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THE ROLE OF E-CADHERIN EXPRESSION IN THE TREATMENT OF WESTERN UNDIFFERENTIATED EARLY GASTRIC CANCER: CAN A BIOLOGICAL FACTOR PREDICT LYMPH NODE METASTASIS?

Tesi di Dottorato di Ricerca

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SUMMARY

BACKGROUND

Gastric cancer (GC) incidence has decreased in western countries due to the use of eradication therapy for H. Pylori and the improvement in food preservation methods.

However, GC is still the fifth solid cancer for frequency and the third cause of cancer-related death (over 934,000 new cases and 720,000 deaths per year) worldwide [1].

Early gastric cancer (EGC) is defined as a gastric cancer (GC) confined to the mucosa (pT1a) or the submucosa (pT1b) irrespective of lymph node metastasis (LNM). It bears a more favorable prognosis after conventional gastrectomy with nodal dissection compared to advanced gastric cancer cases [2].

For small EGC, endoscopic treatment such as mucosal resection (EMR) and submucosal dissection (ESD) has been widely adopted as an alternative to conventional surgery, because it preserves gastric function and consequently leads to an improved quality of life [3-4].

The Japanese Gastric Cancer Associations guidelines [5] proposed two different sets of endoscopic resections: standard and expanded (Table 1).

Table 1. Tumor's indication for endoscopic resection according the Japanese Gastric Cancer Associations guidelines [5]

Standard criteria for EMR/ESD	Expanded criteria for EMR/ESD
pT1a, tumor size < 2 cm, differentiated type, UL (-)	pT1a, tumor size > 2 cm, differentiated type, UL (-) pT1a, tumor size < 3 cm, differentiated type, UL (+) pT1a, tumor size < 2 cm, undifferentiated type, UL (-) pT1b (SM1, < 500 μm), tumor size < 3 cm

UL (-) = Ulcerative component negative
EMR = Endsocopic mucosal resection
ESD = Endoscopic submucosal dissection
SM1 = Tumor distant < 500 μm from muscularis mucosae

Both procedures are considered curative when all of the following conditions are met: en-bloc resection, negative horizontal margin (HM0), negative vertical margin (VM0), and absence of lympho-vascular infiltration. However, these organ-sparing approaches do not involve lymph node dissection. In the Eastern world, endoscopic resection is being increasingly utilized to treat also small undifferentiated EGC according to the extended criteria.

However, studies in the Western world (USA and Europe) reported in these tumors a rate of nodal metastasis ranging between 5% and 20% [6], that is higher of those observed in Eastern counterparts (not exceeding 5%) [7-9].

This significant variability of LNM between geographic areas could be secondary to the different biological behavior of these tumors.

Therefore, the National Comprehensive Cancer Network (NCCN) [10] proposed EMR/ESD for only small-sized (< 2 cm) and well differentiated EGC.

Thus, in order to safely perform conservative endoscopic resection (EMR/ESD) also in western undifferentiated EGC, the risk of concurrent nodal metastasis should be accurately investigated.

E-Cadherin

E-cadherin is a single-pass trans-membrane glycoprotein (120-kDa protein) that belongs to a family of highly conserved trans-membrane glycoproteins called cadherins whose function is to assist with calcium-dependent cell adhesion to form organized tissues by complexing with another set of cytosolic proteins called catenins.

The E-cadherin glycoprotein consists of three structural domains: a cytosolic domain, a single trans-membrane domain and an extracellular calcium-dependent domain.

The cytoplasmatic domain binds proteins that regulate endocytosis, recycling and degradation, and mediate signal transduction, gene transcription, and local control of actin cytoskeleton. Within the cytoplasmic domain there are two binding sites (CBD), which comprise a juxtamembrane domain (JMD) of 94-amino acid that binds to P-120 catenin and an extensive C-terminal region that binds to β- and α-catenins. The extracellular calcium-dependent domain consists of five tandem repeat domains. The extracellular motif binds to homophilic cadherin molecule from adjacent cells and this adhesion requires calcium ions, which act at a hinge and prevent the domain from flexing, conferring rigidity.

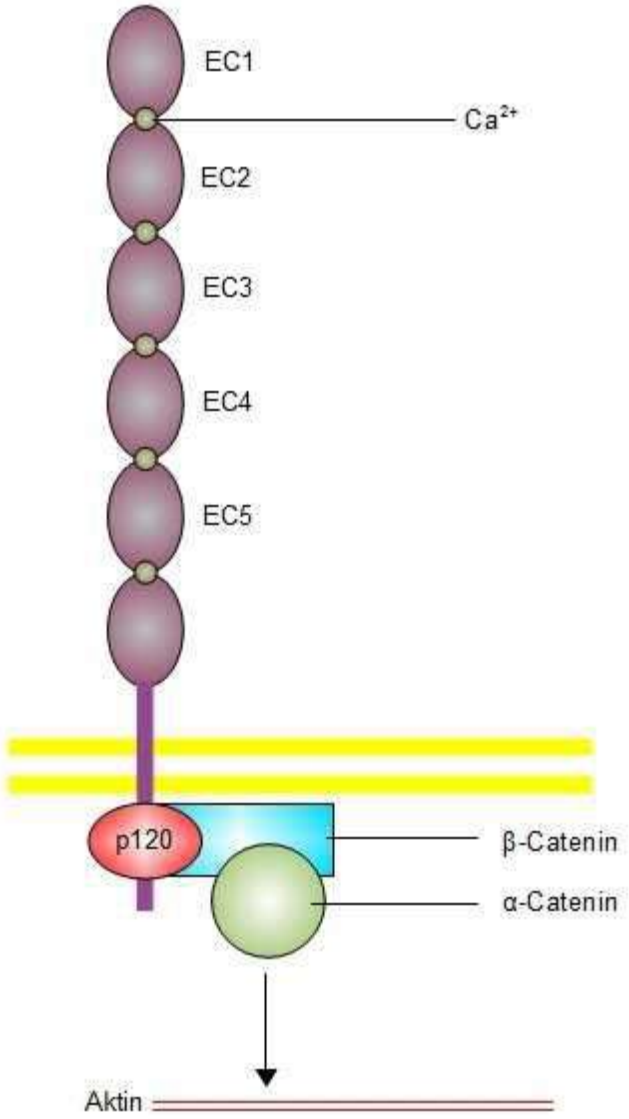


Figure 1. E-cadherin (endothelium calcium-dependent adhesion) is a class of type 1 trans-membrane proteins that links to β-catenins to form E-cadherin/β-catenin complex which is further linked to the actin cytoskeleton.

β-catenin

β-catenin is a cytoplasmatic protein made by 13 repetitions of a typical "armadyllus" domain that form a triple α-helix.
β-catenin binds to the C-16 terminal cytoplasmatic domain of E-cadherin and the affinity of this interaction is extremely high.
On the other side, β-catenin binds to α-catenin with low affinity, which allows interaction with cytoskeleton proteins like actin and actin-binding proteins such as vinculin and α-actinin.

E-cadherin/β-catenin complex

The structural integrity of the E-cadherin/β-catenin complex is regulated by protein kinases and phosphatases. Three Serine residues within the E-Cadherin cytoplasmic domain are phosphorylated by GSK3 β (Glycogen Synthase Kinase β), a process that leads to an increased number of interactions and affinity for β-catenin.
On the other hand, if phosphorylation, by the action of Src, takes place on tyrosine residues, the E-cadherin/β-catenin bond is disrupted.
Also the bond between p120 catenin and the juxtamembrane region of the cytoplasmatic domain of E-cadherin is regulated by phosphorylation, which results in an increased binding affinity of E-cadherin itself. P120 catenin increases retention of the E-cadherin complex to the plasma membrane and prevents internalization and degradation of E-cadherin. The loss of this stability is correlated to tumor progression and invasion.

Epithelial–mesenchymal transition

Epithelial–mesenchymal transition (EMT) is a process through which epithelial cells are converted into mesenchymal cells.

This process comprises several changes, such as the loss of cell–cell adhesion, loss of cell polarity and gain of migrating and invading properties [11] (Figure 2).

EMT defines a series of event through which cells lose their epithelial phenotype and acquire a mesenchymal phenotype. It is a finely regulated, dynamic process, which is fundamental to pluricellular organisms to allow formation of different organs and tissues.

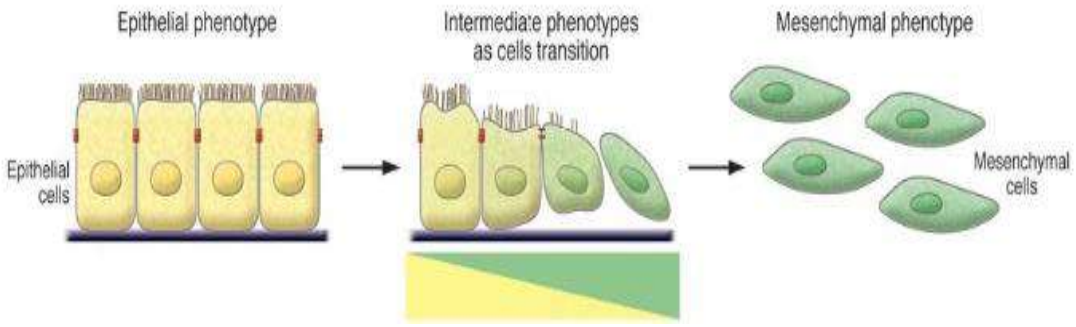


Figure 2. Epithelial–mesenchymal transition (EMT) [11].

The first in vitro descriptions of this process date back to 1982, and the first discoveries took place in the context of embryonic development studies. Further studies demonstrated that EMT is not only crucial to embryogenesis and organogenesis, but also to adulthood, in multiple physiologic situations.

Moreover, EMT plays a key role in certain pathological events, such as tumoral progression and fibrogenesis, which share several steps despite having different results [12].

Cells that form simple epithelium bear specific morphologic and functional figures. They organize themselves to form continuous laminae in which cell-to-cell adhesion is guaranteed by several junctional mechanisms, which confer the epithelium its mechanical properties.

Epithelial cells are characterized by polarity and display different specialization at the level of the apical, lateral and basal portions, resulting in a polarity of ultrastructural and functional cytoplasmatic organization. Epithelial cells are scarcely mobile: movements are allowed only within the tissue they form.

On the other side, mesenchymal cells do not bear morphological or functional polarity.

They do not arrange to form laminar structures and do not establish stable junctional complex between each other; they only display localized focal adhesion sites.

Mesenchymal cells have a fusiform or stellate shape and are characterized by high mobility which allows migration within the surrounding interstitium through different mechanisms based on emission of pseudopods and phyllopods.

During EMT, epithelial cells lose their phenotype and acquire the mesenchymal phenotype [13].

During this transition, they rearrange so as to lose some epithelial markers and express other markers typical of the newly acquired mesenchymal phenotype.

Therefore, an epithelial molecular “reprogramming” takes place, with new instructions for the cell. It can be defined as “epithelial–mesenchymal transition proteome”.

The metastatic capacity of cancer cells originates from the acquired ability to lose the normal adhesions with the adjacent structures

(homing) and to spread into lymphatic or blood stream with eventual distant organ invasion.

The loss of intercellular adhesions is likely to be the first step toward the metastatic phase [14]. Cellular adhesion properties depend on several membrane proteins with cadherins considered as the main actors.

The down regulation and loss of E-cadherin area necessary, but not sufficient, condition for the occurrence of EMT.

The loss of adhering junctions and, subsequently, of cytoskeletal interaction appears to be the key event that favors the realization of the entire process.

Significant changes generally occur during EMT, including the down regulation of epithelial markers such as E-cadherin, and up regulation of other mesenchymal markers such as alpha smooth muscle actin (αSMA), vimentin, matrix metalloproteinases 2 and 9 (MMP-2, MMP-9) and N-cadherin [15].

The up-regulation of these proteins allows cancer cells to invade the surrounding microenvironment and give rise to distant metastases.

EMT is therefore configured as a prerequisite for originating cancer cells capable of infiltrating the surrounding tissues and of metastasizing. However, tumoral cells constitute a highly heterogeneous cell population and, in some cases, many invasive and metastatic carcinomas are not characterized by a complete transition to a mesenchymal phenotype, but rather have the molecular and morphological characteristics typical of a well-differentiated epithelium, expressing high levels of E-cadherin and presenting epithelial junctions and apical-basolateral polarity [16].

This may be due to the fact that malignant carcinoma cells can initiate a partial EMT and return from mesenchymal to epithelial phenotype in

distal metastasis sites according to a process called MET (mesenchymal-epithelial transition) [17].

In fact, these secondary tumors in the distal sites maintain the same histopathological characteristics of the primary tumor, without showing mesenchymal phenotype.

Another explanation compatible with the maintenance of a differentiated epithelial phenotype and the high invasiveness of carcinoma cells may lie in collective migration, through which some invasive carcinomas invade the surrounding tissues under the form of multicellular aggregates or clusters. In the latter case, the cells located inside the aggregate are protected from the external environment and therefore also from immunological attacks [16]. In addition, epithelial junctions in well-differentiated metastatic carcinomas can form a physical barrier that restricts the access of drugs to the tumor site, thus decreasing therapeutic efficacy [16].

These evidences show that the transition to an aggressive malignant phenotype is not an "all or nothing" event.

AIM OF THE STUDY

The aim of the study is to improve knowledge in the treatment of GC, an issue of relevant value due to the still high frequency and mortality of this neoplasm in Western countries.

Starting from an update review of the English literature, we performed a preliminary retrospective study about patients underwent to surgery for undifferentiated early gastric cancer.

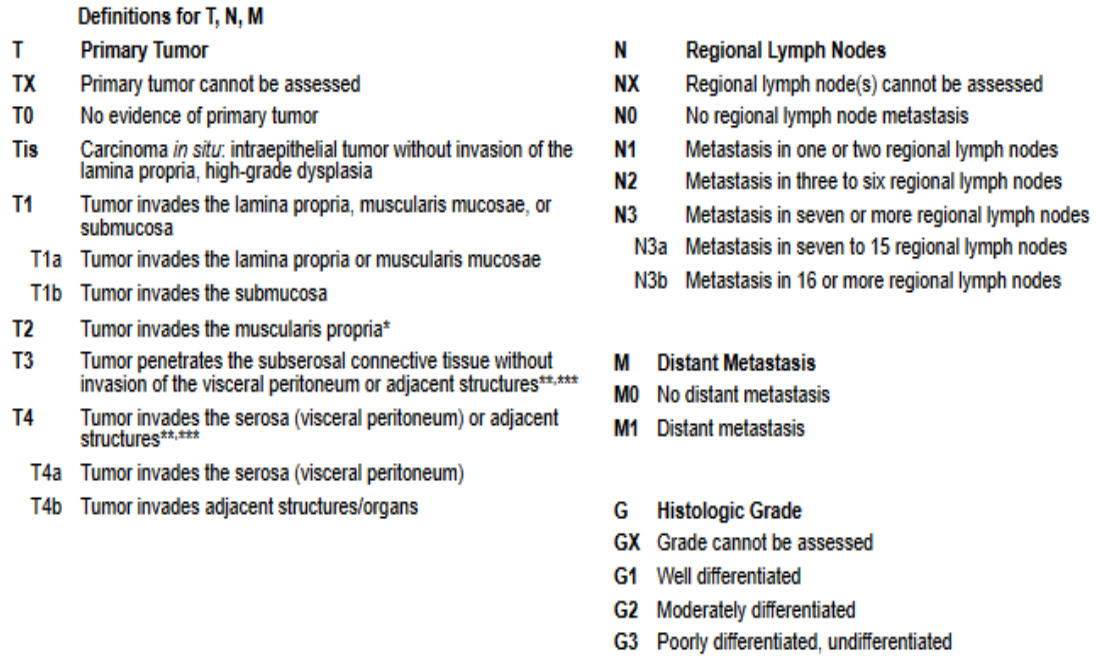
We propose the use of E-cadherin expression as a possible early biological factor predictive of lymph nodes involvement, selecting patients that could be effectively treated in the future with low impact modalities, as the endoscopic resection (EMR/ESD), and those who will required more invasive and aggressive approach.

MATERIALS AND METHODS

Study design

We retrospectively reviewed (from October 2015 to October 2019) the medical records of all the patients who were treated for gastric cancer at two large referral institutions (Department of Surgery of the University of Catania and the Department of Surgery of the Main Hospital of Lodi). All the patients with undifferentiated ECG pT1a or pT1b (SM1 < 500 μm from the muscularis mucosae according the AJCC 8^{th}) were included in the study and their histological specimens were tested for E-cadherin expression profile (Figure 3).

Figure 3. TNM Staging Classification for Carcinoma of the Stomach, American Joint Committee on Cancer AJCC 8th.



Definitions for T, N, M

T	**Primary Tumor**
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma *in situ*: intraepithelial tumor without invasion of the lamina propria, high-grade dysplasia
T1	Tumor invades the lamina propria, muscularis mucosae, or submucosa
T1a	Tumor invades the lamina propria or muscularis mucosae
T1b	Tumor invades the submucosa
T2	Tumor invades the muscularis propria*
T3	Tumor penetrates the subserosal connective tissue without invasion of the visceral peritoneum or adjacent structures**,***
T4	Tumor invades the serosa (visceral peritoneum) or adjacent structures**,***
T4a	Tumor invades the serosa (visceral peritoneum)
T4b	Tumor invades adjacent structures/organs

N	**Regional Lymph Nodes**
NX	Regional lymph node(s) cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in one or two regional lymph nodes
N2	Metastasis in three to six regional lymph nodes
N3	Metastasis in seven or more regional lymph nodes
N3a	Metastasis in seven to 15 regional lymph nodes
N3b	Metastasis in 16 or more regional lymph nodes

M	**Distant Metastasis**
M0	No distant metastasis
M1	Distant metastasis

G	**Histologic Grade**
GX	Grade cannot be assessed
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated, undifferentiated

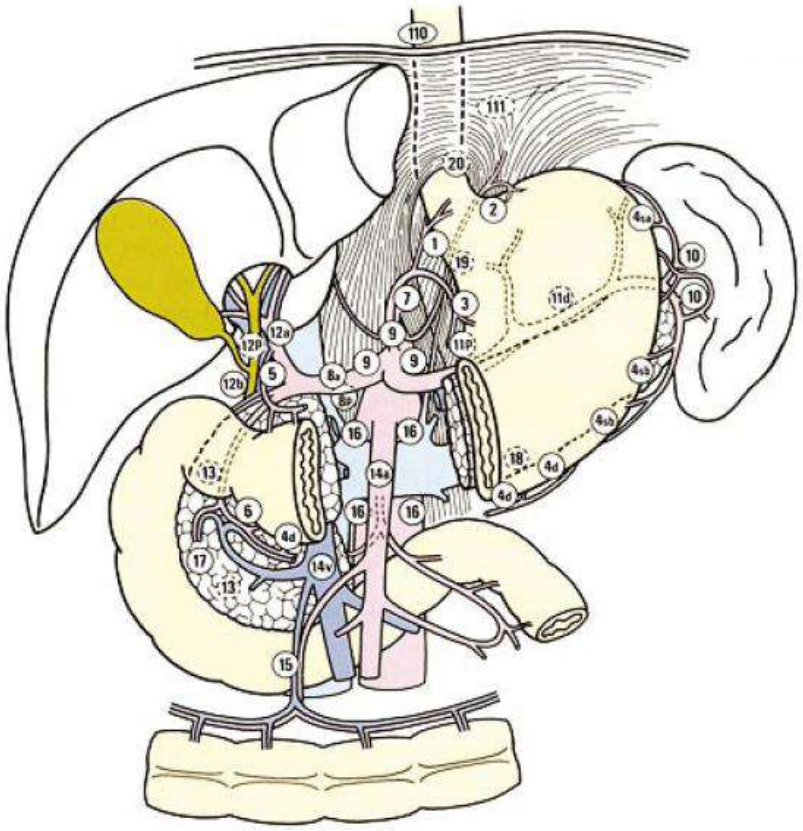
The primary endpoint was the evaluation of the prevalence of LNM and the correlation with the degree of E-cadherin expression. The overall surgical outcomes were also analyzed and reported.

