadherin-motif tandem repeat subdomains that have putative calcium binding sites (21). The cytoplasmic domain interacts with catenin molecules, including β -catenin or plakoglobin (also called γ -catenin), to mediate its binding to actin filaments of the cytoskeleton (22). Either β -catenin or plakoglobin may bind α -catenin, giving rise to two distinct cadherin-catenin complexes. α -catenin then links these two complexes to actin filaments. This anchorage has been speculated to be regulated by tyrosine phosphorylation (23) (Fig. 1). β -catenin may also bind to the cytoplasmic domain of the epidermal growth factor receptor (EGFR) (24).

E-cadherin downregulation is associated with certain malignant characteristics, including tumor progression, loss of differentiation, invasion and metastasis (25,26). E-cadherin may be inactivated in cancer by mechanisms including mutations, epigenetic silencing, and increased endocytosis and proteolysis (22). Studies on the loss of heterozygosity in chromosome 16q21-22 have linked E-cadherin downregulation to gastric, prostate, hepatocellular and esophageal carcinomas (27). Despite this, E-cadherin mutations are rare in carcinomas of the bladder, colon, endometrium, lung, esophagus, ovary and thyroid, and in intra-hepatic cholangiocarcinoma (28).

Promoter hypermethylation is an important epigenetic event associated with the loss of E-cadherin expression (29). Several suppressors of E-cadherin transcription have been associated with the progression of multiple cancer types. For example, increased *SNAIL* expression, as is common in ductal breast carcinoma, is strongly associated with reduced E-cadherin gene expression (30).

Other mechanisms may disturb normal E-cadherin function under pathological conditions. E-cadherin is removed from the plasma membrane by endocytosis and recycled to the sites of novel cell-cell contacts. Abnormal activation of proto-oncogenes, including *Src* and EGFR, results in increased phosphorylation of tyrosine residues in the cytoplasmic domain of E-cadherin (Fig. 2), leading to the recruitment of the E3-ubiquitin-protein ligase Hakai and subsequently enhancing the endocytosis and ubiquitin-dependent degradation of E-cadherin (31).

Matrix metalloproteinases (MMPs; Fig. 3), including stromelysin-1, matrilysin, MMP9 and MT1-MMP, cleave E-cadherin ectodomains close to the plasma membrane (32). Several other proteases, including serine protease kallikrein-7, may be involved. Pancreatic adenocarcinomas frequently overexpress kallikrein-7, which facilitates tumor cell invasiveness via the cleavage and release of soluble E-cadherin (33). Another example of E-cadherin disturbance contributing to tumor malignancy is peritoneal metastasis in advanced epithelial ovarian cancer, where calpain-mediated E-cadherin fragmentation appears to promote intraperitoneal cancer progression (34). In the human colon cancer cell line HT-29, syndecan-2, a cell-surface heparan sulfate proteoglycan induces extracellular shedding of E-cadherin and supports the acquisition of a fibroblast-like morphology by regulating MMP-7 expression (35).

Post-translational modifications of E-cadherin have also been described. The *O*-mannosylation of E-cadherin is crucial for its adhesive functions in homeostasis. Carvalho *et al* (36) demonstrated that E-cadherin underwent a decrease in *O*-mannosylation in gastric carcinoma that resulted in the impairment of its function through interference with its cell membrane localization and, subsequently, with the assembly and competence of adherens junctions.

Recently, Petrova *et al* (37) demonstrated that the metastasis of an E-cadherin-expressing mammary cell from the mammary gland to the lung is dependent on reduced E-cadherin adhesive function. An activating monoclonal antibody against E-cadherin that induced a high-adhesive state significantly reduced the number of cells that metastasized to the lung. Thus, stimulating the activity of E-cadherin on the cell surface inhibits metastatic progression, suggesting that the downregulation of adhesion in these tumor cells contributes to their metastatic potential.

4. E-cadherin and colon cancer

Recent studies have identified that the loss of expression of E-cadherin and colon cancer invasiveness are associated (38); however, this is rarely attributed to E-cadherin gene mutation (39). The mechanism for downregulation is more frequently post-translational modifications, as suggested by Kitadai *et al* (40).

Table I. Examples of biomarkers for colorectal cancer.

