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Immunoexpression of E-cadherin, CD44 and Claudin 7 in gastric adenocarcinomas

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Abstract

Gastric adenocarcinomas represent frequent malignant tumors in the digestive tract, with a high and constant mortality rate in last decades. The disturbance of the adhesion molecules expression, which normally is essential in maintaining epithelial homeostasis, has a critical role in the initiation and progression of tumors. In this study, we analyzed the immunoexpression of E-cadherin, cluster of differentiation 44 (CD44), and Claudin 7 in 58 cases of gastric adenocarcinomas, in relation to the histopathological parameters of the lesions' aggressiveness. Increased E-cadherin immunoexpression was observed in tubular adenocarcinomas, those of low grade and in stages I–III. CD44 presented high scores in discohesive, hepatoid, tubular, and tubulopapillary adenocarcinomas, those of high grade and in advanced stages. Claudin 7 associated increased scores for tubular, tubulopapillary and micropapillary tumors, those of low grade and mainly in stage I. The markers used in the study can be useful for assessing the aggressiveness of gastric adenocarcinomas, in the context of specific adapted therapy.

Keywords: E-cadherin, CD44, Claudin 7, gastric adenocarcinomas.

Introduction

Gastric adenocarcinomas are among the most frequent malignant tumor lesions encountered in the digestive tract and represent approximately 95% of the malignant tumors with this localization [1]. Gastric adenocarcinomas are responsible for 8.8% of the deaths caused by cancer worldwide and continue to represent a major problem globally [2, 3].

The multifactorial etiology and heterogeneous clinico-pathological character are determinants for the aggressive biological behavior of the lesions [4, 5]. Although the screening programs have decreased the incidence of the lesions, and the methods of diagnosis and treatment of gastric adenocarcinomas have been improved in recent years, the prognosis remains reserved and the mortality rate high. In the context of frequently discrete symptoms, most patients are diagnosed in advanced stages [5, 6]. Therefore, there is a permanent concern for improving the prognosis of patients, existing numerous studies that have analyzed the biomolecular mechanisms involved in tumor initiation and progression. Among these, an important role in recent years was occupied by the intercellular adhesion system, which is regulated by numerous classes of proteins, including cadherins, claudins and cluster of differentiation 44 (CD44) [7–10].

E-cadherin and its role in carcinogenesis have been intensively studied in recent years, both in the context of the alteration of intercellular adhesion and of the epithelial and mesenchymal phenotype [11, 12]. Numerous studies conducted on gastric adenocarcinomas have concluded that E-cadherin immunoexpression is strongly associated with the type, tumor grade and tumor stage, being considered an efficient prognostic marker [13–15]. On the contrary, other studies reported the absence of significant associations between the E-cadherin expression and histopathological (HP) parameters [16, 17].

CD44 is a transmembrane glycoprotein expressed on the surface of various cells and described as an important marker involved in proliferation, differentiation, cell migration and angiogenesis [18, 19]. Although there are numerous studies that support the association of CD44 expression with the initiation and progression of gastric cancer and the fact that it has an important role in the diagnosis and prognosis of the disease, there are authors who have not identified a notable association [18, 20].

Claudin 7 plays a crucial role in maintaining epithelial integrity, recent studies indicating the presence of immuno-expression in different types of carcinomas [21, 22]. At the same time, the studies that analyzed Claudin 7 immuno-expression in gastric tumors are limited and inconsistent [21, 22].

In the context in which the most of studies that analyzed the intercellular adhesion proteins in gastric adenocarcinomas were conducted on the Lauren classification, the role of E-cadherin, CD44 and Claudin 7 in gastric carcinogenesis and the utility for the tumors assessment remains controversial, research in this direction being of actuality.

Aim

In this study, we analyzed the immunoexpression of E-cadherin, CD44 and Claudin 7 in relation to the HP parameters of the gastric adenocarcinoma aggressiveness.

Materials and Methods

The study included a number of 58 cases of gastric adenocarcinomas from patients admitted to the Departments of General Surgery, Emergency County Hospital, Craiova, Romania, over a period of four years (2017–2020), and which were diagnosed in the Department of Pathology of the same Hospital.

The biological material was represented by surgical specimens of total gastrectomy fixed in 10% neutral buffered formalin, processed by the usual technique of paraffin embedding and standard stained with Hematoxylin–Eosin (HE). The inclusion criterion in the study was the diagnosis of primitive gastric adenocarcinoma, without other tumoral history or chemo-, radio- and immunotherapy.

The classification of lesions was done in accordance with the latest classification of the digestive system tumors, developed by the *World Health Organization* (WHO) *Working Group* [23]. The HP study followed the main aggressiveness parameters of gastric adenocarcinomas represented by the HP type, tumor grade, and the tumor stage in relation to specific markers of intercellular adhesion represented by E-cadherin, CD44 and Claudin 7 (Table 1).