Preoperative assessment included a complete medical history, physical examination, endoscopic ultrasound (EUS) to measure the depth of invasion, and upper gastrointestinal endoscopy with biopsies.

Abdominal and chest CT scans were performed to assess the presence of local infiltration to adjacent organs, regional and distant nodal disease and lung, liver and/or peritoneal metastases.

The goal of surgical procedure was a complete resection (R0) of the tumor. Distal sub-total gastrectomy was performed in cases of tumors located in the lower and middle third of the stomach, if a proximal margin of at least 5 cm was feasible to achieve.

Lymphadenectomy involved the systematic removal of perigastric lymph node stations (n° 1–7), and those along the celiac axis (n° 9), hepatic artery (n° 8a), hepatoduodenal ligament (n°12a) and splenic artery (n°11p/d). Lymph nodes at the splenic hilum were removed by splenectomy only when macroscopically involved (Figure 4).



Location / Lymph node station		LMU / MUL MLU / UML	LD / L	LM / M / ML	MU / UM	U	E+
No. 1	rt paracardial	1	2	1	1	1	
No. 2	lt paracardial	1	M	3	1	1	
No. 3	lesser curvature	1	1	1	1	1	
No. 4sa	short gastric	1	M	3	1	1	
No. 4sb	lt gastroepiploic	1	3	1	1	1	
No. 4d	rt gastroepiploic	1	1	1	1	2	
No. 5	suprapyloric	1	1	1	1	3	
No. 6	infrapyloric	1	1	1	1	3	
No. 7	lt gastric artery	2	2	2	2	2	
No. 8a	ant comm hepatic	2	2	2	2	2	
No. 8b	post comm hepatic	3	3	3	3	3	
No. 9	celiac artery	2	2	2	2	2	
No. 10	splenic hilum	2	M	3	2	2	
No. 11p	proximal splenic	2	2	2	2	2	
No. 11d	distal splenic	2	M	3	2	2	
No. 12a	lt hepatoduodenal	2	2	2	2	3	
No. 12b,p	post hepatoduod	3	3	3	3	3	
No. 13	retropancreatic	3	3	3	M	M	
No. 14v	sup mesenteric v.	2	2	3	3	M	
No. 14a	sup mesenteric a.	M	M	M	M	M	
No. 15	middle colic	M	M	M	M	M	
No. 16a1	aortic hiatus	M	M	M	M	M	
No. 16a2,b1	paraaortic, middle	3	3	3	3	3	
No. 16b2	paraaortic, caudal	M	M	M	M	M	
No. 17	ant pancreatic	M	M	M	M	M	
No. 18	inf pancreatic	M	M	M	M	M	
No. 19	infradiaphragmatic	3	M	M	3	3	2
No. 20	esophageal hiatus	3	M	M	3	3	1
No. 110	lower paraesophag	M	M	M	M	M	3
No. 111	supradiaphragmatic	M	M	M	M	M	3
No. 112	post mediastinal	M	M	M	M	M	3

Figure 4. Lymph nodes station numbers.

The American Society of Anesthesiologists (ASA) score was used to stratify patients according to their perioperative risk.

Tumor specimens were classified according to the Macroscopic Classification of the Japanese Gastric Cancer Association [5] (Figure 5).

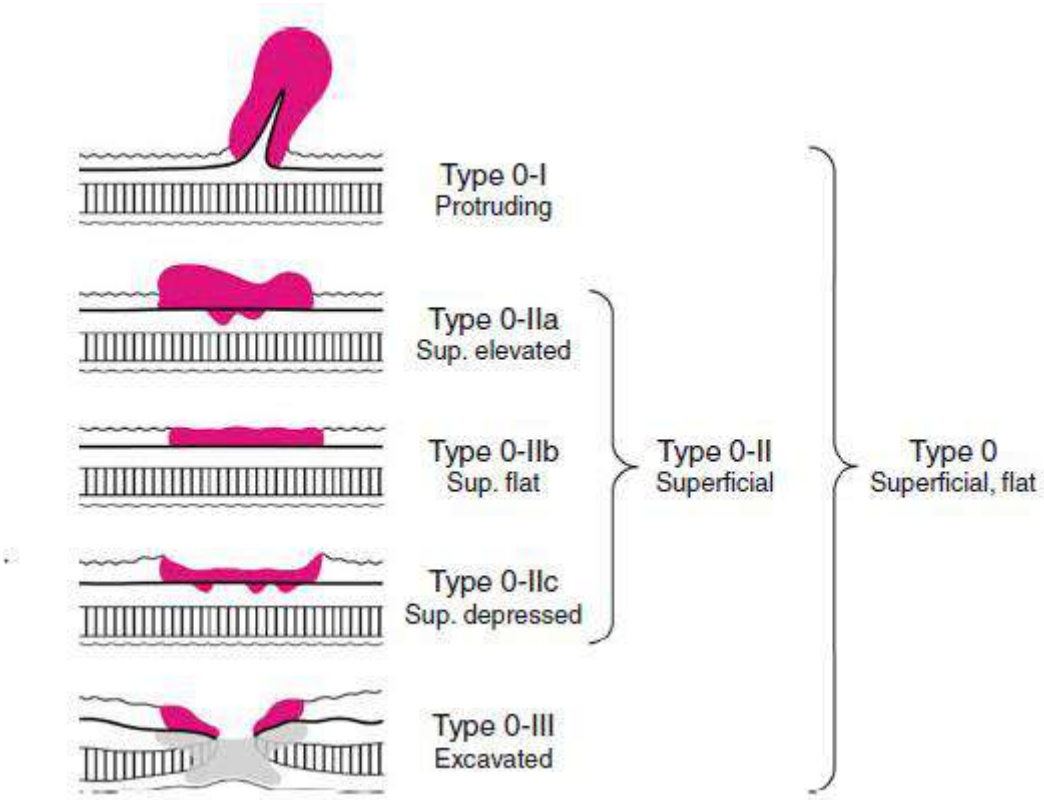


Figure 5 Macroscopic classification of early gastric cancer according to the Japanese Gastric Cancer Association [5].

Immunohistochemistry (IHC)

A representative paraffin block was obtained from each case pT1a and pT1b sm1 (< 500 μm from muscolaris mucosae) gastric cancer (Figure 6-7).

Immunohistochemistry (IHC) examination was performed using an automatic immune-stainer (DAKO OMNIS).

Subsequently, the slides were incubated for one hour with the corresponding monoclonal antibody (clone 36 B5).

Each immunohistochemical staining was evaluated through a photomicroscope (Olympus1).

Image acquisition was performed by Nano Zoomer-XR C12000 series (Hamamatsu Photonics).

The E-cadherin (E-cad) expression profile was stratified according to the grading system described by Chu et al. [18]:

• Absent (0): staining in fewer than 10% of tumor cells;

• Low (1+): weak staining in only 10%-50% of tumor cells;

• Low-intermediate (2+): moderate staining in 50%-75% of tumor cells;

• High (3+): strong staining of more than 75% of tumor cells.

(Figure 8-11)

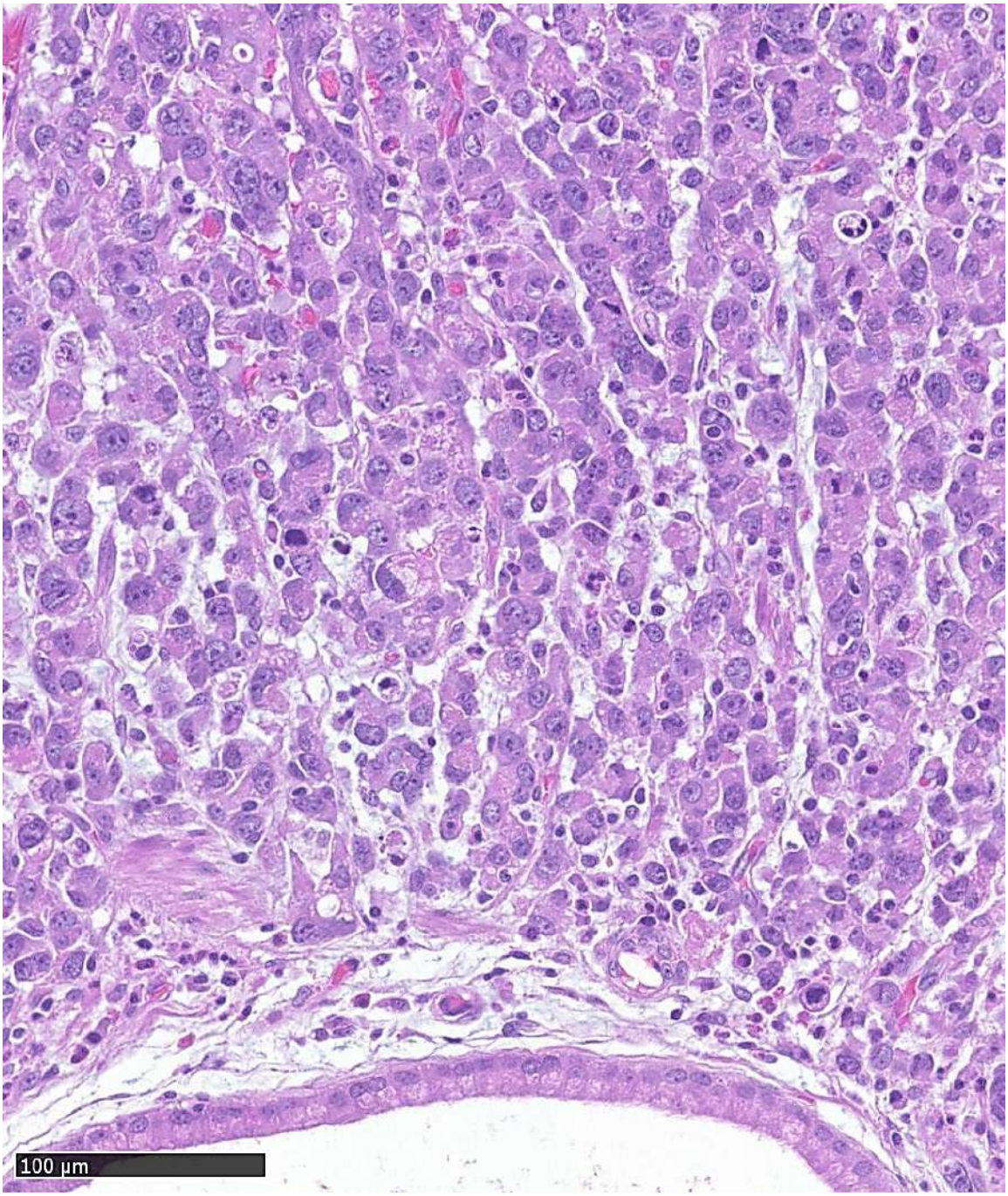


Figure 6. pT1a gastric cancer.

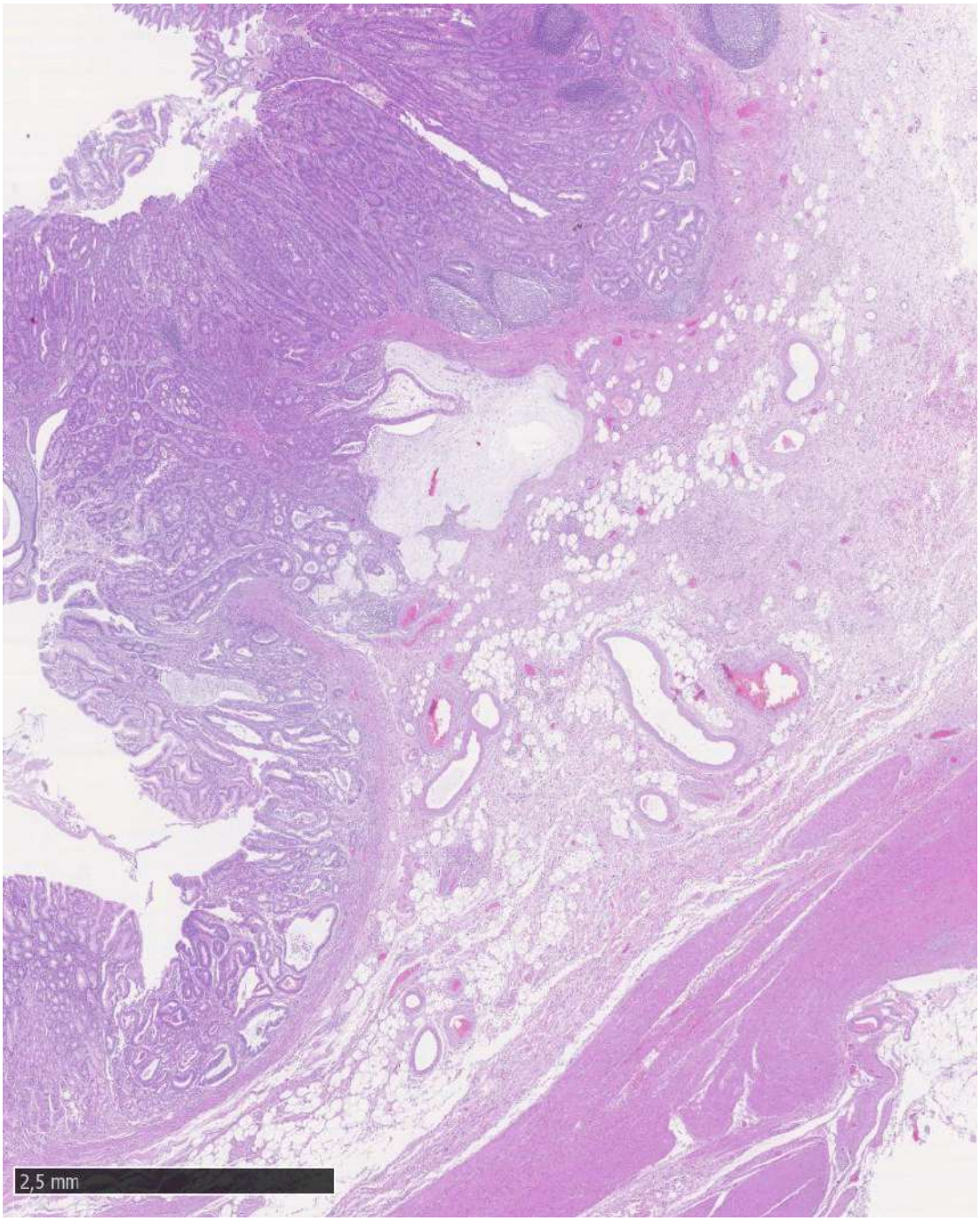


Figure 7 pT1b sm1 gastric cancer.

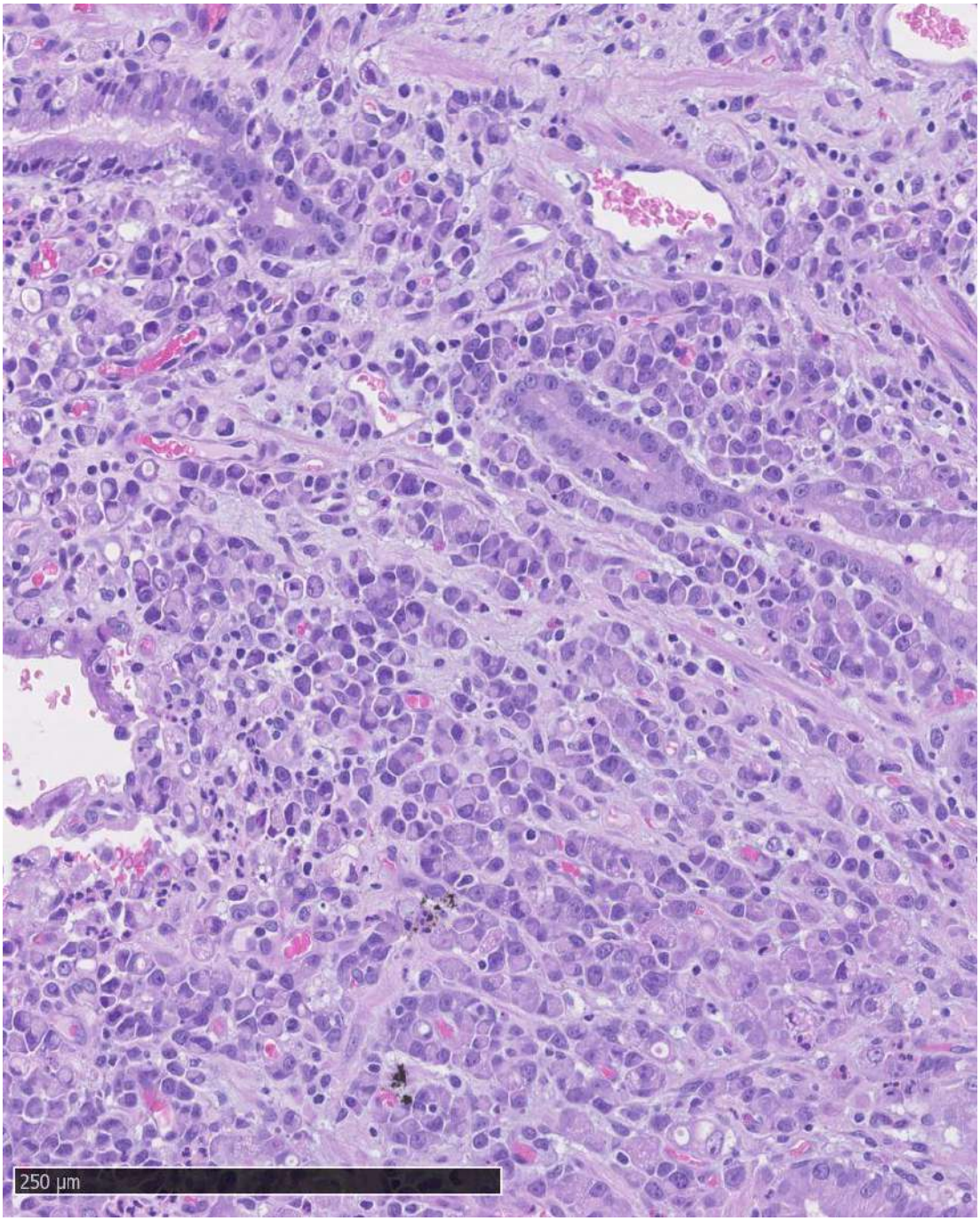


Figure 8 A. Hematoxylin-eosin slide.