Author (year)	Marker	Sample type	(Refs.)
Yörüker <i>et al</i> (2016)	DNA integrity	Serum/plasma	(8)
Fan <i>et al</i> (2014)	α -2-HS glycol protein	Serum	(9)
Newton <i>et al</i> (2012)	Carcinoembryonic antigen	Serum	(10)
Wang <i>et al</i> (2012)	MicroRNA-21	Serum/plasma	(11)
Cohen <i>et al</i> (2009)	Circulating tumor cells	Peripheral blood	(12)
Taback <i>et al</i> (2006)	Microsatellite instability	Serum/plasma	(13)
Bazan <i>et al</i> (2006)	KRas mutations	Serum/plasma	(14)

KRas, Kirsten rat sarcoma.

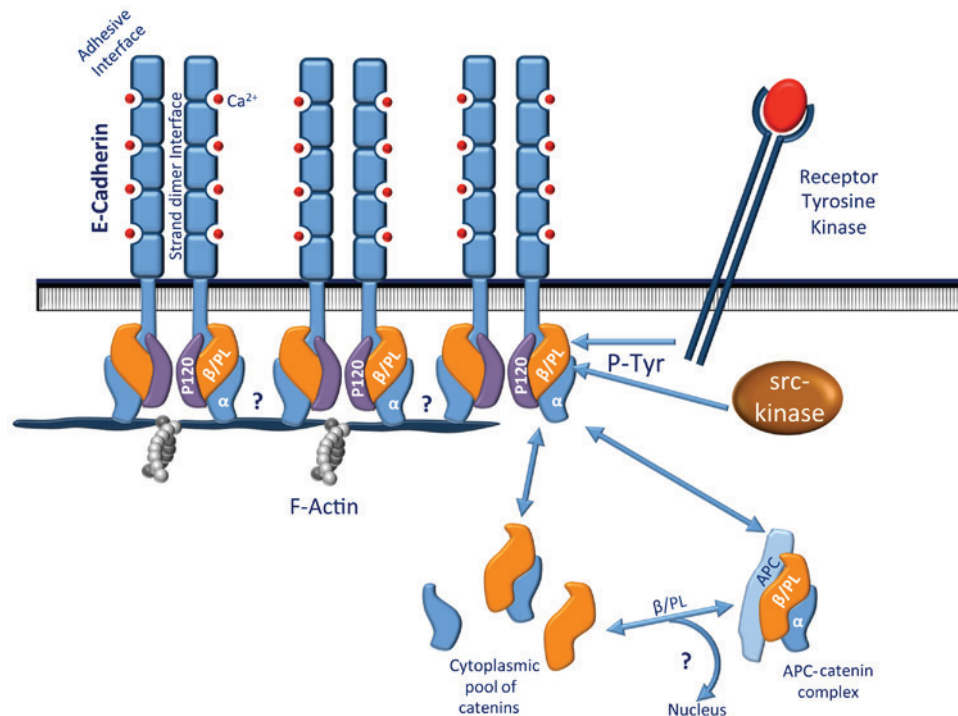


Figure 1. Interaction of the E-cadherin-catenin complex. The cadherin adhesion system is linked to the actin-based cytoskeleton via catenins. The figure illustrates protein interactions between different catenin pools, E cadherin and p120-catenin (p120), regulating the dynamic assembly and disassembly of the cadherin-catenin complex and its function in signal transduction. β/PL, β-catenin or plakoglobin; α, α-catenin; P-Tyr, tyrosine phosphorylation; APC, adenomatosis polyposis coli-Wnt signaling pathway regulator.

A number of studies have identified E-cadherin as a good biomarker for CRC prognosis. In the earliest study, tissues samples were examined. Invasion and metastasis were revealed to be associated with the reduction of α-catenin alone or with reduction of α-catenin and E-cadherin co-expression, not purely with E-cadherin expression alone (41). More recent studies were performed on sera samples. In a study by Velikova *et al* (42), soluble E-cadherin concentrations in patients with CRC were not significantly increased compared with those of the control group. In another study conducted with 36 patients with CRC, E-cadherin was demonstrated to be a good marker of CRC. However, it lacked the required specificity to predict tumor progression; concentrations of E-cadherin were higher for patients with CRC as well as for patients with benign tumors (43). In another study, Weiss *et al* (44) compared soluble E-cadherin