Table 1 – ***Antibodies used and immunostaining data***

Antibody	Clone	Dilution	Pretreatment	External positive control
E-cadherin	NCH 38	1:50	Boiling in citrate solution (HIER), pH 6	Mammary gland
CD44	DF1485	1:50	Boiling in citrate solution (HIER), pH 6	Tegument
Claudin 7	Polyclonal	1:150	Boiling in citrate solution (HIER), pH 6	Kidney

CD44: Cluster of differentiation 44; HIER: Heat-induced epitope retrieval.

For the immunohistochemical (IHC) reactions, 3 μm serial sections were obtained from the paraffin blocks, that were mounted on with poly-L-lysine coated slides. After deparaffinization in xylene, the sections were rehydrated and exposed to endogenous enzyme blocking with hydrogen peroxide, nonspecific site blocking with bovine serum albumin (BSA), and antigen retrieval by microwaving for 20 minutes, according to the protocols indicated by the manufacturers. The working system for the polymeric amplification was represented by EnVision™ FLEX+ System (code K8002, Dako). The visualization of reactions was realized with 3,3'-Diaminobenzidine (DAB) tetrahydrochloride chromogen. In this study were used positive external controls to validate the reactions. Finally, the sections were counterstained with Hematoxylin.

For the semiquantitative evaluation of IHC reactions, we used a final staining score (FSS) obtained by multiplying the percentage of marked cells on a 40× microscopic field (MF) by the intensity of the reaction. There were analyzed 10 MFs for each case. The score assigned for the number of marked cells was 1 (5–25% cells), 2 (26–50% cells), 3 (>50% cells), while the score for the intensity of reactions was 1 (weak), 2 (moderate) and 3 (high). FSS had values between 1–9, scores 1–4 being considered low, 6–9 high. The positivity threshold value was given by the presence of at least 5% immunostained tumor cells, below which the reactions were considered negative. The assessment of the reactions was done in parallel by two pathologists (OIC and AES), the results being later compared and adjusted. The images were obtained by using the Motic Panthera DL microscope, equipped with Motic Images Plus 3.0 ML software.

For the statistical analysis were used comparison tests represented by $\chi 2$ (*chi*-squared) and Pearson within the Statistical Package for the Social Sciences (SPSS) 10 software, the results being considered significant for values of $p<0.05$. In this study, for the calculation of average values and standard deviations, there were used numerical values of the obtained immunostainings for all the cases, including the negative ones.

In the scientific research, the ethical aspects were respected, based on the informed consent of the patients, the study being approved by the Local Ethics Commission (No. 151/24.09.2021).

Results

In this study, there were investigated 58 patients diagnosed with gastric adenocarcinoma, with the age between 40 and 86 years, with an average diagnosis age of 68.3±10.4 years, the majority being of male gender (65.5%). Most cases were represented by tubular type adenocarcinomas (39.7%) and poorly cohesive carcinomas with signet-ring cell (PCC-SRC) (15.5%), most being of high grade (60.3%) and classified in tumor stage III (51.7%) (Table 2).

Table 2 – ***Cases distribution according to the investigated clinicopathological parameters***

Parameter	Variable	No. of cases
Age [years]	<50	2
	50–70	31
	>70	25
Gender	Male	38
	Female	20
Histopathological type	Tubular	23
	Tubulopapillary	3
	PCC-NOS	6
	PCC-SRC	9
	Mixed	6
	Mucinous	7
	Micropapillary	2
	Hepatoid	2
Tumor grade	Low	23
	High	35
Tumor stage	I	4
	II	18
	III	30
	IV	6

F: Female; M: Male; PCC-NOS: Poorly cohesive carcinomas non-signet-ring cell; PCC-SRC: Poorly cohesive carcinomas with signet-ring cell.

Immunoexpression of E-cadherin

E-cadherin was identified in 81% of investigated gastric adenocarcinomas, the negative cases belonging to poorly cohesive carcinomas non-signet-ring cell (PCC-NOS) type, PCC-SRC, mucinous and hepatoid carcinomas, of high grade and in advanced stages. The reactions were identified in tumor cells membrane. For the entire analyzed group, the average number of labeled cells was 19.2±20.5, the reactions presented variable intensity, the FSS having an average value of 2.1.