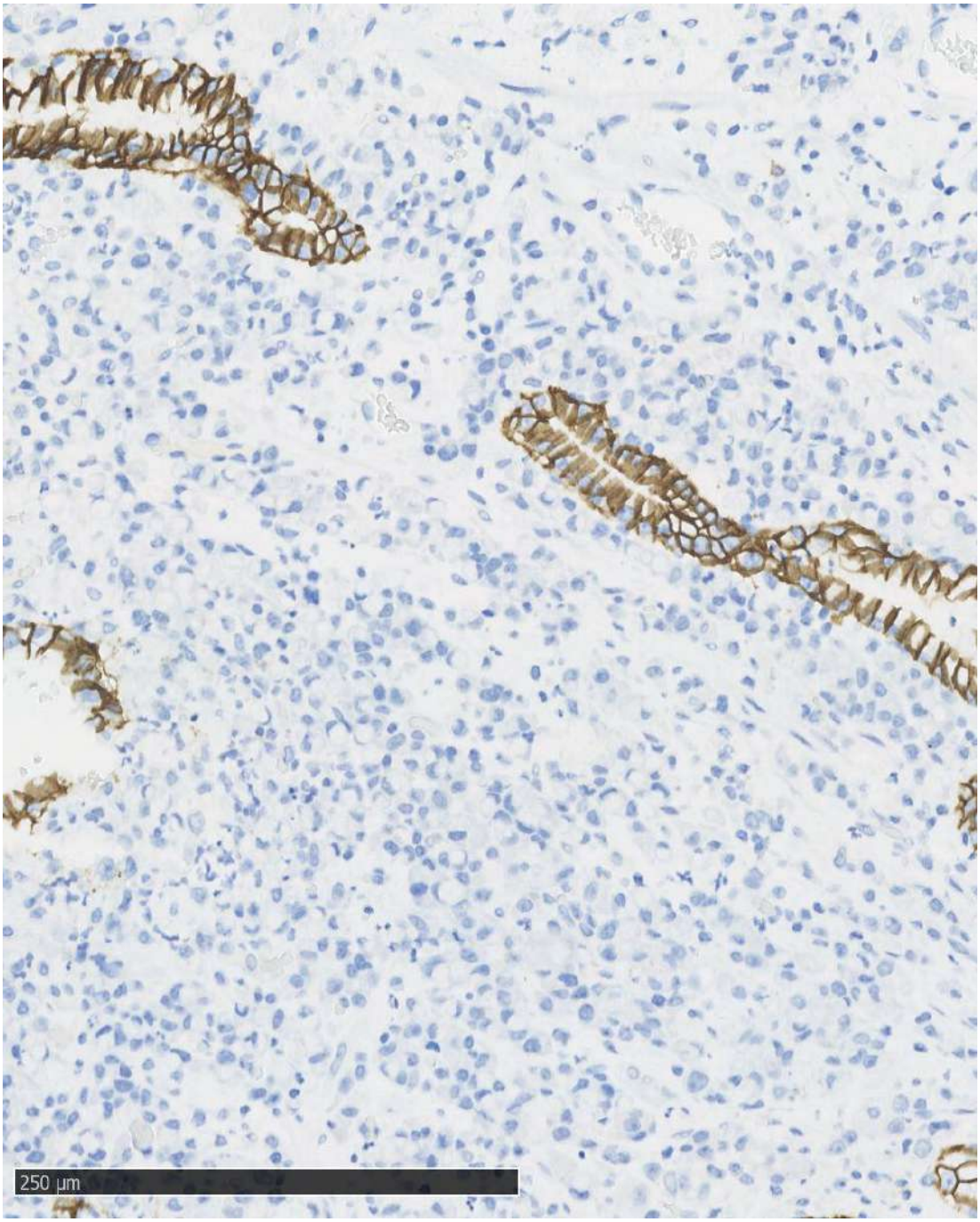


Figure 8 B. Absent (0) expression of E-cad.

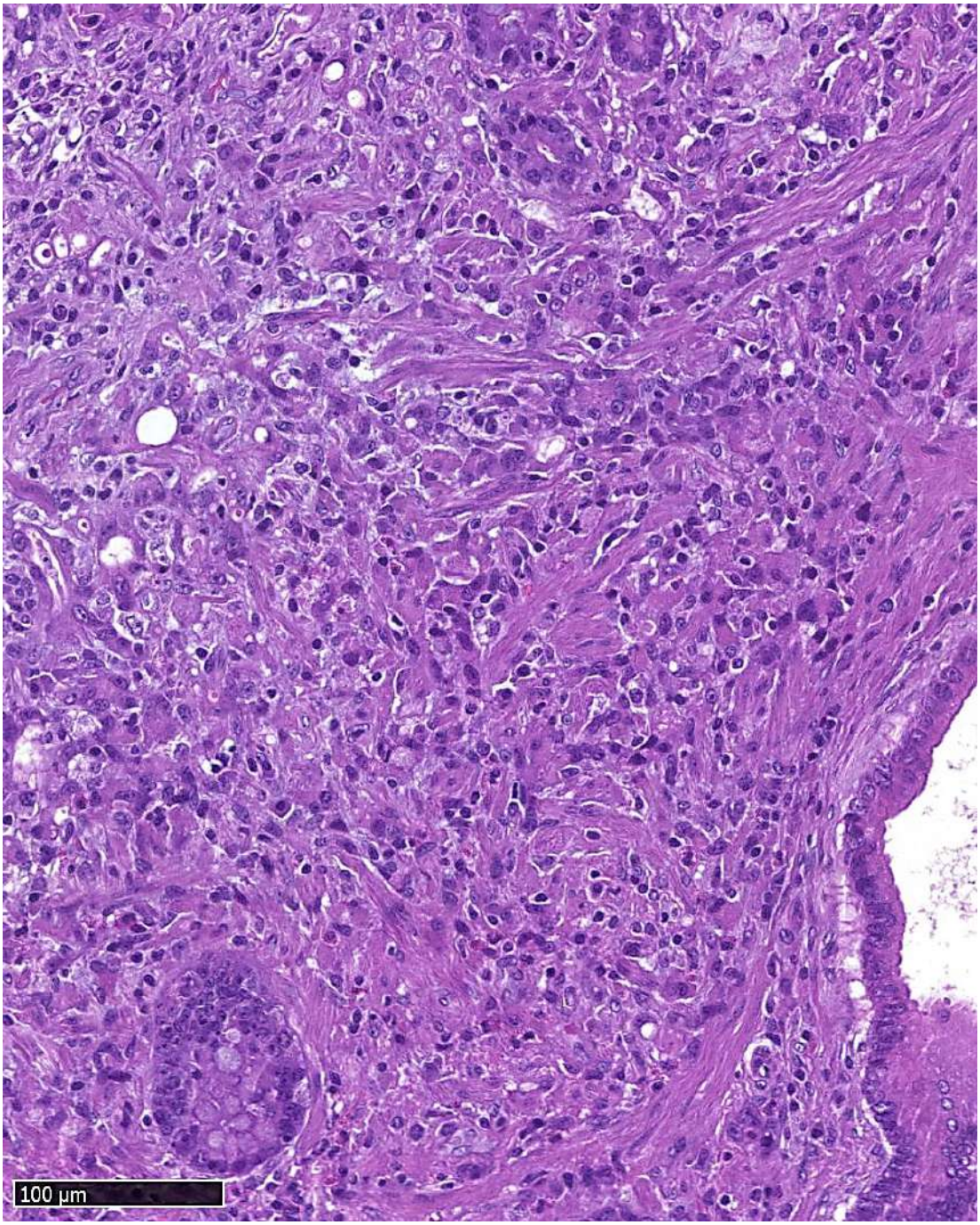


Figure 9 A. Hematoxylin-eosin slide.

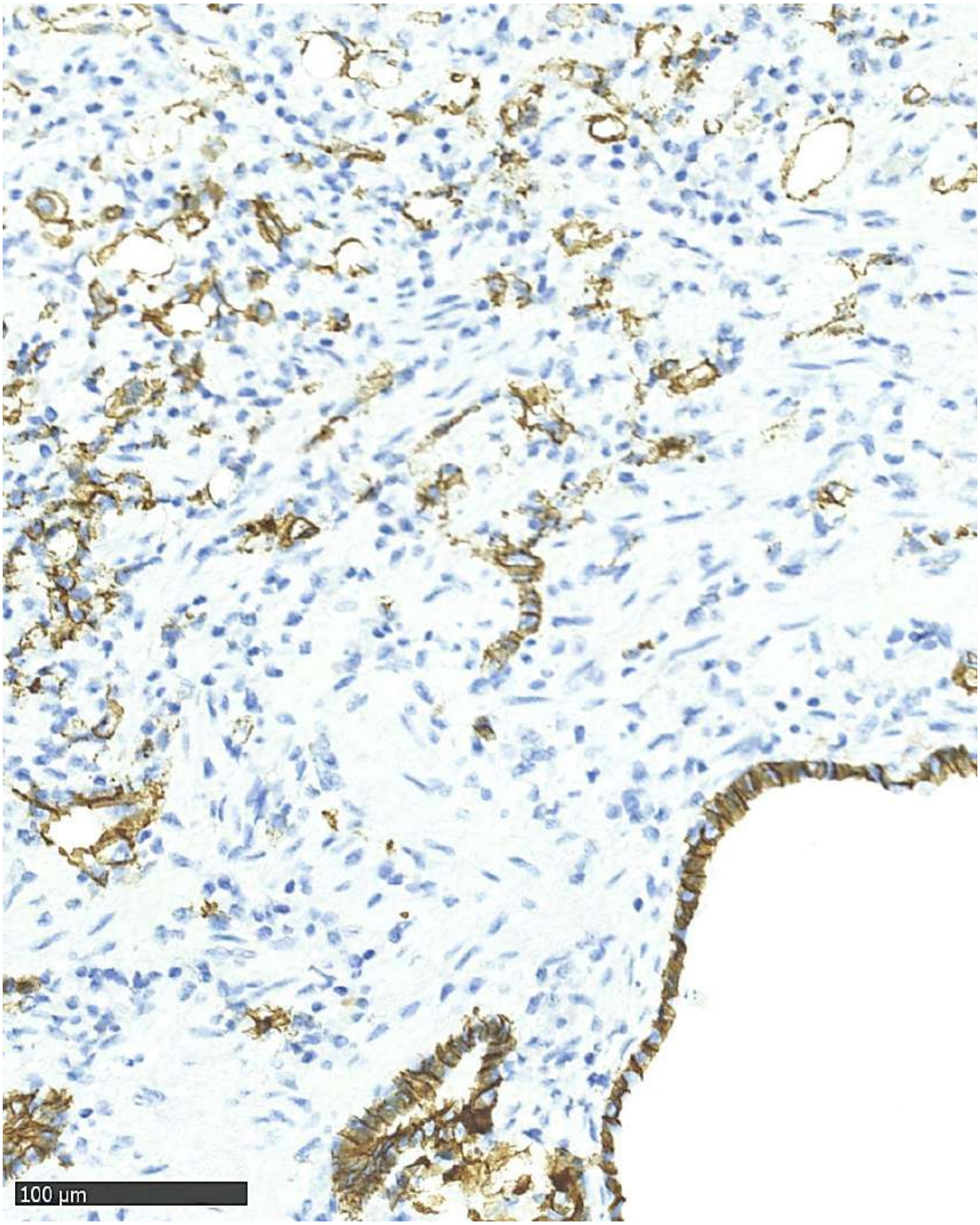


Figure 9 B. Low (1+) expression of E-cad.

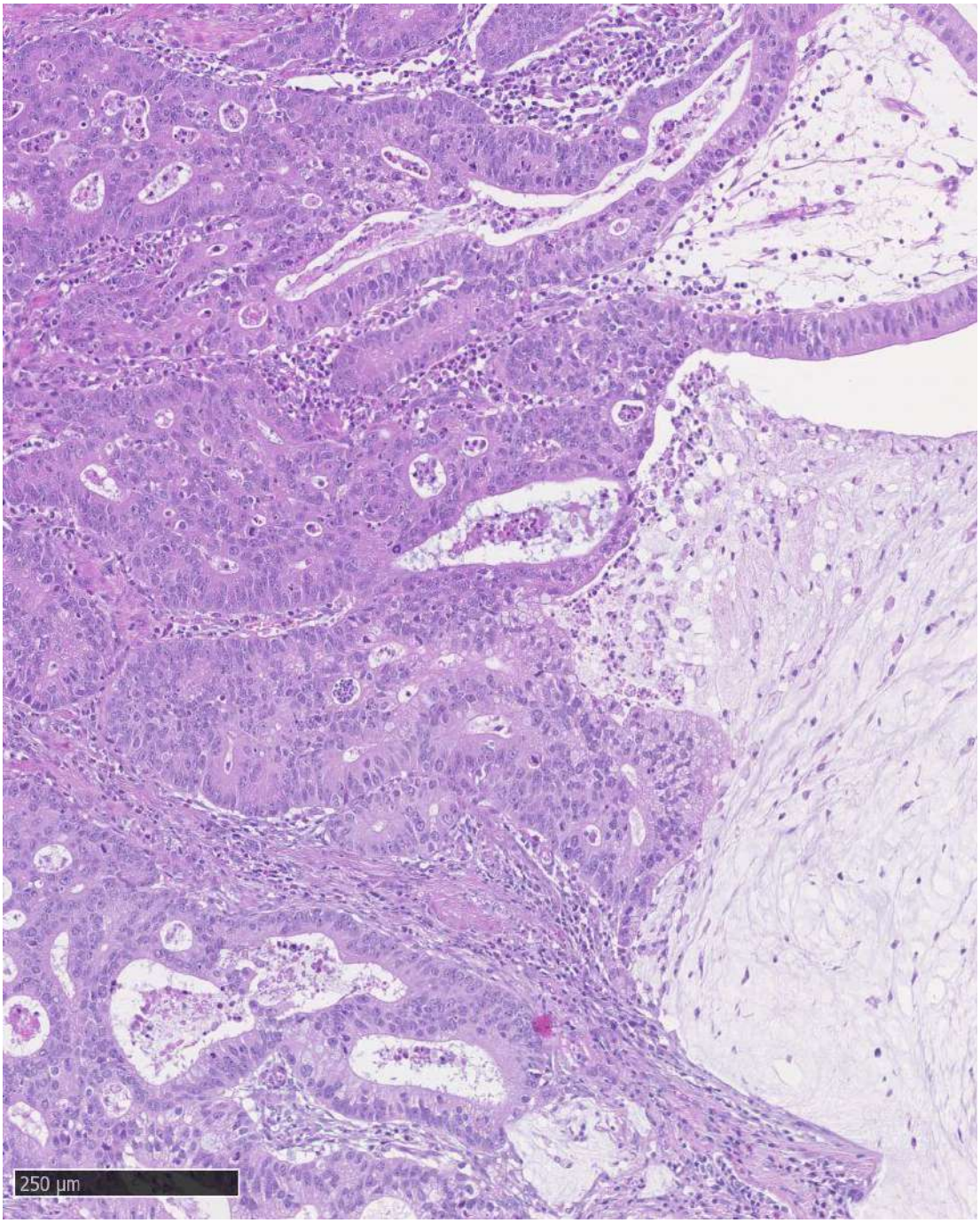


Figure 10 A. Hematoxylin-eosin slide.

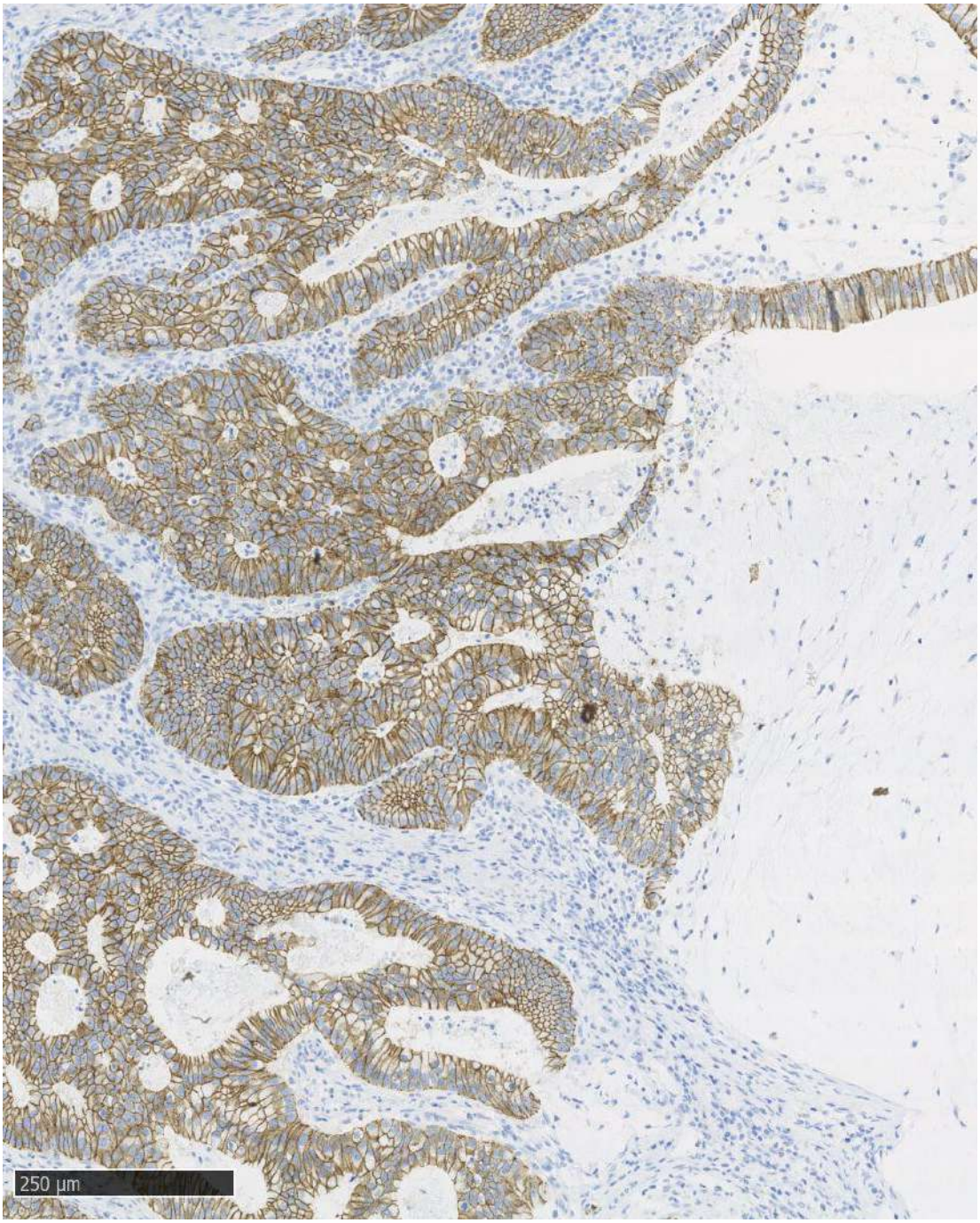


Figure 10 B. Low-intermediate (2+) expression of E-cad.