levels in 59 patients with CRC to the levels in patients with other conditions, including colorectal adenomas, inflammatory bowel disease or familial adenomatous polyposis. There was a significant elevation of soluble E-cadherin levels in patients with advanced CRC (stages III and IV). This study suggested a potential application for soluble E-cadherin as a diagnostic marker for monitoring disease in patients with CEA-negative tumors (44). The largest study was conducted on 186 patients with CRC; a preoperatively elevated soluble E-cadherin level was associated with a worse prognosis (38). The study demonstrated that E-cadherin was a metastasis prediction marker and a pre-therapeutic prognostic marker for patients with CRC and hepatic metastases. All these data confirmed that soluble E-cadherin levels increase with advancing tumor stage (38,41-44).

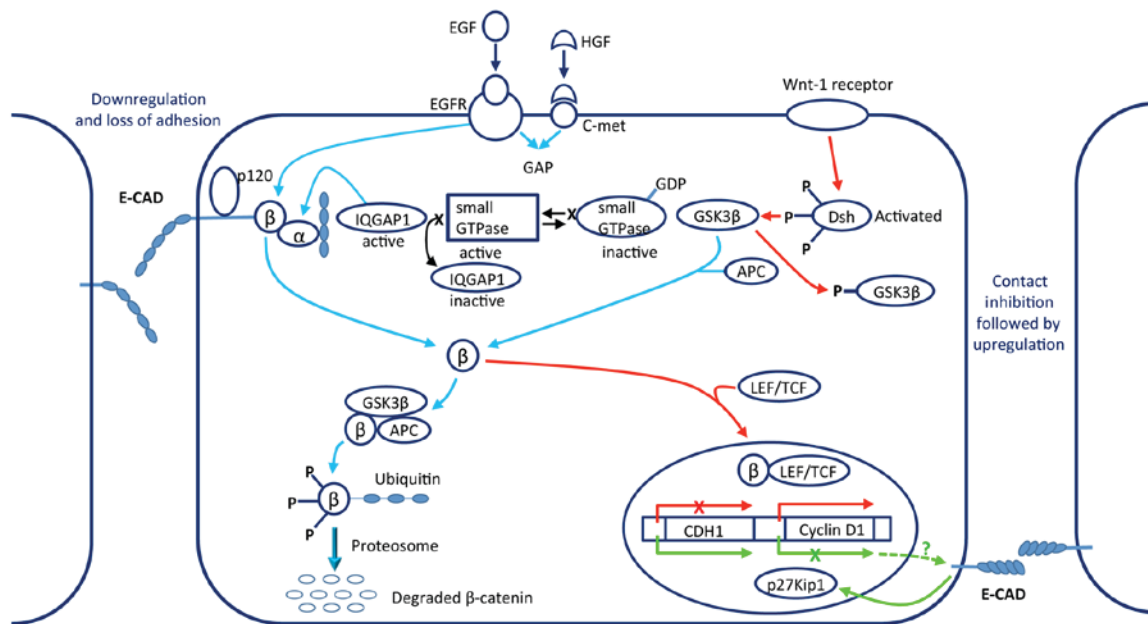


Figure 2. Operating mode of the E-cadherin-catenin complex in tumor metastasis. Stimulation of EGFR and c-Met receptors led to the translocation of β -catenin into the cytosolic pool where it can be degraded. If the Wnt1 pathway is activated at the same time as the other two pathways, the degradation of β -catenin is inhibited and it is translocated to the nucleus to combine with LEF/TCF, causing transcription of the cyclin D1 gene and downregulation of the *CDH1* gene. Reversal by way of contact inhibition is poorly understood. E-CAD, E-cadherin; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; c-Met, hepatocyte growth factor receptor; GAP, GTPase activating protein; β , β -catenin; α , α -catenin; IQGAP1, IQ motif-containing GTPase activating protein 1; GDP, guanosine diphosphate; GSK3 β , glycogen synthase kinase 3 β ; APC, adenomatosis polyposis coli Wnt signaling pathway regulator; P, phosphorylation group; Dsh, disheveled; LEF/TCF, lymphoid enhancer factor/T-cell factor family of transcription factors; CDH1, cadherin 1 (E-cadherin gene); p27Kip1, cyclin-dependent kinase inhibitor 1B.