In relation to the type of adenocarcinomas, the strongest reactions were observed in the case of tubular type adenocarcinomas, with a number of marked cells of 39.1±18.8, variable intensity, and an average FSS of 4 (Figure 1A). These were followed by mixed, tubulopapillary and micropapillary types of adenocarcinomas, with a positive cell number of 14.5±6.1, 11.6±5.7 and 7.5±3.5, with weak and moderate intensity, and a mean FSS of 1.8, 1.3 and 1.5, respectively (Figure 1B). PCC-SRC and PCC-NOS presented a positive cell number of 4.8±3.2 and 4.1±3.7, weak intensity and a mean FSS of 0.7 and 0.6 (Figure 1C). In the case of mucinous type adenocarcinomas, the number of labeled cells was 1.7±2.9, the reactions being weak and with a mean FSS of 0.2 (Table 3) (Figure 1D).

Depending on the tumor grade, we observed that the low-grade lesions presented higher values, respectively a number of labeled cells of 38±20.4, with variable intensity and an average score of 4 (Figure 1E). Comparatively, the high-grade tumors presented an immunopositive cell number of 6.9±6.6, with weak and moderate intensity and a mean FSS of 0.8 (Table 3) (Figure 1F).

Table 3 – ***E-cadherin, CD44 and Claudin 7 immunoexpression in relation to HP parameters***

Parameter / *p*-value		E-cadherin	CD44	Claudin 7
HP type	Tubular	4	4	4.4
	Tubulopapillary	1.3	3.3	4.6
	PCC-NOS	0.6	4.6	0.8
	PCC-SRC	0.7	7.6	0.7
	Mixed	1.8	2	3
	Mucinous	0.2	1.8	4
	Micropapillary	1.5	1.5	5
	Hepatoid	0	7.5	3
	p-value	0.001	0.006	0.049
Tumor grade	Low	4	4.6	4.9
	High	0.8	3.8	2.2
	p-value	0.001	0.351	0.008
Tumor stage	I	5	6	7
	II	2.2	4.3	2.9
	III	1.9	4.2	3.1
	IV	1	2.3	2.6
	p-value	0.056	0.869	0.158

HP: Histopathological; PCC-NOS: Poorly cohesive carcinomas non-signet-ring cell; PCC-SRC: Poorly cohesive carcinomas with signet-ring cell.

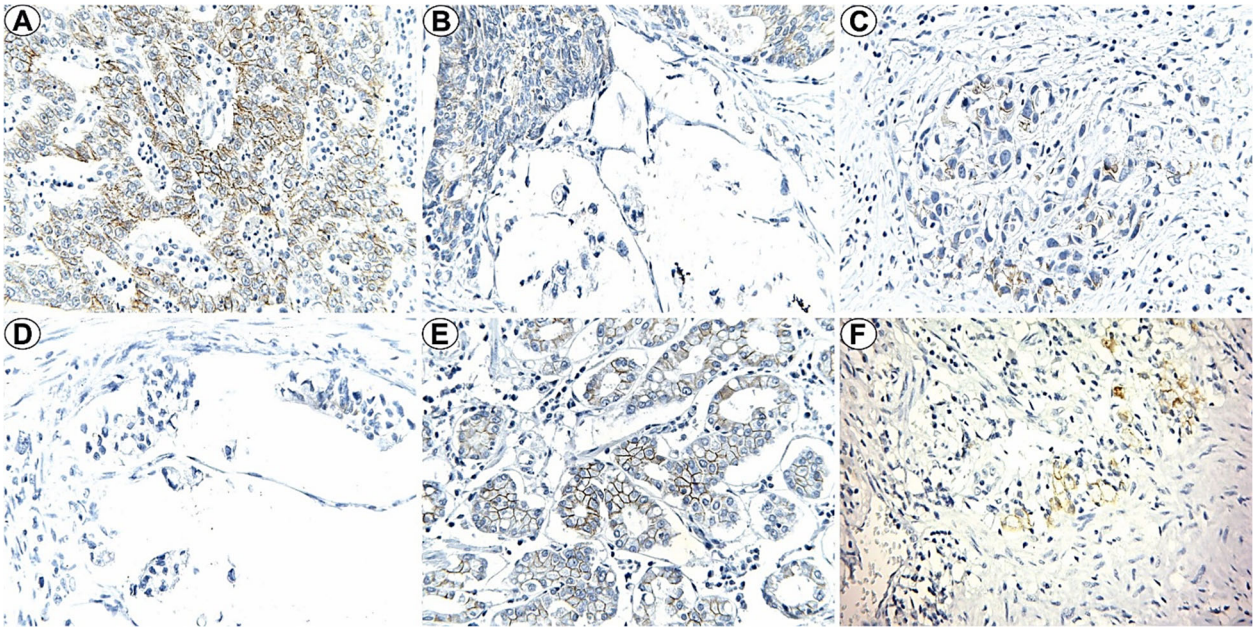

Figure 1 – ***Gastric adenocarcinoma, E-cadherin immunostaining (×40): (A) Tubular type; (B) Mixed type; (C) PCC-NOS type; (D) Mucinous type; (E) Low-grade adenocarcinoma; (F) High-grade adenocarcinoma. PCC-NOS: Poorly cohesive carcinomas non-signet-ring cell.***

For tumor stage I, the number of marked cells was 48±29.7, the intensity of reactions was variable, and average FSS of 5. In stages II, III and IV, the number of positive cells were 20.7±18.9, 16.5±18.1 and 19.1±17.7, with intensity of the reactions predominantly weak and moderate, and the average FSS values of 2.2, 1.9, and 1, respectively (Table 3).