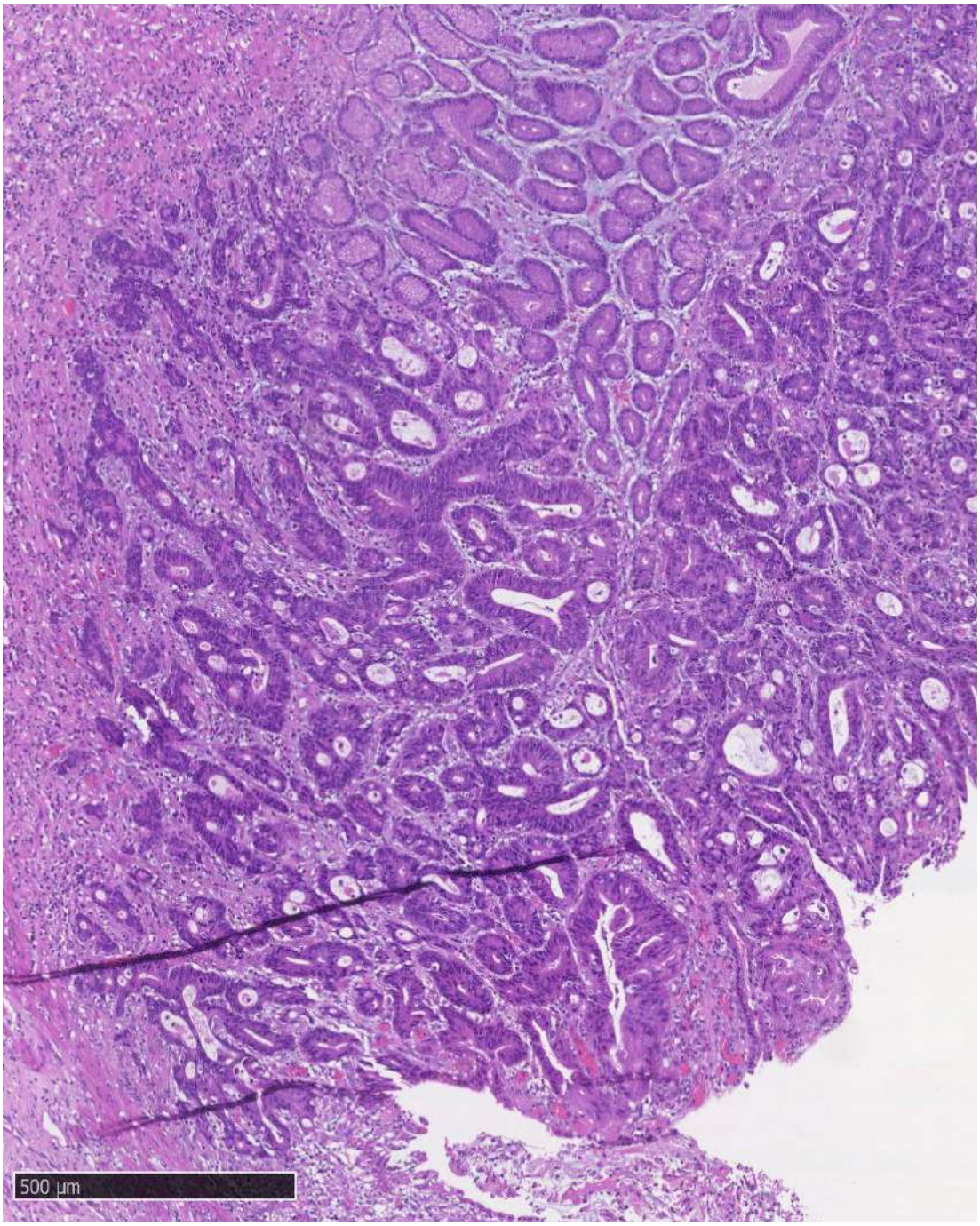


Figure 11 A. Hematoxylin-eosin slide.

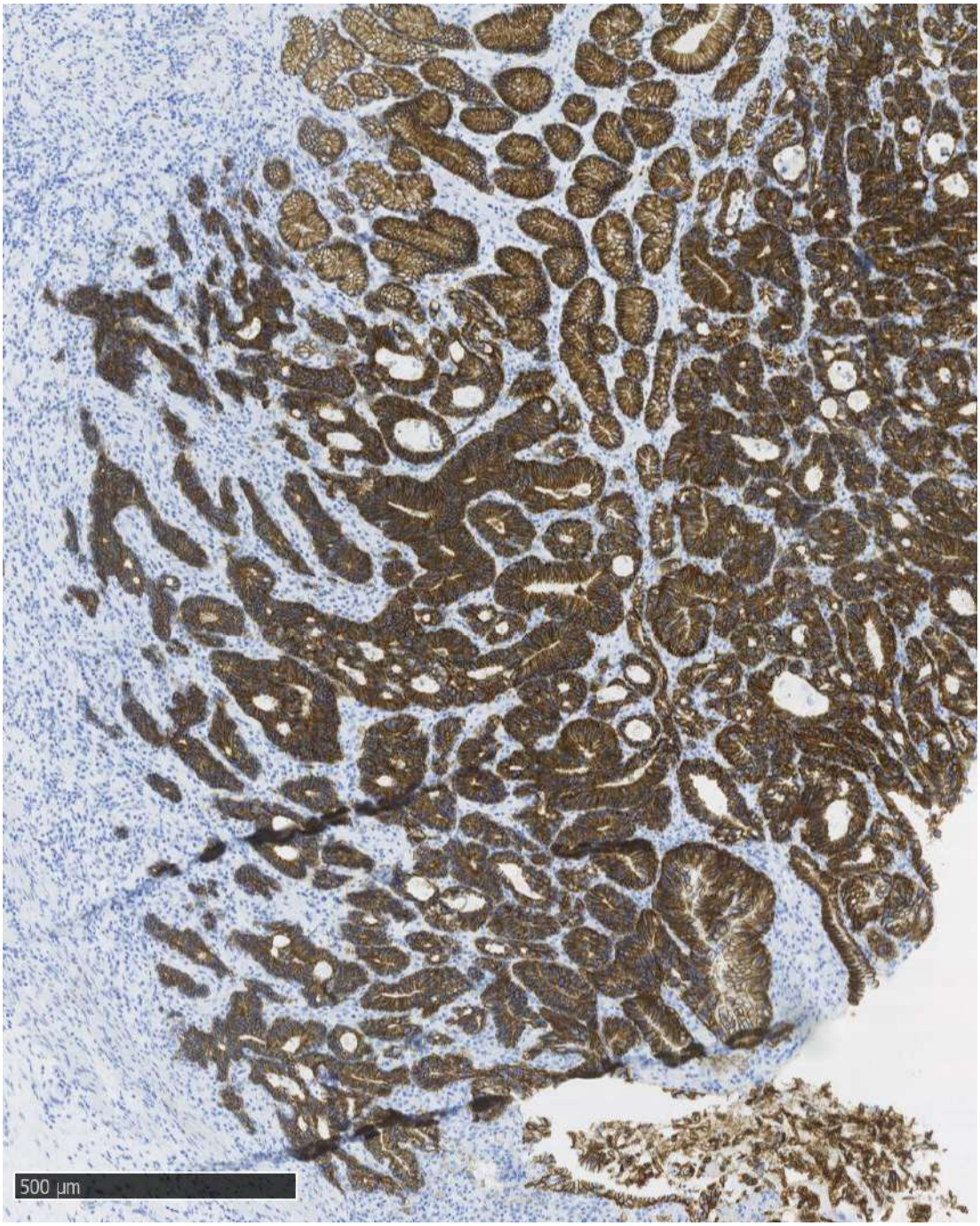


Figure 11 B. High (3+) expression of E-cad.

Ethics statement

Patients were not required to give consent for this study, due to the retrospective nature of the study. All analyzed data was anonymzed without identifiers. The study was reviewed and approved by the Institutional Review Board and by both the Ethics Committee of the General Surgery Department of Catania and the Ethics Committee of ASST of Lodi .

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0. Data were represented as absolute frequency. For the univariate analysis, Mann- Whitney U tests were used. A p-value <0.05 was considered significant with confidence intervals (CI) of 95%. For the multivariate analysis, we used Cox-logistic regression analysis.

RESULTS

A total of 39 patients with early gastric cancer met the inclusion criteria, of which 16 (41%) pT1a, and 23 (58.9%) pT1b SM1.

Thirty-two (82%) patients underwent subtotal gastrectomy, whereas total gastrectomy was performed in only seven cases (17.9%). Patient's characteristics are summarized in Table 2.

Table 2. Patient's characteristics of the study

Patients	**n/total (%)**
Sex	
Male	16/39 (41%)
Female	23/39 (59%)
Age (years)	62,7± 9,2 years-old (range 45 -75 yrs)
ASA score	1/39 (2.6%)
1	11/39 (28.2%)
2	25/39 (64.1%)
3	2/39 (5.1%)
4	
Gastrectomy	32/39 (82%)
Subtotal	7/39 (18%)
Total	

EGC was classified according to the Macroscopic Classification of the Japanese Gastric Cancer Association [4].

The majority of tumors were Type-0-IIa (33.3%) (superficial elevated) or Type 0-III (30.8%) (excavated).

Six cases were Type-0-I (15.4%) (protruding), four were Type-0-IIb (10.2%) (superficial flat) and four Type-0-IIc (10.2%) (superficial depressed).

Ulcerative findings (UL +) were present in 21/39 patients (53.8%). Undifferentiated EGC included pure undifferentiated (PU-type; 56.4%) and predominantly or mixed undifferentiated cases (MU-type; 43.6%). Among PU-type tumors, there were 16 signet-ring cell carcinomas (SRC; 72.7%), five poorly solid or non-cohesive differentiated adenocarcinomas (poor; 22.7%) and one mucinous tumor (muc; 4.5%).

All patients underwent D2 lymphadenectomy and the mean number of lymph nodes retrieved was 15.47 (range 4–23).

According to the 8th AJCC nodal involvement classification [11], our series included 31 (79.5%) pN0 cases (no regional lymph node metastasis) and eight (20.5%) pN1 cases (metastasis in one or two regional lymph nodes) (Table 3).

Table 3. Tumors characteristics

Patients	n/total (%)
pT Stage	
pT1a	16/39 (41%)
pT1b (SM1)	23/39 (60%)
Macroscopic Type	
Type-0- I	6/39 (15.4%)
Type-0-IIa	13/39 (33.3%)
Type-0- IIb	4/39 (10.2%)
Type-0-IIc	4/39 (10.2%)
Type-0- III	12/39 (30.8%)
Histological type	
MU-Type	17/39 (43.6%)
PU-Type	22/39 (56.4%)
SRC	16/22 (72.7%)
Poor	5/22 (22.7%)
Muc	1/22 (4.5%)
Grading	
G1	10/39 (25.6%)
G2	18/39 (46.1%)
G3	11/39 (28.2%)
pN	
pN0	31/39 (79.5%)
pN1	8/39 (20.5%)

MU = mixed undifferentiated
PU = pure undifferentiated
SRC = signet-ring cell carcinoma
Poor = poorly solid adenocarcinoma
Muc = mucinous tumor

We analyzed the relationship between E-cad expression and some clinic-pathological features: histotype, depth of invasion, grading, tumor size and N status. All cases enrolled in our study were classified into two groups: low E-cadherin expression (E-cad 0/1+) and high E-cadherin expression (E-cad 2+/3+). On univariate analysis (Table 4), we found an association between low E-cadherin expression and low tumor grading ($p = 0.019$), pure undifferentiated histotype (PU-type) ($p = 0.014$) and lymph node involvement (N+) ($p < 0.001$).

Table 4. Univarite analysis between low E-cadherin expression (E-cad 0 / +1) and high E-cadherin expression (E-cad +2 / +3).

	N° Positivity to E- cad 0/+1	N° Positivity to E-cad +2/+3	Mann-Whitney U-Test (P)
Histotype			P = 0.014
MU-Type	1	16	
PU-Type	9	13	
Depth of invasion			P = 0.122
pT1a	2	14	
pT1b sm1	8	15	
Grading			P = 0.019
G1	1	9	
G2	3	15	
G3	6	5	
Size			P = 0.415
< 1 cm	1	6	
1<= 2 cm	3	12	
>2 cm	6	11	
N Status			P < 0.001
N0	2	29	
N+	8	0	

MU = mixed undifferentiated
PU = pure undifferentiated

The association between low E-cadherin expression and lymph node metastasis (LNM) was confirmed by multivariate analysis (OR = 14.5, 95% CI 3.46–60.76, p < 0.001) (Table 5).

Table 5. Multivariate analysis between low E-cadherin expression (E-cad 0 / +1) and high E-cadherin expression (E-cad +2 / +3).

N Status	N° Positivity to E- cad 0/+1	N° Positivity to E-cad +2/+3	OR	(95% CI)	P
N0	2	29	14.5	3.46-60.76	P <0.001
N+	8	0			

DISCUSSION

Chapter 1 - REGULATION OF E-CADHERIN EXPRESSION

The loss of E-cadherin expression may be caused by several mechanisms [19-23]. Mutation or deletion of CDH1 (E-cadherin gene) leads to missed production of a functioning protein or production of a non-functioning one. These mutations can be found in some hereditary gastric cancers [24] but are also common in familial cases [25].

On the other hand, in sporadic gastric cancer (GC), somatic mutation of CDH1 is extremely rare and is more frequently associated with diffuse phenotype GC [26].

In many cases there are no structural mutations and the loss of E-cadherin is due to epigenetic alterations such as promoter hypermethylation or activation of transcriptional repressors. Also, microRNAs (non-coding RNAs, including miR-200 and miR-101 family) and long non-coding RNAs modulate CDH1 through the regulation of transcription factors of E-cadherin (ZEB1 and ZEB2) [27].

1.1 Germline mutation of CDH1 gene: hereditary gastric cancers

Hereditary diffuse gastric cancer (HDGC) is due to heterozygous germline mutations in the E-cadherin gene (CDH1). The prevalence of HDGC ranges from 1% to 3% of all gastric cancers [28-29].

Diffuse gastric cancer was initially observed and described in 1964, in a multigenerational Maori family from New Zealand [30].

Subsequently, similar CDH1 germline mutations were reported in diffuse gastric cancer in families of Northern European origin [31].

In 2015, the International Gastric Cancer Linkage Consortium (IGCLC) defined the following criteria for identification of HDGC families [32].

Full criteria: two or more documented cases of GC at any age in first- or second-degree relatives, with at least one confirmed DGC; or personal history of DGC before the age of 40 years; or personal or family history (first- or second-degree relatives) of DGC and lobular breast cancer, one diagnosed before the age of 50 years.

Supporting criteria: families with bilateral or multiple cases of lobular breast cancer before the age of 50 years; or families with clustering of DGC and cleft lip/cleft palate; or any patient that is diagnosed with in situ signet ring cells and/or pagetoid spread of signet ring cells.

In 30–50% of the patients with HDGCs, the cause is mutated germline alleles of E-cadherin (CDH1). In the remaining families, the factors driving susceptibility remain unknown [29].

Eighty percent of these CDH1 germline mutations are truncating, resulting in complete loss of E-cad protein due to occurrence of premature stop codons [33-36].

Twenty percent of the mutations are missense type (resulting in an E-cad protein with an amino acid substitution) also known as VUS (variants of unknown significance) [32,36].

The functional impact of these missense-type mutations is not clear and remains under investigation.

CDH1 is a tumor suppressor gene and therefore both alleles have to be silenced to induce protein loss. A second hit is required for the inactivation of the gene and tumorigenesis.

The mechanisms by which the second allele of CDH1 is inactivated may include hyper-methylation of the CDH1 promoter site causing epigenetic silencing, somatic mutation and loss of heterozygosity.

The penetrance of HDGC gene is incomplete. Approximately 30-50% of HDGC families may harbor this mutation [37].

In a recent series of mutation carriers, the cumulative incidence of gastric cancer was 70% (95% CI, 59-80%) for males and 56% (95% CI, 44-69%) for females and the risk of lobular breast cancer was 42% (95% CI, 23-68%) [38]. In the same study, patients who tested negative for CDH1 mutation had mutations in closely related HDGC susceptibility genes, such as CTNNA1, BRCA2, STK11, SDHB, PRSS1, ATM, MSR1, and PALB2. The majority of these patients present with diffuse gastric cancer in their mid-thirties (range 14–69 years) and are more commonly females [38]. The risk of lobular breast cancer (LBC) in these families approaches 42% by age 80 years, compared with the 12.5% lifetime risk for sporadic breast cancer [39-40]. The average age for clinical presentation in HDGC is 38 years and is generally asymptomatic in the majority of patients. When specific symptoms do appear, the disease is typically at advanced metastatic stages and has a poor prognosis [41-42]. Patients with positive pathogenic germline mutation should consider prophylactic total gastrectomy regardless of endoscopic findings [43]. Analysis on the prophylactic post-gastrectomy specimens in CDH1 mutant variants describes multifocal, isolated nests of neoplastic signet ring cells at the base of the glands along with pagetoid infiltrative pattern of spread under a histologically normal-appearing mucosa [43]. The optimal age to perform prophylactic gastrectomy has to be individualized; however, most authors recommend it being performed during early adulthood between 20 and 30 years of age [44].

For patients who decline prophylactic gastrectomy, screening and surveillance with upper endoscopy following Cambridge protocol should be offered [42-45]. Screening should begin 5–10 years prior to the youngest family member's diagnosis. The current recommendations are performing semiannual or annual detailed 30 min high-definition white light upper endoscopy with biopsy of any visible suspicious lesions, including pale mucosa. In addition, multiple random deep biopsies should be obtained from pre-pyloric area, antrum, transitional zone, body, fundus and cardia. A minimum of 30 biopsies is recommended. The stomach should be inflated to check for ease of distention.

If the stomach appears rigid and there is suspicion of linitis plastica, an endoscopic ultrasonography or a CT scan should be performed to evaluate the layers of the wall.

Post-gastrectomy evaluation of surgical specimens demonstrated signet ring cancer in 45–60% of those with negative endoscopic evaluations [45]. Given the rarity of lobular breast cancer (LBC) in the general population, there are insufficient data regarding best surveillance practices for early detection of breast cancer in female patients with CDH1 mutation carriers. LBC often presents as sheets of malignant cells that do not form a well-defined mass as compared with invasive ductal cancer The sensitivity of a mammogram for detecting LBC is therefore suboptimal. Screening recommendations include bilateral breast MRI beginning at age 30 [47-48]. Some patients may consider preventative mastectomy as an option, but this is not routinely recommended for CDH1 mutation carriers.

1.2 Familial gastric cancers

Familial clustering of gastric cancer is observed in about 10% of cases [49-50]. In familial aggregation of gastric cancer, several situations can be identified: cases in which the histopathology of the tumors is unknown, simply designated as familial gastric cancer (FGC); and cases in which it is possible to have information on the histopathological type of one or more gastric cancers. The latter group encompasses the following specific syndromes/diseases: familial diffuse gastric cancer (FDGC) [51] and familial intestinal gastric cancer (FIGC) [52].

1.3 Somatic mutation and epigenetic alteration of CDH1 gene

Somatic mutation of CDH1 is extremely rare in sporadic GCs and is more frequent in diffuse phenotype tumors both in Caucasian and Japanese populations. The predominant defects in diffuse type tumors are splice mutations causing skipping in exon 8 or 9, which accounts for in-frame deletions, whereas missense and truncating mutations are less frequent [53]. Moreover, intragenic polymorphisms arise from changes in the third (wobble) position of the respective codons and are more frequent in codons 692 and 751. Liu YC et al. [54] analyzing diffuse type tumors, reported respectively five and four cases of intragenic polymorphisms in codon 692 and codon 755. Only one of 38 diffuse type tumors had a truncated codon 699 mutation.