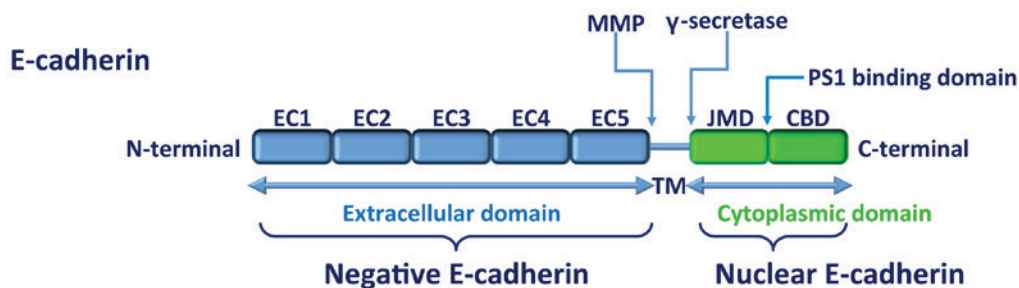


Figure 3. Schematic representation of E-cadherin protein. The protein contains ECs, a JMD and a CBD, together with cleavage sites for MMPs and γ -secretase, and a binding domain for PS1. EC, extracellular domain; JMD, juxtamembrane domain; CBD, catenin-binding domain; MMP, matrix metalloproteinase; PS1, presenilin-1; TM, transmembrane domain.

E-cadherin serves a crucial role in cell-cell adhesion and maintaining epithelial morphology. Loss of E-cadherin leads to the loss of epithelial differentiation and the acquisition of a motile and invasive phenotype (45). A recent study (46) enrolled patients with signet ring cell carcinoma, a rare type of colorectal adenocarcinoma with a worse prognosis than classical colorectal adenocarcinoma. The study demonstrated that patients with a loss of tumor E-cadherin expression had a lower survival time. Loss of E-cadherin expression was a significant, independent predictor for poor prognosis. The implications of E-cadherin in CRC progression has also been recently demonstrated (47): The expression of E-cadherin in 108 patients with CRC metastasis was lower than that in normal adjacent tissues, and was associated with tumor differentiation, invasion depth, lymph node metastasis and tumor stage. Furthermore, expression of E-cadherin prolonged the survival time of mice with patient-derived CRC xenografts (47).

As a consequence of this association, E-cadherin is a potential target for anticancer treatment. Chen *et al* (48) illustrated this; dimethoxy curcumin inhibited cell growth and enhanced E-cadherin expression in two CRC cell lines (HT-29 and SW480). In a CRC liver metastasis mouse model, the combination of a vascular disrupting agent with the anti-angiogenic drug sunitinib increased treatment efficacy; it reduced the number of viable tumor cells and prolonged animal survival time. E-cadherin staining was lower for the treated cells than for controls. The surviving tumor cells underwent a redistribution of E-cadherin from the cell junctions to the cytoplasm and nucleus (49) exhibiting epithelial-mesenchymal transition (EMT) and contributing to therapy resistance. Regorafenib (Stivarga[®]) targets protein tyrosine phosphatase and is approved for the pharmacotherapy of CRC metastasis. Regorafenib also targets EMT; it directly activates SH2-domain-containing

phosphatase 1 (SHP-1) to inhibit EMT. SHP-1 expression is positively correlated with E-cadherin expression, and is significantly correlated with the overall survival time of patients with CRC (50).

Furthermore, by upregulating E-cadherin expression, CRC risk may be reduced. E-cadherin expression was increased in patients supplemented with calcium and vitamin D (51). Calcium and vitamin D may thus be chemopreventive agents against CRC.

5. Conclusion

E-cadherin protein expression is associated with CRC tumor progression. The detection of soluble E-cadherin may allow the early detection of CRC and the monitoring of tumor progression. However, current tests lack sensitivity and specificity. Studies on larger cohorts may be illuminating in this area. Another possibility is to compare the levels of E-cadherin with those of other key molecules in CRC progression. A combination of key molecule levels may be more effective than one molecule alone.

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