The statistical analysis of E-cadherin immunoexpression revealed a significant association in relation to the HP type (p=0.001, χ^2 test) and the tumor grade (p=0.001, χ^2 test), as well as values at the limit of significance in relation to the tumor stage (p=0.056, χ^2 test), the highest values of E-cadherin scores being observed in tubular carcinomas, those of low grade and in stage I–III (Figure 2, A–C).

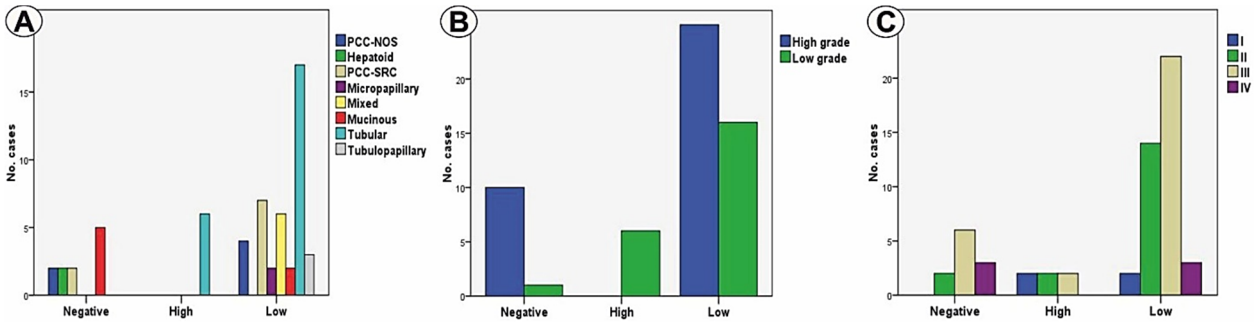

Figure 2 – ***Cases distribution depending on E-cadherin scores and histopathological type (A), tumor grade (B), and tumor stage (C). PCC-NOS: Poorly cohesive carcinomas non-signet-ring cell; PCC-SRC: Poorly cohesive carcinomas with signet-ring cell.***

CD44 immunoexpression

CD44 was identified in 94.8% cases of gastric adenocarcinomas, being identified a negative immunoreaction in three cases (mixed, mucinous, and tubular types), of high grade and in advanced stages tumors. The immunoreactions were identified in the membrane and apical cytoplasm of tumor cells, as well as in some stromal elements represented by macrophages, fibroblasts, and lymphocytes. For the entire analyzed group, the average number of marked cells was 39±21.9, the reactions presented variable intensity, the FSS having an average value of 4.1.

Regarding the HP type, strong reactions were identified in the case of PCC-SRC, with a number of positive cells of 70.5±5.2, moderate and high intensity and a mean FSS of 7.6, these being followed by the hepatoid type, with a number of 52.5±10.6 of marked cells, high intensity and a mean final score of 7.5 (Figure 3, A and B). The immunostaining was moderate and high in PCC-NOS, with 40.8±7.3 positive tumor cells, and FSS of 4.6. For tubular and tubulopapillary tumors, the values were 40±20.1 and 23.3±7.6, with variable or moderate/high intensity and mean FSS of 4 and 3.3, respectively (Figure 3C). In the mixed type was identified a number of 21.6±14.0 of labeled cells, with weak and moderate intensity and the FSS was 2. For mucinous and micropapillary adenocarcinomas were observed a number of 20±10.4 and 10±7 positive cells, with weak/moderate intensity and average FSS values of 1.8 and 1.5, respectively (Table 3) (Figure 3D).

Low-grade gastric adenocarcinomas presented 44.3±17.6 positive tumor cells, with variable intensity, and a mean FSS of 4.6 (Figure 3E). In comparison, the high-grade adenocarcinomas had a number of immunopositive cells of 35.5±23.9, with variable intensity, and a mean FSS value of 3.8 (Table 3) (Figure 3F).