In many cases the loss of E-cadherin is due to epigenetic alterations such as promoter hypermethylation, loss of heterozygosity (LOH) or activation of transcriptional repressors such as Snail, Slug, ZEB1, ZEB2

and Twist. Hypermethylation is present in more than 50% of diffuse type somatic tumors but not in intestinal ones, in both Caucasian and Japanese populations [54]. Methylation of the CDH1 promoter has also been documented as the 'second hit' responsible for the development of both hereditary and sporadic diffuse GCs [55-56]. LOH is a major mechanism for CDH1 gene inactivation. The frequency of LOH ranges is similar between diffuse and intestinal type tumors (39% vs 36%) [54]. In addition, E-cadherin can be regulated by activation of transcriptional repressors of CHD1, such as Snail, Slug, ZEB1, ZEB2 and Twist. These zinc finger family proteins inhibit the expression of genes containing E-boxes in the promoter regions. The activation of these repressors leads to epigenetic transcriptional silencing through de-acetylation, which is performed by the HDAC (histone deacetylase) recruited near the promoter of E-cadherin [57-58].

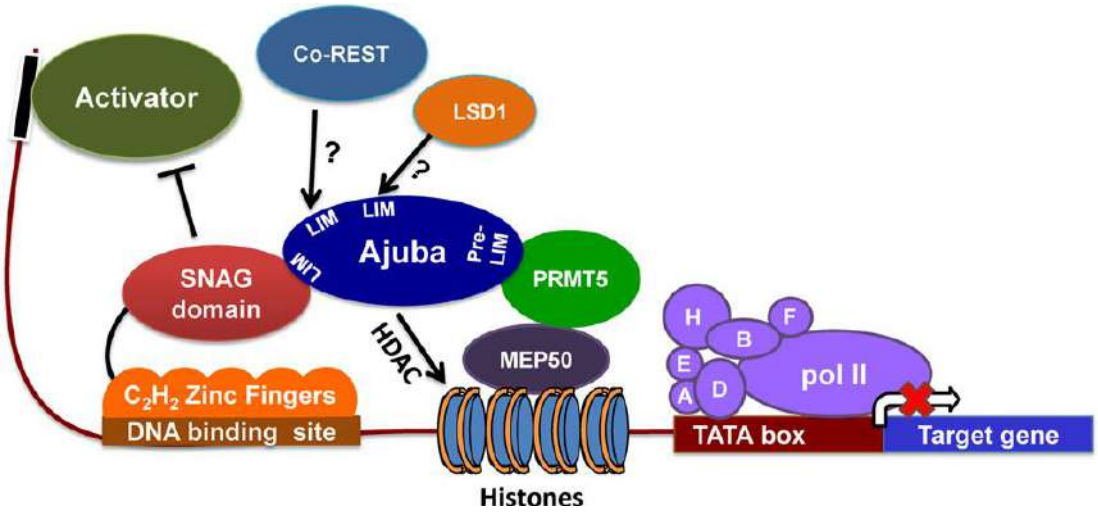


Figure 11. Zinc fingers bind to promoter and SNAG domain interacts with Ajuba (multiple LIM domains protein), which recruits histone deacetylase (HDAC) to condense local chromatin structure and prevent transcription initiation [58].

E-cadherin can also be regulated at post-transcriptional level. The transport of newly synthesized proteins to the plasma membrane can be inhibited through O-glycosylation. Alternatively, E-cadherin can be degraded by proteolysis or endocytosis.

Fragments of E-cadherin obtained through proteolytic degradation can act as intra-nuclear signaling molecules. For instance, the cutting mediated by gamma-secretase protein produces a C-terminal cytoplasmatic fragment (CTF2) that is transported to the nucleus in a p120-dependent manner. Within the nucleus, CTF2 modulates the interaction between p120 catenin and a transcriptional repressor (Kaiso), modifying cell survival [60]. Also CFT1 and CFT2 are two most important fragment of E-cadherin. The former derives from MMPs proteolytic activity at the level of the intra- and extra-cellular protein region interface, while the latter represents the C-terminal fragment of low molecular weight that results from the action of caspase-3 protein. Yang et al. [61] suggest that the caspase-3/E-cadherin pathway can be involved in the apoptosis of gastric epithelial cells induced by H. pylori

Chapter 2 - E-CADHERIN AND CELLULAR PATHWAYS

Recent studies have shown that CDH1 also plays an important role as tumor suppressor gene in gastric carcinogenesis [62-64]. Important signaling interactions between E-cadherin and cellular pathways include the RTK/EGFR/MAPK pathway, Rho/RAC pathway, β-catenin/Wnt pathway and unbound P-120 [29].

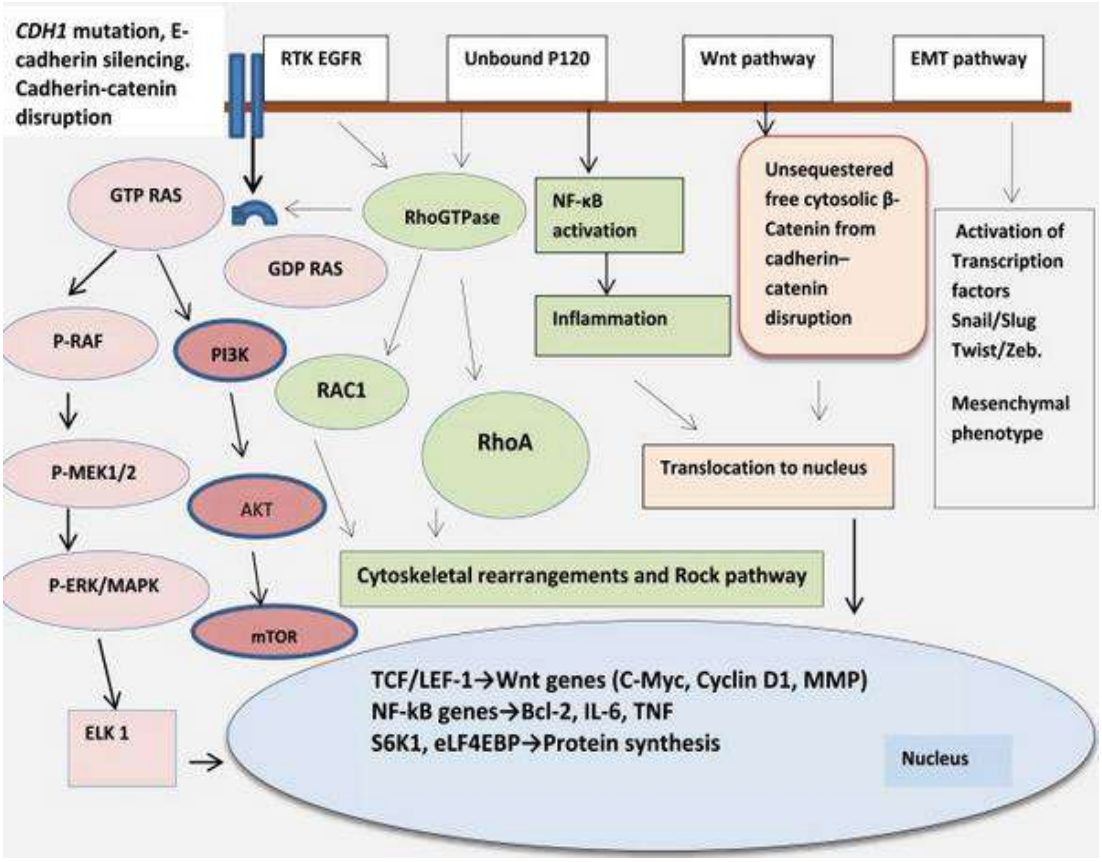


Figure 12. E-cadherin and cellular pathways

2.1 RTK/EGFR/MAPK pathway

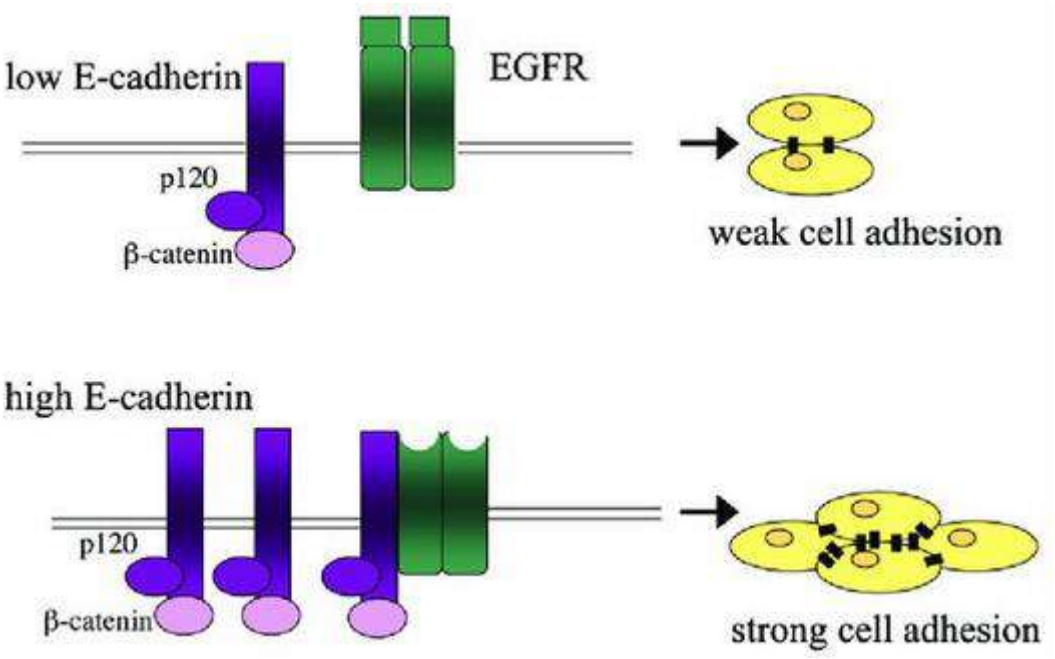


Figure 13. E-cadherin and epidermal growth factor receptor (EGFR)

Another function of E-cadherin is its co-localization with EGFR at cell-cell contact to inhibit epidermal growth factor receptor (EGFR) pathways. Mutation of E-cadherin is associated with ligand-dependent activation of EGFR and downstream effectors through RAS/RAF/MEK pathways and other pro-tumorigenic pathways such as FAK/c-Src and PI3K/AKT/MTOR, thus contributing to enhanced cell proliferation and motility [65-67]. In addition, the loss of E-cadherin is associated with increased nuclear translocation of β-catenin into the nucleus. This translocation of β-catenin represses the expression of PTEN, a tumor suppressor and a critical regulator of the PI3K/AKT/MTOR pathway [68-69].

2.2 Rho GTPases pathway

The Rho family of GTPases is a subfamily of the RAS superfamily (Rho, Ras, Rab, Ran and Arf) [70]. They act as binary molecular switches and regulate many aspects of intracellular cytoskeleton dynamics, such as motility and cell polarity. Recently, it has been discovered that RhoGTPases also act on gene transcription and cell proliferation [71]. Their activity is regulated by factors that control their ability to bind to and hydrolyze guanosine triphoshate (GTP) to guanosine diphosphate (GDP). When they are bound to GTP, they are “on” and when they are bound to GDP, they are “off”.

There is a bilateral relation between E-cadherin and the GTPase signaling pathway [71].

E-cadherin regulates the activity of both RhoA and Rac: the latter is activated by E-cadherin, while RhoA is suppressed.

E-cadherin recruits and activates PI3K at the sites of cell–cell contact. Through PI3K, guanine nucleotide exchange factors (GEFs) promote the exchange of GDP with GTP, activating the Rac GTPase pathway (Figure 14 A); on the other hand, the presence of E-cadherin on cell membrane inhibits RhoA activation (Figure 14 B).

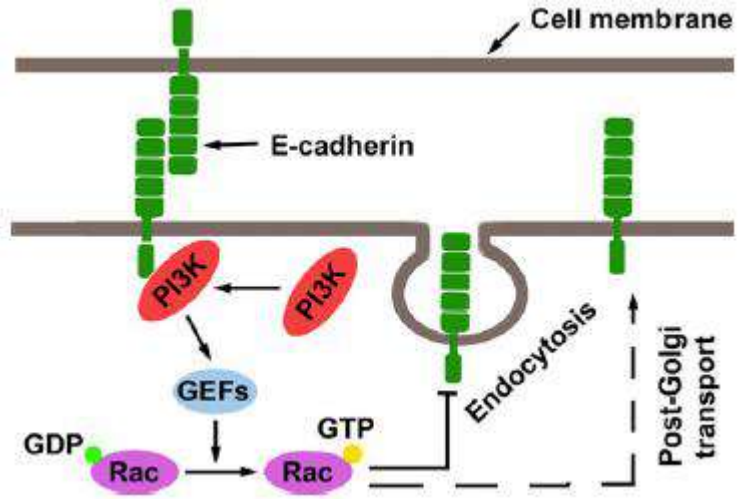


Figure 14 A. Effects of E-cadherin on the RAC signalling. E-cadherin-mediated cell–cell contacts activate Rac through PI3K, and the activated Rac prevents endocytosis of E-cadherin and promotes the post-Golgi transport of E-cadherin.

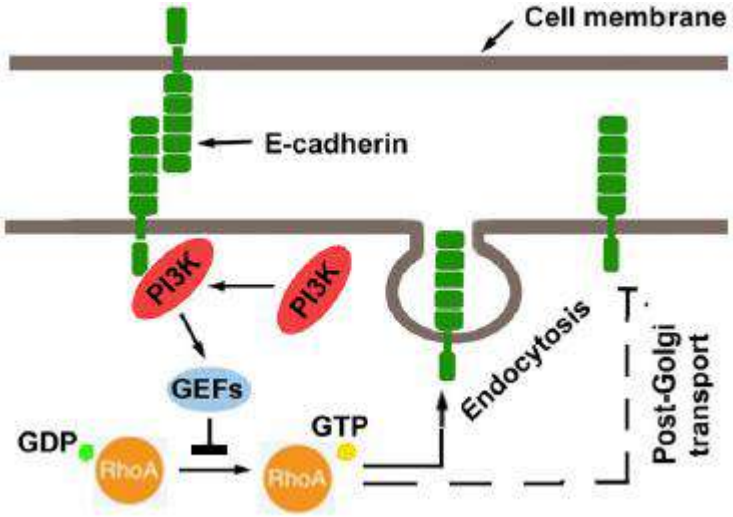


Figure 14 B. Effects of E-cadherin on the RhoA signalling. E-cadherin-mediated cell–cell contacts inhibits RhoA activation. On the other hand, activated RhoA stimulates enodcytosis of E-cadherin through p120 dissociation and indirectly decreases post-Golgi transport and membrane localization .

Activated Rac prevents endocytosis of E-cadherin and promotes the post-Golgi transport of E-cadherin, while activated RhoA indirectly decreases E-cadherin membrane localization through p120 dissociation (Figure 15). Activated RhoA also inhibits anoikis, a form of programmed cell death occurring when there is loss of cell architecture and polarity [72].

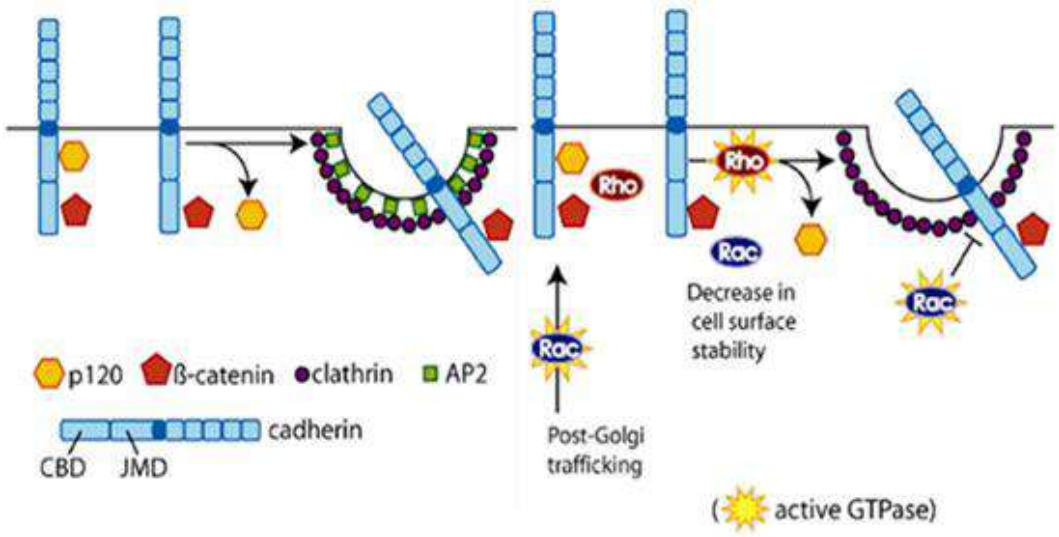


Figure 15. Effect of activated Roc and RhoA on E-cadherin expression.

2.3 NF-kB signaling pathway

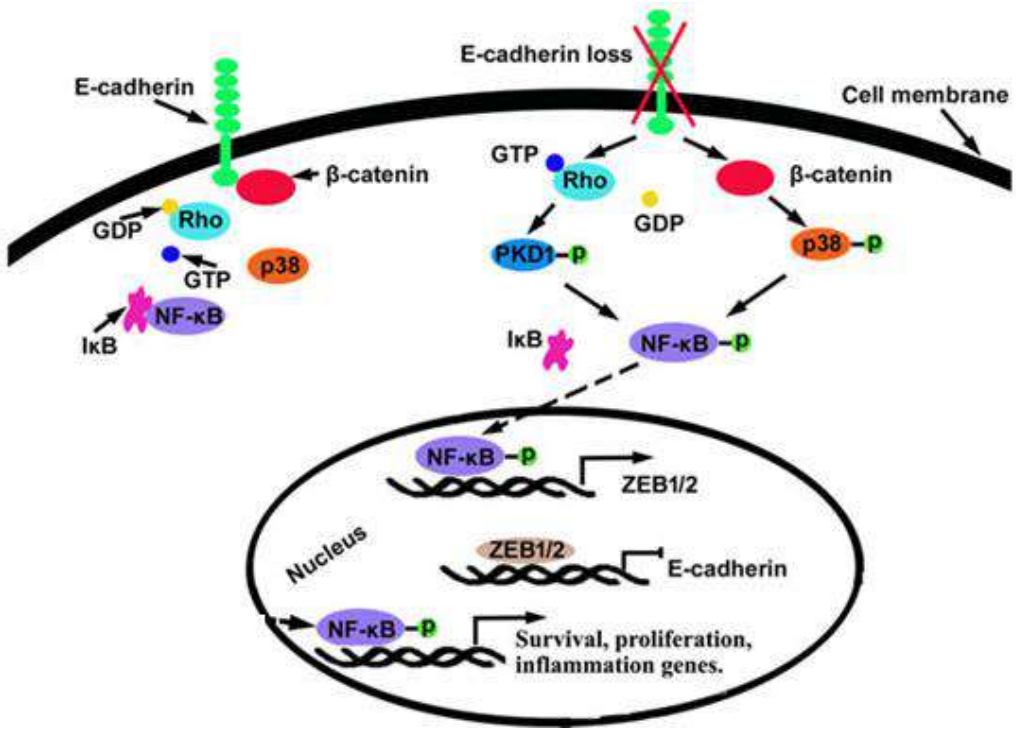


Figure 16. NF-kB signaling pathway.

In normal conditions, overexpression of E-cadherin suppresses the activity of NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells). While, the loss of E-cadherin or the loss of E-cadherin-mediated cell–cell contact activates NF-kB signaling through two pathways of signal transduction [73]. The first includes the activation of cytoplasmic β-catenin, which subsequently induces P38-mediated NF-kB activation. The second one involves the activation of the RhoA pathway, which then activates protein kinase D1 (PKD1), a downstream target of RhoA, that leads to the activation of NF-kB. On the other hand, activated NF-kB inhibits the expression of E-cadherin by elevating transcriptional repressors, such as Snail and ZEB1/2 [74-75]. These data suggest the presence of a feedback regulation mechanism between E-cadherin and NF-kB signaling.

2.4 β-catenin/Wnt pathway

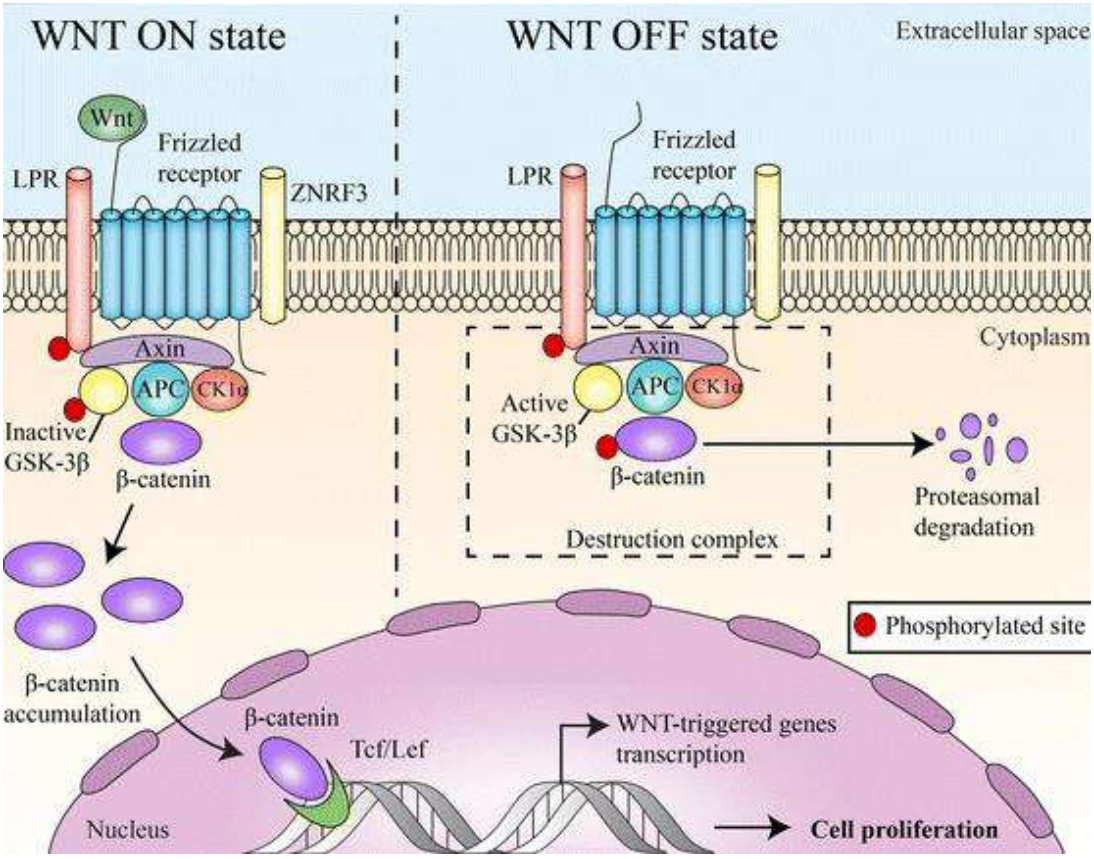


Figure 17. β-catenin/Wnt pathway

When the adhering zonule is disassembled for E-cadherin down regulation, β-catenin is released from the E-cadherin/β-catenin complex into the cytoplasm [76]. It can then translocate to the nucleus and interact with transcriptional processes. Under physiological conditions, cytoplasmic β-catenin remains in an inactive state by being bound to the tertiary-degradation complex formed by axin/adenomatous polyposis coli/glycogen synthase kinase-3β/CK1 (Axin/APC/GSK3β/CK1).

Wnt signaling hinders this degradative process by phosphorylating and inhibiting the GSK3β complex [77]. This raises the critical threshold of β-catenin in the cytoplasm required for translocation into the nucleus.

Under permissive conditions which amplify aberrant Wnt signaling such as paracrine factors from tumor environment, cytokines from stromal cells and TNF-α from macrophages, β-catenin translocates into the nucleus and binds to T cell factor/lymphoid enhancer factor-1 (TCF/Lef1) to induce Wnt target genes such as c-Myc, cyclins, and MMP. This leads to uncontrolled cell proliferation and growth.

2.5 Unbound p120

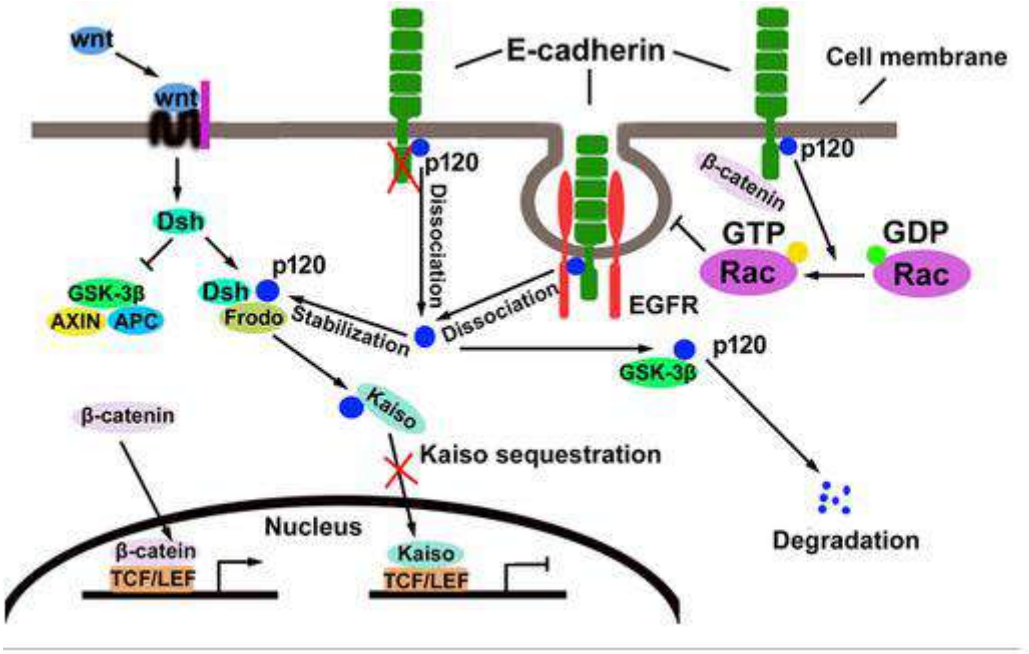


Figure 18. P120 and E-cadherin-mediated cell pathways.

P120 catenin, a member of the catenin family, binds to the cytoplasmic region of E-cadherin and helps to maintain cell-cell contact by preventing the endocytosis of E-cadherin and stabilizing the cadherin-catenin complex [78]. P120 has been found to play an important role in the cross-talk between members of E-cadherin-mediated cell signaling. Certain signaling pathways have been shown to regulate the expression

and function of E-cadherin through p120. EGF promotes the endocytosis of E-cadherin by regulating p120 activity and, thus, decreasing E-cadherin levels in the cell membrane.

The activation of Wnt signaling stabilizes p120 and inhibits Kaiso translocation to the nucleus by forming a p120–Kaiso complex in the cytoplasm [79].

E-cadherin also affects the distribution and function of p120.

On the other hand, p120 itself has been documented to regulate E-cadherin-mediated signaling pathway through both GTPase and β-catenin activity [80].

P120 is able to promote or suppress Rho GTPase directly and indirectly regulating Rho GEFs.

Furthermore, GTPase regulation could occur either at the site of E-cadherin-mediated cell–cell contact or in the cytoplasm.

When associated with E-cadherin, usually p120 protein binds to E-cadherin and stabilizes adherens junctions, suppressing both RhoA (thus activating RAS/RAC/MAPK) and NF-κB pathways (BCL, IL-6, TNF); On the other hand E-cadherin-mediatedcell–cell contact activates Rac. Upon E-cadherin silencing, this negative regulation of RhoA is lost and p120 promotes cell growth by activating RhoGTPase and inflammatory NF-κB pathways.

Once dissociated from E-cadherin, p120 can diffuse into the cytoplasm and activate GTPases.

P120 can enter the nucleus to regulate gene transcription directly, like β-catenin, through an Arm-repeat domain.

In the nucleus, p120 was reported to interact with the zinc finger transcriptional repressor.

Nuclear p120 was also shown to interact with the BTB/POZ transcriptional repressor Kaiso, inhibiting Kaiso transcriptional activity [81-82].

Kaiso is an inhibitor of the Wnt signaling pathway, directly inhibiting the transcription of Wnt11 and the expression of Wnt signaling targets, such asc-Myc, cyclin D1 and matrilysin (MMP-7), through competitive binding of TCF/LEF to β–catenin.

Therefore, there might be a positive feedback circuit between p120 and Wnt signaling activity. P120 may play a positive role in activation of the Wnt signaling pathway.

On the other hand, Wnt signaling activation stabilizes p120, which in urn promotes Kaiso sequestration or removal from the nucleus and elevated Wnt signaling.

2.6 Post-transcriptional microRNA silencing gene

Lauren's classification distinguishes two gastric cancer subtypes based on histological and clinical features: intestinal-type (IGC) and diffuse-type gastric cancer (DGC). These different histotypes represent distinct disease entities with different epidemiology, etiology, carcinogenesis and biological behaviors. The role of germline mutations or epigenetic and structural alterations of CHD1, which encodes E-cadherin, is well established in DGC cancerogenesis. On the other hand, CDH1 status is not as extensively studied in IGC as in DGC. However, as reported by many studies, CDH1 expression is also down regulated in IGC.

In recent years, microRNAs (miRs) and long non-coding RNAs have emerged as promoters and suppressors of carcinogenesis and metastasis in many types of cancer, including IGC [83].

This non coding RNA trascripts are capable of modifying E-cadherin expression through epigenetic control, negatively regulating gene expression and orchestrating pathways involved in cell-cycle control, proliferation, apoptosis, angiogenesis and metastasis.

With regard to IGC, a wide range of miRs have been associated with Helicobacter Pylori (HP-related infection), a well-established event in intestinal gastric cancer carcinogenesis. Interestingly, E-cadherin down regulation has been described in concomitance with HP-derived neutrophil infiltration [85-86].

Several miRs are involved in epithelial-mesenchymal transition (EMT), modulating E-cadherin expression by directly targeting CDH1 or acting on one or more of its trascriptional repressors, including histone-methyltransferase enzyme (EZH2) or zinc finger proteins such as ZEB1, ZEB2 and Slug.

miR-101 has been reported to act as a tumor supressor by targeting CDH1 inhibitors, such as ZEB1/ZEB2 and EZH2. Low levels of mR-101 in plasma have been reported to be associated with gastric cancer progression and HP-induced inflammation [87-90].

miR26b is expressed at low levels in gastric cancer and its down regulation is associated with a higher TNM staging and shorter suvival. Several studies have shown that miR26b, like miR-101, inhibits EZH2 expression leading to CDH1 downregulation [91-92]. Also miR-200 family members act as tumor suppressors: they are markedly down regulated during EMT with a concomitant decrease in E-cadherin, and their lower expression has been associated with poor prognosis [93-93].

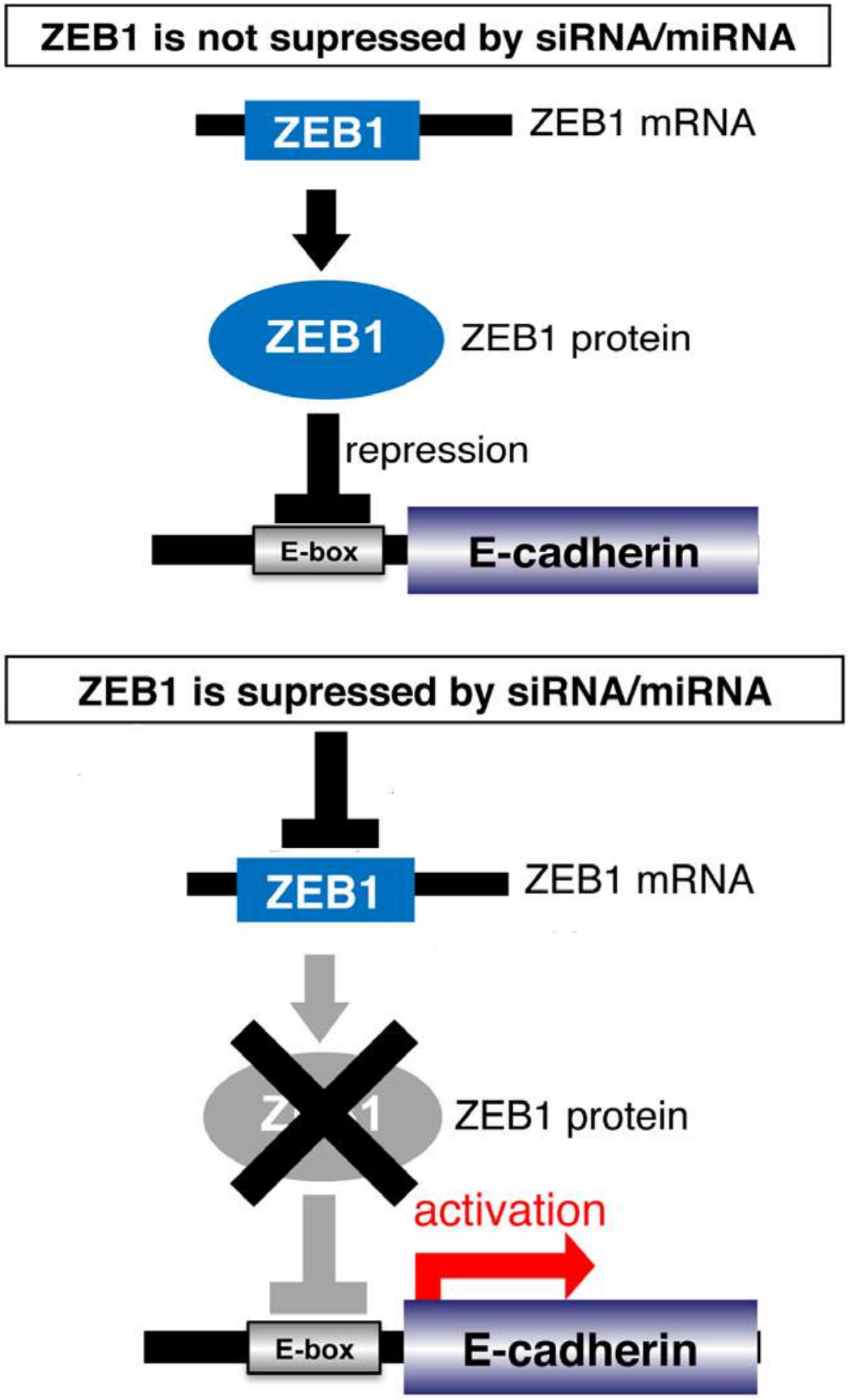


Figure 19. Post-transcriptional microRNA regulation of E-Cadherin through ZERB1.

Chapter 3 - MOLECULAR CLASSIFICATION OF GASTRIC CANCER

In the last few years, many new GC classifications have been proposed. **The Cancer Genome Atlas** (**TCGA**) identified 4 genomic subtypes: Epstein–Barr virus (EBV), microsatellite instability (MSI), chromosomal instability (CIN) and genomically stable (GS) [97]. **The Asian Cancer Research Group** (**ACRG**) classified GC into microsatellite instable (MSI) and microsatellite stable (MSS) types [98]. Secondarily, MSS were further divided into epithelial–mesenchymal transition (EMT), TP53+ and TP53− groups. Following the introduction of molecular classifications, great research efforts have been conducted, in order to clarify their potential impact in clinical decision-making and treatment of GC.

This is particularly true for the ACRG classification, because in theirstudy some clinically relevant features were attributed to the molecular subgroups [98].

Distinct groups, indeed, showed peculiar clinical–pathological characteristics (such as age, tumor location, invasion and stage). Importantly, different prognosis was attributed to the four groups, with MSI showing the best survival rate, and EMT bearing the worst prognosis.

Patients with EMT show very high propensity to peritoneal dissemination, as well as nodal metastases, and may benefit from prophylactic HIPEC and extended lymphadenectomy when confirmed in prospective trials.

3.1 MSI group (TGCA and ACRG classifications)

Patients with MSI present with specific molecular characterization such as MLH1 silencing, hypermutations of KRAS, PI3K–PTEN–mTOR pathway, ALK, and ARID1A.

The subgroup of GC with microsatellite instability is probably the most studied to date.

It is present in both TGCA and ACRG classifications and was previously extensively investigated and its results reported in several papers, although with heterogeneous and some what conflicting results. In a recent meta-analysis of 48 studies (18.612 subjects) [99], patients with MSI on average accounted for about 9% of the total cases. The pooled analysis indicated that women had a significantly increased risk of MSI compared with men, and the mean age was 66 years.

Most MSI tumors were of the intestinal type according to Lauren, and were located in the distal stomach. Importantly, the risk of nodal metastases was lower than in MSS cases (OR 0.70,95% CI 0.57–0.86, P< 0.001), and also tumor stage was less advanced.

In addition, overall survival was greater in patients with MSI gastric cancer compared to MSS cases (OR 0.69, 95% CI 0.56–0.86, $p < 0.001$).

In a recent paper including 472 patients [100], cancer-related 5-year survival was significantly higher in MSI-H versus MSS group (67.6% vs.35%), and this prognostic impact was confirmed by multivariate Cox regression analysis. The authors observed also a linear correlation between advanced age and the rate of MSI; indeed, the percentage of MSI increased gradually with increasing age, accounting for 48% of patients over age 85. Furthermore,the prognostic effect of MSI status

was more evident in elderly compared to younger patients. The highest differencein survival was seen between MSI and MSS groups of patients older than 65 years, while no statistical difference was observed in younger groups, and multivariate analysis confirmed MSI status as a significant factor in patients aged over 70 years (HR 1.82, P = 0.013). These findings support the evidence that MSI may act as a significant predictor of better prognosis above all in the elderly. In Italian study [101], a detailed analysis of lymph nodal spread in MSI vs. MSS GC was performed in a total of 361 patients. All patients were submitted to an extended (D2) or super-extended (D2 plus) lymphadenectomy; the different lymph node stations were divided and classified according to the JGCA criteria, and single nodes were retrieved from fresh specimens. MSI tumors showed a lower rate of lymph node metastases (46% vs. 70% of MSS group), a lower mean number of involved nodes (1 vs. 5), a lower number of involved node stations, and a lower propensity to spread to second and third compartment nodes. Furthermore, no skip metastases were observed in the MSI group. These data, when validated in other experiences and in preoperative endoscopic biopsies, may be useful in tailoring lymphadenectomy for GC, allowing a less extended dissection in MSI tumors, above all when facing high-risk patients with relevant comorbidities. Finally, an interesting paper reported a post hoc analysis of patients included in the MAGIC trial [102]; patients were treated with surgery alone or perioperative chemotherapy plus surgery for operable gastroesophageal cancer, and the association between MSI status and long-term survival was investigated. Results revealed that MSI status was associated with a positive prognostic effect in patients treated with surgery alone, whereas in patients treated with neo-adjuvant chemotherapy the prognostic effect was negative. If confirmed, these

results could change indications to NAC in the subgroup of patients with cancer the esophago-gastric junction (EGJ) with MSI.