In relation to the tumor stage, the adenocarcinomas in both stage I and stage II had a higher number of marked cells, 53.7±28.6 and 41.1±20.2, respectively, the intensity being moderate and high, respectively variable, and the average FSS were 6 and 4.3, respectively. In contrast, in stages III and IV, the number of immunomarked cells were 38.3±21.7 and 26.6±21.6, with variable intensity, respectively weak and moderate, and the mean FSS value of 4.2 and 2.3, respectively (Table 3).

The statistical analysis of CD44 immunoexpression indicated a significant association in relation to the HP type (p=0.006, χ^2 test), without other associations with the tumor grade (p=0.351, χ^2 test) and tumor stage (p=0.869, χ^2 test). Thus, the carcinomas that associated high CD44 scores were represented by numerous high-grade tumors, in advanced stages, of discohesive and hepatoid types, but also in tubular or tubulopapillary types (Figure 4, A–C).

Claudin 7 immunoexpression

Claudin 7 was identified in 93.1% of the studied gastric adenocarcinomas, the negative cases being represented by PCC-NOS and PCC-SRC, of high grade and in advanced stages. The reactions were identified in the tumor cell membrane. For the entire analyzed group, the average number of marked cells was 33±20.3, the reactions presented variable intensity and the FSS mean value was 3.2.

In relation to the HP type, in tubular and tubulopapillary adenocarcinomas, the number of marked cells was 45.2±17.9 and 40±10, with variable intensity, respectively moderately/high and mean FSS of 4.4 and 4.6, respectively (Figure 5A). Micropapillary and mucinous types had a number of positive cells of 40±7 and 39.2±8.8, weak/moderate intensity, respectively variable and mean FSS of 5 and 4 (Figure 5B). In hepatoid and mixed types, the immunomarked cells were in number of 40±14.1 and 38.3±6, with weak/moderate intensity and average FSS of 3 (Figure 5C). In the case of PCC-NOS and PCC-SRC, the number of labeled cells was 6.6±6 and 5.5±3.9, with weak/moderate intensity and average FSS of 0.8 and 0.7, respectively (Table 3) (Figure 5D).

Reported to the tumor grade, we observed that low-grade adenocarcinomas presented a number of marked cells of 47.8±16.7, with variable intensity and an average score of 4.9 (Figure 5E). Comparatively, for high-grade adenocarcinomas was identified a reduced number of immunopositive cells, respectively 23.2±16.3, with variable intensity and a mean FSS of 2.2 (Table 3) (Figure 5F).

In relation to the stage of gastric adenocarcinomas, for stage I was observed a superior number of marked cells compared to the other tumor stages, respectively 60±21.2, moderate and high intensity and mean FSS value of 7. Stages II and III presented a number of positive cells of 31.1±18.7, respectively 31.3±20.2, the intensity of the reactions was variable and the average FSS of 2.9 and 3.1, respectively. In gastric adenocarcinomas stage IV was identified a number of 29.1±16.2 marked cells, variable intensity and FSS value of 2.6 (Table 3).

The statistical analysis of Claudin 7 immunoexpression revealed a significant association in relation to the HP type (p=0.049, χ^2 test) and tumor grade (p=0.008, χ^2 test), without relation to the tumor stage (p=0.158, χ^2 test). High scores of Claudin 7 were associated with tumors with tubular/papillary/micropapillary architecture, of low grade and mostly in stage I (Figure 6, A–C).