3.2 MSS and EMT group (ACRG classification)

The group of tumors with MSS and epithelial-to-mesenchymal transition (EMT) according to the ACRG classificationis is also remarkably interesting from a clinical point of view.

EMT is a process where epithelial cells are transformedinto cells with mesenchymal phenotype, characterized byloss ofcellular polarity and adhesion and enhanced invasive and migratory properties.

Epithelial markers, such as E-cadherin, are repressed, and mesenchymal markers, such as vimentin and fibronectin, are up regulated.

These alterations, together with microenvironment remodeling, facilitate GC aggressiveness, invasion, migration, metastasis and chemoresistance. EMT phenotype correlates, other than with adiffuse type and poorly differentiated histology, with an advanced TNM stage and poor prognosis. According to the ACRG report [98], the MSS/EMT group accounts for about 15% of cases, and is associated with younger age (53 years in median), location in the middle third (45.6%) or entire stomach (6.5%), diffuse type (80.4%) and signet ring cell histology (43.5%). Inaddition, this subgroup is associated with more advanced pT stage, lymph node metastasis, TNM stage and perineural invasion.

Importantly, this group of GC showed the worst prognosis when compared with other groups, and when analyzing the pattern of relapse, 77% of MSS/EMT cases inthe ACRG cohort recurred in the peritoneum

(vs. less than 20% of other groups); on the other hand, none of the cases had liver metastases [98].

3.3 Chromosomal instability (CIN) group (TGCA classification)

The CIN subtype represents about 50% of total GC cases. About 80% of cases in this group are of the intestinal type, and the main location is the fundus/body or EGJ/cardia [97].

This group is particularly interesting in view of a potential targeted therapy; indeed, CIN tumors present amplification in oncogene pathways such as RTK/RAS/MAPK signaling, including HER2, BRAF, epidermal growth factor (EGFR), MET, FGFR2, and RAS. Further studies are necessary to elucidate the clinical implications of this group, with special reference to a multimodality approach.

3.4 Genomic stability (GS) group (TGCA classification)

The molecular subtype with GS represents about 20% of cases in the TCGA report. Most of these tumors are of diffuse histotype (about 60% of diffuse type cases are included in this group), and a peculiar characteristic is the predominance of poorly cohesive type tumors in this class. Tumors are equally distributed in the stomach. The main somatic genomic alterations involve CDH1, ARID1A and RHOA [103]. CDH1 mutations have been reported to be a significant predictor of poor prognosis after radical surgery for GC, and this may have clinical implications that deserve further studies.

4.5 EBV-associated group (TGCA classification)

The molecular group with EBV represents about 9% of cases according to the TCGA report. In the ACRG classification, EBV is for the majority present in the MSS/TP53+ subgroup. The largest international pooled analysis on 4599 gastric cancer patients by Camargo et al [104]. proved that this group is strongly overrepresented by males. Additionally, positive EBV GCs were early stage gastric cancer, with cardia localization, diffuse histotype according to Laurén classification, and poor differentiation. The mediansurvival for EBV GC was 8.5 years vs. 5.3 years for non-EBVGC ($p = 0.0006$). At multivariate analysis, EBV status wasone of the statically significant predictors of survival. Currently, ongoing trials are trying to identify a group of patients that will respond to immunological therapy.

CONCLUSIONS

Small undifferentiated EGC (<2 cm) have risk of lymph nodes involvement and E-cadherin may be a possible prediction factor.

Detection of E-cad on the bioptic sample of the primary cancer could be a feasible method to predict which patients should undergo endoscopic resection and which ones should be submitted to surgery with extended lymphadenectomy.

In these cases of low or absent expression of E-cadherin, although the tumoral stage is low and no histological LNM is found, extensive lymphadenectomy and micrometastatic research should be performed.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-E386 [PMID: 25220842 DOI:10.1002/ijc.29210]

2. Wang YW, Zhu ML, Wang RF, Xue WJ, Zhu XR, Wang LF, Zheng LZ. Predictable factors for lymph node metastasis in early gastric cancer analysis of clinicopathologic factors and biological markers. Tumor Biol. (2016) 37:8567–8578 [PMID: 26733174 DOI: 10.1007/s13277-015-4721-3]

3. Ono H, Kondo H, Gotoda T, Shirao K, Yamaguchi H, Saito D, Hosokawa K, Shimoda T, Yoshida S. Endoscopic mucosal resection for treatment of early gastric cancer. Gut. 2001;48:225–9. [PMID: 11156645 DOI: 10.1136/gut.48.2.225]

4. Gotoda T, Yamamoto H, Soetikno RM. Endoscopic submucosal dissection of early gastric cancer. J Gastroenterol. 2006;41:929–42. [PMID: 17096062 DOI:10.1007/s00535-006-1954-3]

5. Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2010 (ver. 3). Gastric Cancer. 2011 Jun;14(2):113-23. [PMID: 21573742 DOI: 10.1007/s10120-011-0042-4]

6. Pokala SK, Zhang C, Chen Z, Gamboa AM, Cristofaro SL, Keilin SA, Cai Q, Willingham FF. Lymph node metastasis in early gastric adenocarcinoma in the United States of America. Endoscopy. 2018 May;50(5):479-486. [PMID: 29228402 DOI: 10.1055/s-0043-122379]

7. Park YD, Chung YJ, Chung HY, Yu W, Bae HI, Jeon SW, Cho CM, Tak WY, Kweon YO. Factors related to lymph node metastasis and the feasibility of endoscopic mucosal resection for treating poorly differentiated adenocarcinoma of the stomach. Endoscopy 2008; 40: 7-10 [PMID: 18210339 DOI: 10.1055/s-2007-966750]

8. Odagaki T, Suzuki H, Oda I, Yoshinaga S, Nonaka S, Katai H, Taniguchi H, Kushima R, Saito Y. Small undifferentiated intramucosal gastric cancer with lymph-node metastasis: case report. World J Gastroenterol. 2013 May 28;19(20):3157-60. [PMID: 23716998 DOI: 10.3748/wjg.v19.i20.3157]

9. Nasu J, Hori S, Asagi A, Nishina T, Ikeda Y, Tanimizu M, Iguchi H, Aogi K, Kurita A, Nishimura R. A case of small undifferentiated intramucosal gastric cancer with lymph node metastasis. Gastric Cancer. 2010 Nov;13(4):264-6. [PMID: 21128063 DOI: 10.1007/s10120-010-0562-3]

10. National Comprhensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Gastric Cancer. Version 3. 2019 — November 18, 2019.

11. Kalluri R, Weinberg RA. The Basics of Epithelial-Mesenchymal Transition. J Clin Invest. 2009 Jun;119(6):1420-8. [PMID: 19487818 DOI: 10.1172/JCI39104]

12. Thiery JP. Epithelial-mesenchymal Transitions in Tumour Progression**.** Nat Rev Cancer 2002 Jun;2(6):442-54. [PMID: 12189386 DOI: 10.1038/nrc822]

13. Thiery JP, Sleeman JP. Complex Networks Orchestrate Epithelial-Mesenchymal Transitions. Nat Rev Mol Cell Biol 2006 Feb;7(2):131-42. [PMID: 16493418 DOI: 10.1038/nrm1835]

14. Pastushenko I, Blanpain C. EMT Transition States During Tumor Progression and Metastasis. Trends Cell Biol 2019 Mar;29(3):212-226. [PMID: 30594349 DOI: 10.1016/j.tcb.2018.12.001]

15. Lamouille S, Xu J, Derynck R. Molecular Mechanisms of Epithelial-Mesenchymal Transition. Nat Rev Mol Cell Biol 2014 Mar;15(3):178-96. [PMID: 24556840 DOI: 10.1038/nrm3758]

16. Christiansen JJ, Rajasekaran AK. Reassessing Epithelial to Mesenchymal Transition as a Prerequisite for Carcinoma Invasion and Metastasis. Cancer Res 2006 Sep 1;66(17):8319-26. [PMID: 16951136 DOI: 10.1158/0008-5472.CAN-06-0410]

17. Ceausu AR, Ciolofan A, Cimpean AM, Magheti A, Mederle O, Raica M. The Mesenchymal-Epithelial and Epithelial-Mesenchymal Cellular Plasticity of Liver

Metastases With Digestive Origin. Anticancer Res. 2018 Feb;38(2):811-816. [PMID: 29374706 DOI: 10.21873/anticanres.12288]

18. Chu YQ, Ye ZY, Tao HQ, Wang YY, Zhao ZS. Relationship between cell adhesion molecules expression and the biological behavior of gastric carcinoma. World Journal of Gastroenterology. 2008;14(13):1990-6. [PMID: 18395897 DOI: 10.3748/wjg.14.1990]

19. Berx G, Becker KF, Hofler H, van Roy F. Mutations of the human E-cadherin (CDH1) gene. Hum Mutat 1998;12:226–37. [PMID: 9744472 DOI:10.1002/(SICI)1098-1004(1998)12:4<226::AID- HUMU2>3.0.CO;2-D]

20. Brooks-Wilson AR, Kaurah P, Suriano G, Leach S, Senz J, Grehan N, Butterfield YS, Jeyes J, Schinas J, Bacani J, Kelsey M, Ferreira P, MacGillivray B, MacLeod P, Micek M, Ford J, Foulkes W, Australie K, Greenberg C, LaPointe M, Gilpin C, Nikkel S, Gilchrist D, Hughes R, Jackson CE, Monaghan KG, Oliveira MJ, Seruca R, Gallinger S, Caldas C, Huntsman D. Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. J Med Genet 2004;41:508–17. [PMID: 15235021 DOI: 10.1136/jmg.2004.018275]

21. Jee YS, Jang TJ, Jung KH. Prostaglandin E(2) and interleukin-1beta reduce E-cadherin expression by enhancing Snail expression in gastric cancer cells. J Korean Med Sci. 2012 Sep;27(9):987-92. [PMID: 22969242 DOI: 10.3346/jkms.2012.27.9.987]

22. Becker KF, Rosivatz E, Blechschmidt K, Kremmer K, Sarbia M, Höfler H. Analysis of the E-cadherin repressor Snail in primary human cancers. Cells Tissues Organs 2007;185:204–12. [PMID: 17587826 DOI: 10.1007/s00428- 005-0118-9]

23. Alves CC, Carneiro F, Hoefler H, Becker KF. Role of the epithelial-mesenchymal transition regulator Slug in primary human cancers. Front Biosci (Landmark Ed). 2009 Jan 1;14:3035- 50. [PMID: 19273255 DOI: 10.2741/3433]

24. Blair V, Martin I, Shaw D, Winship I, Kerr D, Arnold J, Harawira P, McLeod M, Parry S, Charlton A, Findlay M, Cox B, Humar B, More H, Guilford P. Hereditary

Diffuse Gastric Cancer: Diagnosis and Management Clin Gastroenterol Hepatol 2006 Mar;262–275. [PMID: 16527687 DOI:10.1016/j.cgh.2005.12.003]

25. Carneiro F, Oliveira C, Suriano G, Seruca R. Molecular pathology of familial gastric cancer, with an emphasis on hereditary diffuse gastric cancer. J Clin Pathol. 2008 Jan;61(1):25-30. [PMID:17513507 DOI:10.1136/jcp.2006.043679]

26. Liu YC, Shen CY, Wu HS, Hsieh TY, Chan DC, Chen CJ, Yu JC, Yu CP, Harn HJ, Chen PJ, Hsieh CB, Chen TW, Hsu HM. Mechanisms inactivating the gene for E-cadherin in sporadic gastric carcinomas. World J Gastroenterol. 2006 Apr 14;12(14):2168-73. [PMID: 16610016 DOI: 10.3748/wjg.v12.i14.2168]

27. Rossi T, Tedaldi G, Petracci E, Abou Khouzam R, Ranzani GN, Morgagni P, Saragoni L, Monti M, Calistri D, Ulivi P, Molinari C. E-cadherin Downregulation and microRNAs in Sporadic Intestinal-Type Gastric Cancer. Int J Mol Sci. 2019 Sep 10;20(18). [PMID: 31509966 DOI: 10.3390/ijms20184452]

28. Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE. E-cadherin germline mutations in familial gastric cancer. Nature. 1998 Mar 26;392(6674):402-5. [PMID: 9537325 DOI: 10.1038/32918]

29. Santosh S. CDH1 (E-Cadherin) Mutation and Gastric Cancer: Genetics, Molecular Mechanisms and Guidelines for Management. Cancer Manag Res. 2019 Dec 13;11:10477-10486. [PMC6916690 DOI:10.2147/CMAR.S208818]

30. Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, Höfler H. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res. 1994;54 (14):3845–3852. [PMID 8033105]

31. Gayther SA, Gorringe KL, Ramus SJ, Huntsman D, Roviello F, Grehan N, Machado JC, Pinto E, Seruca R, Halling K, MacLeod P, Powell S M, Jackson C E, Ponder B A, Caldas C. Identification of germ-line E-cadherin mutations in gastric cancer families of European origin. Cancer Res. 1998;58(18):4086–4089. [PMID 9751616]

32. Van der Post RS, Vogelaar IP, Carneiro F, Guilford P, Huntsman D, Hoogerbrugge N, Caldas C, Chelcun Schreiber KE, Hardwick RH et al. Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet. 2015 Jun; 52(6): 361–374. [PIMD 25979631 DOI: 10.1136/jmedgenet-2015-103094]

33. Barber M, Murrell A, Ito Y, et al. Mechanisms and sequelae of E-cadherin silencing in hereditary diffuse gastric cancer. J Pathol. 2008;216(3):295–306. [PMID 18788075 DOI:10.1002/path.2426]

34. Oliveira C, Sousa S, Pinheiro H, et al. Quantification of epigenetic and genetic 2nd hits in CDH1 during hereditary diffuse gastric cancer syndrome progression. Gastroenterology. 2009;136(7):2137–2148. [PMID 19269290 DOI:10.1053/j.gastro.2009.02.065]

35. Grady WM, Willis J, Guilford PJ, et al. Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. Nat Genet. 2000;26(1):16–17. [PMID 10973239 DOI:10.1038/79120]

36. Melo S, Figueiredo J, Fernandes MS, et al. Predicting the functional impact of CDH1 Missense mutations in hereditary diffuse gastric cancer. Int J Mol Sci. 2017;18:12. [PMID 29231860 DOI:10.3390/ijms18122687]

37. Fewings E, Larionov A, Redman J, et al. Germline pathogenic variants in PALB2 and other cancer-predisposing genes in families with hereditary diffuse gastric cancer without CDH1 mutation: a whole-exome sequencing study. Lancet Gastroenterol Hepatol. 2018;3(7):489–498. [PMID 29706558 DOI:10.1016/S2468-1253(18)30079-7].

38. Hansford S, Kaurah P, Li-Chang H, et al. Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol. 2015;1 (1):23–32. [PMID 26182300 DOI: 10.1001/jamaoncol.2014.168].

39. Corso G, Intra M, Trentin C, Veronesi P, Galimberti V. CDH1 germline mutations and hereditary lobular breast cancer. Fam Cancer. 2016;15(2):215–219. [PMID: 26759166 DOI: 10.1007/s10689-016-9869-5]

40. Corso G, Figueiredo J, La Vecchia C, Veronesi P, Pravettoni G, Macis D, Karam R, Lo Gullo R, Provenzano E, Toesca A, Mazzocco K, Carneiro F, Seruca R, Melo S, Schmitt F, Roviello F, De Scalzi AM, Intra M, Feroce I, De Camilli E, Villardita MG, Trentin C, De Lorenzi F, Bonanni B, Galimberti V. Hereditary lobular breast cancer with an emphasis on E-cadherin genetic defect. J Med Genet. 2018 Jul;55(7):431-441. [PMID: 29929997 DOI: 10.1136/jmedgenet-2018-105337]

41. Huntsman DG, Carneiro F, Lewis FR, et al. Early gastric cancer in young, asymptomatic carriers of germline E-cadherin mutations. NEJM. 2001;344(25):1904–1909. [PMID: 11419427 DOI:10.1056/NEJM200106213442504]

42. Moran CJ, Joyce M, McAnena OJ. CDH1 associated gastric cancer: a report of a family and review of the literature. Eur J Surg Oncol. 2005 Apr;31(3):259-64. [PMID: 15780560 DOI: 10.1016/j.ejso.2004.12.010]

43. Castro R, Lobo J, Pita I, Videira F, Pedro-Afonso L, Dinis-Ribeiro M, Brandão C. Rev Esp Enferm Dig. 2020 May;112(5):367-372. [PMID: 32338015 DOI: 10.17235/reed.2020.6720/2019]

44. Chen Y, Kingham K, Ford JM, Rosing J, Van Dam J, Jeffrey RB, Longacre TA, Chun N, Kurian A, Norton JA. A prospective study of total gastrectomy for CDH1-positive hereditary diffuse gastric cancer. Ann Surg Oncol. 2011 Sep;18(9):2594-8. [PMID: 21424370 DOI: 10.1245/s10434-011-1648-9]

45. Fitzgerald RC, Hardwick R, Huntsman D, Carneiro F, Guilford P, Blair V, Chung DC, Norton J, Ragunath K, Van Krieken JH, Dwerryhouse S, Caldas C, International Gastric Cancer Linkage C. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet 2010;47:436–44. [PMID 20591882 DOI: 10.1136/jmg.2009.074237]

46. Kumar S, Long JM, Ginsberg GG, Katona BW. The role of endoscopy in the management of hereditary diffuse gastric cancer syndrome. World J Gastroenterol. 2019;25(23):2878–2886. [PMID PMID: 31249446 DOI:10.3748/wjg.v25.i23.2878]

47. Dossus L, Benusiglio PR. Lobular breast cancer: incidence and genetic and non-genetic risk factors. Breast Cancer Res. 2015;17:37. [PMID DOI:10.1186/s13058-015-0546-7]

48. Knuttel FM, Menezes GL, van den Bosch MA, Gilhuijs KG, Peters NH. Current clinical indications for magnetic resonance imaging of the breast. J Surg Oncol. 2014;110(1):26–31. [PMID DOI:10.1002/jso.23655]

49. Caldas C, Carneiro F, Lynch HT et al (1999) Familial gastric cancer: overview and guidelines for management. J Med Genet 36:873–880. [PMID: 10593993]