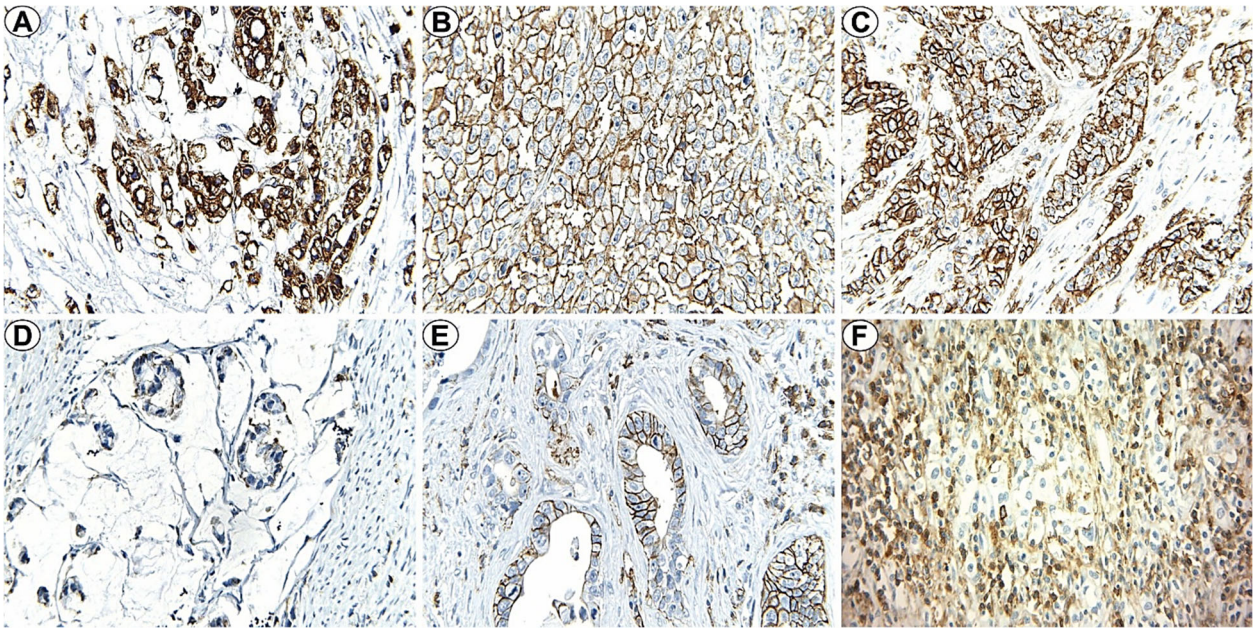

Figure 3 – ***Gastric adenocarcinoma, CD44 immunostaining (×40): (A) PCC-SRC type; (B) Hepatoid type; (C) Tubular type; (D) Mucinous type; (E) Low-grade adenocarcinoma; (F) High-grade adenocarcinoma. CD44: Cluster of differentiation 44; PCC-SRC: Poorly cohesive carcinomas with signet-ring cell.***

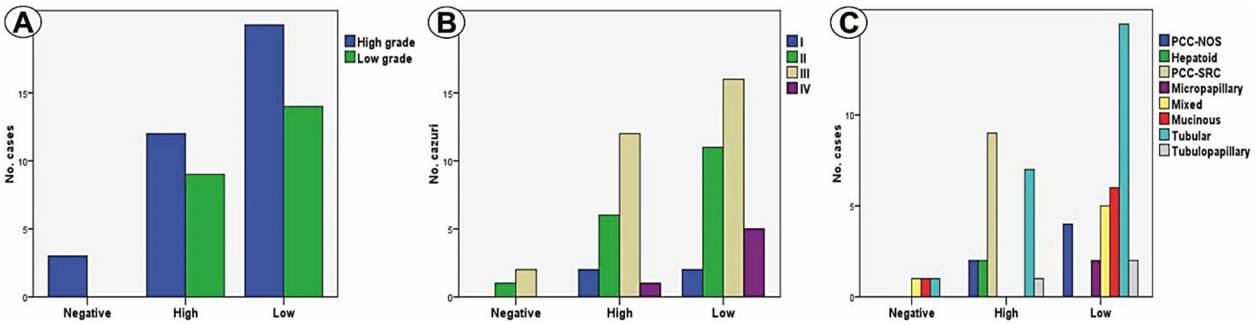

Figure 4 – ***Cases distribution depending on CD44 scores and tumor grade (A), tumor stage (B), and histopathological type (C). CD44: Cluster of differentiation 44; PCC-NOS: Poorly cohesive carcinomas non-signet-ring cell; PCC-SRC: Poorly cohesive carcinomas with signet-ring cell.***

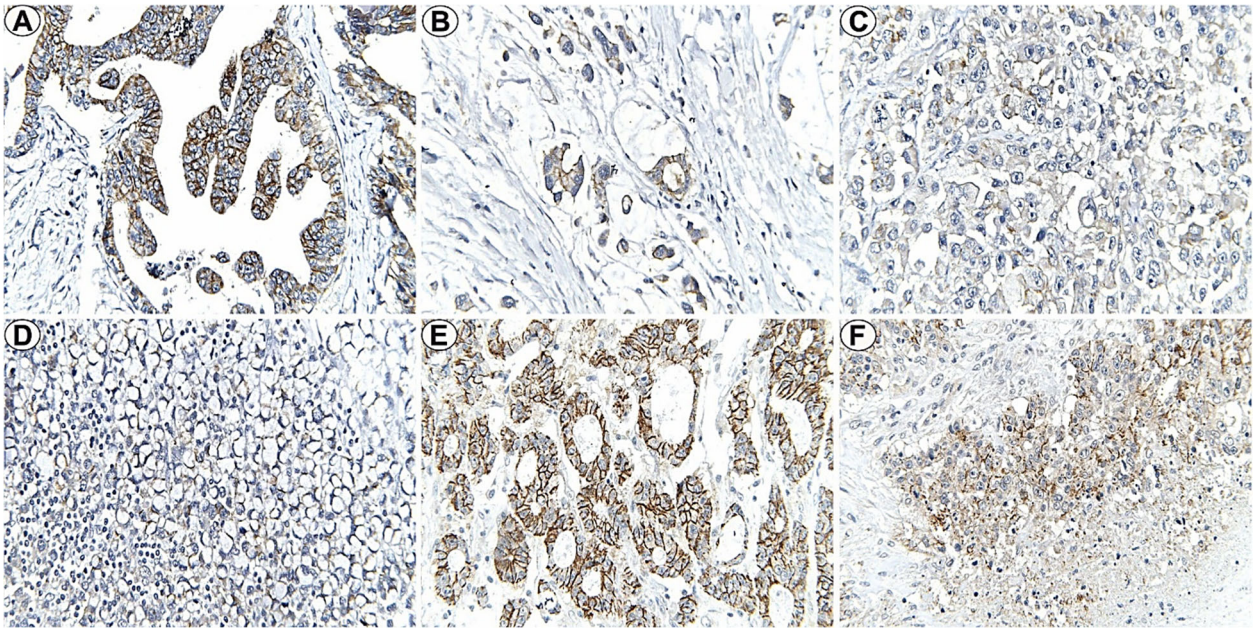

Figure 5 – ***Gastric adenocarcinoma, Claudin 7 immunostaining (×40): (A) Tubulopapillary type; (B) Mucinous type; (C) Hepatoid type; (D) PCC-SRC type; (E) Low-grade adenocarcinoma; (F) High-grade adenocarcinoma. PCC-SRC: Poorly cohesive carcinomas with signet-ring cell.***

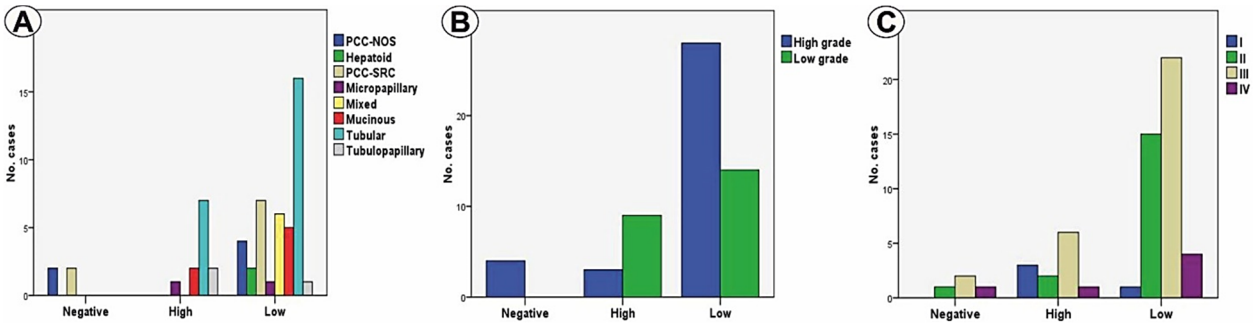

Figure 6 – ***Cases distribution depending on Claudin 7 scores and histopathological type (A), tumor grade (B), and tumor stage (C). PCC-NOS: Poorly cohesive carcinomas non-signet-ring cell; PCC-SRC: Poorly cohesive carcinomas with signet-ring cell.***

Following the analysis of the percentage values for the investigated markers, it was observed a positive linear correlation between E-cadherin and Claudin 7 (p<0.001, Pearson's test), a positive linear relation between E-cadherin and CD44 (p=0.076, Pearson's test), and a negative linear relation between Claudin 7 and CD44 (p=0.377, Pearson's test).

Discussions

Adhesion molecules are surface proteins that promote cellular interaction with the role of maintaining epithelial integrity. Consequently, changes in the expression of these surface proteins alters the epithelial homeostasis. Multiple studies, including from the experience of the study authors, have observed modified immunoexpression of the adhesion molecules in various neoplastic processes, and this aspect has become an essential research direction with potential of applicability in targeted oncological treatment [7, 11, 24, 25].

E-cadherin is a transmembrane adhesion molecule that is part of the cadherin system and plays important roles in intercellular adhesion of the epithelium, in maintaining cell polarity and epithelial stratification [3, 13, 17]. Adherent junctions between cells based on E-cadherin are fundamental for the tissue integrity, thus decreased immunoexpression is associated with tumor initiation, progression, and metastasis [3, 17, 26]. Numerous studies have been conducted to analyze the immunoexpression of E-cadherin in different malignant tumor lesions, including gastric adenocarcinomas [16, 17, 26, 27]. While some authors indicated that the immunoexpression of E-cadherin can be considered an important marker in the evaluation of the patient's prognosis, and its decrease was associated with tumor progression and unfavorable prognosis, other studies did not support these results [16, 17, 26, 27].