50. Kluijt I, Sijmons RH, Hoogerbrugge N, Plukker JT, de Jong D, van Krieken JH, van Hillegersberg R, Ligtenberg M, Bleiker E, Cats A; Dutch Working Group on Hereditary Gastric Cancer. Fam Cancer. 2012 Sep;11(3):363-9. [PMID: 22388873 DOI: 10.1007/s10689-012-9521-y]

51. Graziano F, Ruzzo AM, Bearzi I, Testa E, Lai V, Magnani M. Screening E-cadherin germline mutations in Italian patients with familial diffuse gastric cancer: an analysis in the District of Urbino, Region Marche, Central Italy. Tumori. 2003 May-Jun;89(3):255-8. [PMID: 12908778]

52. Carvalho J, Oliveira P, Senz J, São José C, Hansford S, Teles SP, Ferreira M, Corso G, Pinheiro H, Lemos D, Pascale V, Roviello F, Huntsman D, Oliveira C. Redefinition of Familial Intestinal Gastric Cancer: Clinical and Genetic Perspectives. J Med Genet. 2020 Feb 17:jmedgenet-2019-106346. [PMID: 32066632 DOI: 10.1136/jmedgenet-2019-106346]

53. Berx G, Becker KF, Hofler H, van Roy F. Mutations of the human E-cadherin (CDH1) gene. Hum Mutat 1998; 12: 226-237. [PMID: 9744472 DOI: 10.1002/(SICI)1098-1004(1998)12:4<226::AID-HUMU2>3.0.CO;2-D]

54. Liu YC , Shen CY, Wu HS, Hsieh TS, Chan DC, Chen CJ, Yu JC, Yu CP, Harn HJ, Chen PJ, Hsieh CB, Chen TW, Hsu HM. Mechanisms Inactivating the Gene for E-cadherin in Sporadic Gastric Carcinomas. World J Gastroenterol. 2006 Apr 14;12(14):2168-73. [PMID: 16610016 DOI: 10.3748/wjg.v12.i14.2168]

55. Machado JC, Oliveira C, Carvalho R, Soares P, Berx G, Caldas C, Seruca R, Carneiro F, Sobrinho-Simoes M. E-cadherin gene (CDH1) promoter methylation as the second hit in sporadic diffuse gastric carcinoma. Oncogene 2001; 20: 1525-1528. [PMID: 11313896 DOI: 10.1038/sj.onc.1204234]

56. Grady WM, Willis J, Guilford PJ, Dunbier AK, Toro TT, Lynch H, Wiesner G, Ferguson K, Eng C, Park JG, Kim SJ, Markowitz S. Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. Nat Genet 2000; 26: 16-17. [PMID: 10973239 DOI: 10.1038/79120]

57. Peinado H, Ballestar E, Esteller M, Cano A. Snail Mediates E-cadherin Repression by the Recruitment of the Sin3A/histone Deacetylase 1 (HDAC1)/HDAC2 Complex. Mol Cell Biol. 2004 Jan;24(1):306-19. [PMID: 14673164 DOI: 10.1128/mcb.24.1.306-319.2004]

58. Chiang C, Ayyanathan K. Snail/Gfi-1 (SNAG) Family Zinc Finger Proteins in Transcription Regulation, Chromatin Dynamics, Cell Signaling, Development, and Disease. Cytokine Growth Factor Rev. 2013 Apr;24(2):123-31. [PMID: 23102646 DOI: 10.1016/j.cytogfr.2012.09.002]

59. Zhu W, Leber B, Andrews D W. EMBO J. 2001 Nov 1;20(21):5999-6007. Cytoplasmic O-glycosylation Prevents Cell Surface Transport of E-cadherin During Apoptosis. [PMID: 11689440 DOI:10.1093/emboj/20.21.5999]

60. Ferber EC, Kajita M, Wadlow A, Tobiansky L, Niessen C, Ariga H, Daniel J, Fujita Y. A Role for the Cleaved Cytoplasmic Domain of E-cadherin in the Nucleus. [PMID: 18356166 DOI: 10.1074/jbc.M708887200]

61. Yongmei Yang 1 2 , Jie Du 1 , Fen Liu 3 , Xiaoyan Wang 3 , Xiaohui Li 1 , Yuanjian Li 1. Role of caspase-3/E-cadherin in Helicobacter Pylori-Induced Apoptosis of Gastric Epithelial Cells. Oncotarget. 2017 Jul 22;8(35):59204-59216. [PMID: 28938629 DOI: 10.18632/oncotarget.19471]

62. Cancer genome atlas research network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513(7517):202–209. [PMID: 25079317 DOI: 10.1038/nature13480]

63. Bruner HC, Derksen PWB. Loss of E-Cadherin-dependent cell-cell adhesion and the development and progression of cancer. Cold Spring Harb Perspect Biol. 2018;10(3):a029330. [DOI:10.1101/cshperspect.a029330]

64. Nam S, Kim JH, Lee DH. RHOA in gastric cancer: functional roles and therapeutic potential. Front Genet. 2019;10:438. [DOI:10.3389/fgene.2019.00438].

65. Qian X, Karpova T, Sheppard AM, McNally J, Lowy DR. E-cadherin-mediated adhesion inhibits ligand-dependent activation of diverse receptor tyrosine kinases. EMBO J. 2004;23 (8):1739–1748. [DOIi:10.1038/sj.emboj.7600136]

66. Perrais M, Chen X, Perez-Moreno M, Gumbiner BM. E-cadherin homophilic ligation inhibits cell growth and epidermal growth factor receptor signaling independently of other cell interactions. Mol Biol Cell. 2007;18(6):2013–2025. [DOI:10.1091/mbc.e06-04-0348]

67. Georgopoulos NT, Kirkwood LA, Walker DC, Southgate J. Differential Regulation of Growth-Promoting Signalling Pathways by E-cadherin. PLoS One. 2010 Oct 26;5(10):e13621. [PMID: 21049033 DOI: 10.1371/journal.pone.0013621]

68. Ma J, Guo X, Zhang J, Wu D, Hu X, Li J, Lan Q, Liu Y, Dong W. PTEN Gene Induces Cell Invasion and Migration via Regulating AKT/GSK-3β/β-Catenin Signaling Pathway in Human Gastric Cancer. Dig Dis Sci. 2017 Dec;62(12):3415-3425. [PMID: 29030742 DOI: 10.1007/s10620-017-4764-y]

69. Luongo F, Colonna F, Calapà F, Vitale S, Fiori ME, De Maria R. PTEN Tumor-Suppressor: The Dam of Stemness in Cancer. Cancers (Basel). 2019 Aug; 11(8): 1076. [PMID: 31366089 DOI: 10.3390/cancers11081076]

70. Hall A. Rho Family GTPases. Biochem Soc Trans. 2012 Dec 1;40(6):1378-82. [PMID: 23176484 DOI:10.1042/BST20120103].

71. Etienne-Manneville S, Hall A. Rho GTPases in cell biology. Nature. 2002; 420: 629–35. Rho GTPases in Cell Biology. Nature. 2002 Dec 12;420(6916):629-35. [PMID: 12478284 DOI: 10.1038/nature01148].

72. M Zhan , H Zhao, Z C Han. Signalling Mechanisms of Anoikis. Histol Histopathol. 2004 Jul;19(3):973-83. [PMID: 15168359 DOI: 10.14670/HH-19.973]

73. Cowell CF, Yan IK, Eiseler T, Eiseler T, Leightner AC, Döppler H, Storz P. Loss of cell-cell contacts induces NF-kappaB via RhoA-mediated activation of protein kinase D1. J Cell Biochem. 2009; 106: 714–28. J Cell Biochem. 2009 Mar 1;106(4):714-28. [PMID: 19173301 DOI: 10.1002/jcb.22067]

74. Hu Z, Liu X, Tang Z, Zhou Y, Qiao L. Possible regulatory role of Snail in NF-kappaB-mediated changes in E-cadherin in gastric cancer. Oncol Rep. 2013; 29: 993–1000. [PMID: 23254865 DOI: 10.3892/or.2012.2200]

75. Liu XJ, Chen ZF, Li HL, Hu ZN, Liu M, Tian AP, Zhao D, Wu J, Zhou JN, Qiao L. Interaction Between cyclooxygenase-2, Snail, and E-cadherin in Gastric Cancer Cells. World J Gastroenterol. 2013 Oct 7;19(37):6265-71. [PMID: 24115825 DOI: 10.3748/wjg.v19.i37.6265]

76. Kam Y, Quaranta V. Cadherin-bound betacatenin feeds into the Wnt pathway upon adherens junctions dissociation: evidence for an intersection between beta-catenin pools. PLoS ONE. 2009; 4: e4580. [PMID: 19238201 DOI: 10.1371/journal.pone.0004580]

77. Taelman VF, Dobrowolski, R, Plouhinec JL, Fuentealba LC, Vorwald PP, Gumper I, Sabatini DD, De Robertis EM. Wnt signaling requires sequestration of glycogensynthase kinase 3 inside multivesicular endosomes. Cell 2010 Dec 23;143(7):1136-48. [PMID: 21183076 DOI: 10.1016/j.cell.2010.11.034]

78. Fukumoto Y, Shintani Y, Reynolds AB, Johnson KR, Wheelock MJ. The regulatory or phosphorylation domain of p120 catenin controls E-cadherin dynamics at the plasma membrane. Exp Cell Res. 2008; 314: 52–67. [PMID: 17719574 DOI: 10.1016/j.yexcr.2007.07.024]

79. Park JI, Kim SW, Lyons JP, Ji H, Nguyen HT, Cho K, Barton MC, Deroo T, Vleminckx K, Moon RT, McCrea PD. Kaiso/p120-catenin and TCF/beta-catenin complexes coordinately regulate canonical Wnt gene targets. Dev Cell. 2005; 8: 843–54. 80. [PMID: 15935774 DOI: 10.1016/j.devcel.2005.04.010]

80. Anastasiadis PZ, Reynolds AB. Regulation of Rho GTPases by p120-catenin. Curr Opin Cell Biol. 2001; 13: 604–10. [PMID: 11544030 DOI: 10.1016/s0955-0674(00)00258-1]

81.Kim SW, Park JI, Spring CM, Sater AK, Ji H, Otchere AA, Daniel JM, McCrea PD. Noncanonical Wnt signals are modulated by the Kaiso transcriptional repressor and p120-catenin. Nat Cell Biol. 2004; 6: 1212–20. [PMID: 15543138 DOI: 10.1038/ncb1191]

82.Spring CM, Kelly KF, O'Kelly I, Graham, Crawford HC, Daniel JM. The catenin p120ctn inhibits Kaiso-mediated transcriptional repression of the beta-catenin/TCF target gene matrilysin. Exp Cell Res. 2005; 305: 253–65. [PMID: 15817151 DOI: 10.1016/j.yexcr.2005.01.007]

83. Rossi T , Tedaldi G , Petracci E , Khouzam RA, Ranzani GN , Morgagni P, Saragoni L, Monti M, Calistri D, Ulivi P, Molinari C. E-cadherin Downregulation and microRNAs in Sporadic Intestinal-Type Gastric Cancer. Int J Mol Sci . 2019 Sep 10;20(18):4452. [PMID: 31509966 DOI: 10.3390/ijms20184452]

84. Matsushima K, Isomoto H, Inoue N, Nakayama T, Hayashi T, Nakayama M, Nakao K, Hirayama T, Kohno S. MicroRNA signatures in Helicobacter pylori-infected gastric mucosa. Int. J. Cancer 2011,128,361–370. [PMID: 20333682 DOI: 10.1002/ijc.25348]

85. Zhou X, Xia Y, Li L, Zhang G. MiR-101 inhibits cell growth and tumorigenesis of Helicobacter pylorirelated gastric cancer by repression of SOCS2.Cancer Biol. Ther.2015,16, 160–169. [PMID: 25561270 DOI: 10.4161/15384047.2014.987523]

86. Varambally S, Cao Q, Mani R.S, Shankar S, Wang X, Ateeq B, Laxman, B, Cao X, Jing X, Ramnarayanan K, Brenner JC, Yu J, Kim JH, Han B, Tan P, Kumar-Sinha C, Lonigro RJ, Palanisamy N, Maher CA, Chinnaiyan AM. Genomic Loss of microRNA-101 Leads to Overexpression of Histone MethyltransferaseEZH2 in Cancer.Science2008,322, 1695–1699. [PMID: 19008416 DOI: 10.1126/science.1165395]

87. Guo F, Cogdell D, Hu L, Yang D, Sood AK, Xue F, Zhang W. miR-101 suppresses the epithelial-to-mesenchymal transition by targeting ZEB1 and ZEB2 in ovarian carcinoma. Oncol. Rep.2014,31, 2021–2028. [PMID: 24677166 DOI: 10.3892/or.2014.3106]

88. Dong X, Liu Y. Expression and significance of miR-24 and miR-101 in patients with advanced gastric cancer. Oncol. Lett.2018,16, 5769–5774. [PMID: 30405753 DOI: 10.3892/ol.2018.9324]

89. Imamura T, Komatsu S, Ichikawa D, Miyamae M, Okajima W, Ohashi T, Kiuchi J, Nishibeppu K, Kosuga T, Konishi H, Shiozaki A, Okamoto K, Fujiwara H, Otsuji E. Low plasma levels of miR-101 are associated with tumor progression in gastriccancer. Oncotarget 2017,8, 106538. [PMID: 29290969 DOI: 10.18632/oncotarget.20860]

89. Kurashige J, Kamohara H, Watanabe M, Hiyoshi Y, Iwatsuki M, Tanaka Y, Kinoshita K, Saito S, Baba Y, Baba H. MicroRNA-200b regulates cell proliferation,invasion, and migration by directly targeting ZEB2 in gastric carcinoma. Ann Surg Oncol 2012;19:S656–S664. [PMID: 22311119 DOI:10.1245/s10434-012-2217-6]

90. Carvalho J, van Grieken NC, Pereira PM, Sousa S, Tijssen M, Buffart TE, Diosdado B, Grabsch H, Santos MAS, Meijer G, Seruca R, Carvalho B, Oliveira C. Lack of microRNA-101 causes E-cadherin functional deregulation through EZH2 upregulation in intestinal gastric cancer. J Pathol 2012;228:31–44. [PMID: 22450781 DOI: 10.1002/path.4032]

91. Tsai MM, Huang HW, Wang CS, Lee KF, Tsai CY, Lu PH, Chi HC, Lin YH, Kuo LM, Lin KH. MicroRNA-26b inhibits tumor metastasis by targeting the KPNA2/c-jun pathway in human gastric cancer. Oncotarget2016,7, 39511–39526. [PMID: 27078844 DOI: 10.18632/oncotarget.8629]

92. Deng M, Zhang R, He Z, Qiu Q, Lu X, Yin J, Liu H, Jia X, He Z. TET-Mediated Sequestration ofmiR-26 Drives EZH2 Expression and Gastric Carcinogenesis.Cancer

Res.2017,77, 6069–6082. [PMID: 28923852 DOI: 10.1158/0008-5472.CAN-16-2964]

93. Tang H, Deng M, Tang Y, Xie X, Guo J, Kong Y, Ye F, Su Q, Xie, X. miR-200b and miR-200c as Prognostic Factors and Mediators of Gastric Cancer Cell Progression. Clin. Cancer Res. 2013, 19, 5602–5612. [PMID: 23995857 DOI: 10.1158/1078-0432.CCR-13-1326]

94. Huang, ZS, Guo XW, Zhang G, Liang, LX, Nong, B. The Diagnostic and Prognostic Value of miR-200c in Gastric Cancer: A Meta-Analysis. Dis. Markers 2019, 2019, 1–9. [PMID: 31089400 DOI: 10.1155/2019/8949618]

95. Feng X, Wang Z, Fillmore R, Xi Y. MiR-200, a new star miRNA in human cancer. Cancer Lett. 2014, 344, 166–173. [PMID: 24262661 DOI: 10.1016/j.canlet.2013.11.004]

96. Chang L, Guo F, Huo B, Lv Y, Wang Y, Liu W. Expression and clinical significance of the microRNA-200 family in gastric cancer. Oncol. Lett. 2015, 9, 2317–2324. [PMID: 26137064 DOI: 10.3892/ol.2015.3028]

97. Cancer Genome Atlas Research Network. Comprehensive Molecular Characterization of Gastric Adenocarcinoma. Nature. 2014 Sep 11;513(7517):202-9. [PMID: 25079317 DOI: 10.1038/nature13480]

98. Cristescu R, Lee J, Nebozhyn M, Kim M, Ting JC, Wong SS, Liu J, Yue YG , Wang J, Yu K, Ye XS , Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, 1Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clini-cal outcomes. Nat Med 21(5):449–456. [PMID: 25894828 DOI: 10.1038/nm.3850]

99. Polom K, Marano L, Marrelli D, De Luca R, Roviello G, Savelli V, Tan P, Roviello F (2018) Meta-analysis of microsatellite insta-bility in relation to clinicopathological characteristics and overall survival in gastric cancer. Br J Surg 105(3):159–167. [PMID: 29091259 DOI: 10.1002/bjs.10663]

100. Polom K, Marrelli D, Roviello G, Pascale V, Voglino C, Rho H, Marini M, Macchiarelli R, Roviello F (2017) Molecular key to understand the gastric cancer biology in elderly patients—the role of microsatellite instability. J Surg Oncol 115(3):344–350. [PMID: 27859280 DOI: 10.1002/jso.24513]

101. Polom K, Marrelli D, Pascale V, Ferrara F, Voglino C, Marini M, Roviello F (2017) The pattern of lymph node metastases in micro-satellite unstable gastric cancer. Eur J Surg Oncol 43(12):2341– 2348. [PMID: 28942235 DOI: 10.1016/j.ejso.2017.09.007]

102. Smyth EC, Wotherspoon A, Peckitt C, Gonzalez D, Hulkki-Wilson S, Eltahir Z, Fassan M, Rugge M, Valeri N, Okines A, Hewish M, Allum W, Stenning S, Nankivell M, Langley R, Cunningham D (2017) Mismatch repair deficiency, microsatellite instability, and survival: an exploratory analysis of the medical research council adjuvant gastric infusional chemotherapy (MAGIC) trial. JAMA Oncol 3(9):1197–1203. [PMID: 28241187 DOI: 10.1001/jamaoncol.2016.6762]

103. Garattini SK, Basile D, Cattaneo M, Fanotto V, Ongaro E, Bonotto M, Negri FV, Berenato R, Ermacora P, Cardellino GG, Giovannoni M, Pella N, Scartozzi M, Antonuzzo L, Silvestris N, Fasola G, Aprile G (2017) Molecular classifications of gastric cancers: novel insights and possible future applications. World J Gastrointest Oncol 9(5):194–208. [PMID: 28567184 DOI: 10.4251/wjgo.v9.i5.194]

104. Camargo MC, Kim WH, Chiaravalli AM, Kim KM, Corvalan AH, Matsuo K, Yu J, Sung JJ, Herrera-Goepfert R, Meneses-Gonzalez F, Kijima Y, Natsugoe S, Liao LM, Lissowska J, Kim S, Hu N, Gonzalez CA, Yatabe Y, Koriyama C, Hewitt SM, Akiba S, Gulley ML, Taylor PR, Rabkin CS (2014) Improved survival of gastric cancer with tumour Epstein-Barr virus positivity: an international pooled analysis. Gut 63(2):236–243. [PMID: 23580779 DOI: 10.1136/gutjnl-2013-304531]