Most of the studies that analyzed the E-cadherin immunoexpression related to the HP type of gastric adenocarcinomas indicated a high percentage of labeled cells in the cases of tubular type in contrast to the other types. Karayiannakis *et al.* concluded that the diffuse and undifferentiated type of adenocarcinomas had a decreased immunoexpression, in contrast to the types of gastric adenocarcinomas with glandular pattern where it was well expressed [28]. Thus, was reported that the lowest immunoexpression of E-cadherin was in diffuse and mixed types, while in intestinal type adenocarcinomas the expression was maximum [28]. Arévalo *et al.* demonstrated in a comparative study, a reduction of E-cadherin immunoexpression in signet-ring cell adenocarcinomas, unlike the other types, thus being explained the superior capacity of infiltration [29]. In a similar study was identified a low immunoexpression in micropapillary adenocarcinomas [30]. The study reported high immunoexpression of E-cadherin in the papillary and tubular type, and a considerable decrease to absence was described in the other HP types of gastric adenocarcinoma [31]. The absence of the immunostaining was observed by other authors in signet-ring cell and mucinous adenocarcinomas [31].

In our study, except for the tubular type of gastric adenocarcinoma, in all other types, E-cadherin scores were low.

Multiple studies have reported that E-cadherin immunoexpression decreases in high-grade tumors, aspects that were also identified in this study. Thus, was identified an increased immunoexpression in low-grade adenocarcinomas and decreased or absent in high-grade tumors, E-cadherin immunoexpression being considered by some authors as a marker for tumor differentiation [17, 28, 32, 33].

At the same time, some studies have reported a percentage much reduced of marked tumor cells in advanced stages of adenocarcinomas [17, 31]. Torabizadeh *et al.* reported a notable difference between stage III to stages I and II [34], and Xia *et al.* indicated a marked decrease of E-cadherin in adenocarcinomas in stage II, III and IV compared to stage I [35]. In our study, the lowest values of E-cadherin were identified in most stage II–IV carcinomas.

CD44 is a transmembrane multistructural and multifunctional adhesion molecule, involved in cell proliferation, differentiation, migration, and angiogenesis [36, 37]. This is the main surface receptor for hyaluronic acid, a major component of the extracellular matrix, an important interaction for the connection between epithelial cells and the underlying connective tissue [36].

Most studies indicated that the CD44 immunoexpression is a negative prognostic marker for gastric adenocarcinomas [36–38], although there are authors who do not support this aspect [20, 39]. The studies that analyzed CD44 immunoexpression in relation to the different HP types showed variable results. Thus, some authors reported a high immunoexpression in diffuse type adenocarcinomas compared to intestinal type [20, 40], while others revealed an increased immunoexpression predominantly in the intestinal type [41, 42].

Ahadi *et al.* reported high expression of CD44 in diffuse and mucinous types, followed by mixed, the lowest reactivity being observed in intestinal type [20]. Sanaat *et al.* found

no significant association between CD44 immunoexpression and the HP type of gastric adenocarcinomas [43]. The dual role of CD44 may contribute to the controversial results in the literature; thus, besides the stability given by the anchoring to the underlying matrix of the epithelia, the protein is a marker of normal and neoplastic stem cells, which participates in the active maintenance of the tumor population [44, 45]. On the other hand, the role of the inflammatory microenvironment associated with tumors, with elements positive for CD44, can contribute to the positive or negative evolution of the tumor process, an aspect that is not so investigated [46].

In our study, we found consistent CD44 immunostaining in discohesive and hepatoid aggressive carcinomas, but also in some tubular carcinomas, of high grade and advanced stages.

Studies that analyzed CD44 immunoexpression indicated a significant association with the tumor grade in gastric adenocarcinomas. Increased immunoexpression was identified in poorly differentiated gastric adenocarcinomas compared to well and moderately differentiated ones [20, 37, 38, 40]. However, the results obtained by Dhingra *et al.* contradicts this statement [47].

In some studies, the decrease or loss of CD44 immunoexpression was identified in early stages, while increased immunoexpression was associated with advanced stages [38, 47, 48]. Tongtawee *et al.* reported an increased immunostaining of tumor cells in stages III and IV compared to stages I and II [49]. Jian-Hui *et al.* reported a positive CD44 immunoexpression in stage IV compared to the other stages [50], a result also confirmed by Ghaffarzadehgan *et al.* [42].

In our study, we found no association of CD44 scores with tumor stage and grade.

Claudins are adhesion proteins expressed on the surface of cells with a role in the consolidation of epithelial cells. The decrease in the immunoexpression of these markers is correlated with the destabilization of the epithelial tissue and the promotion of tumor progression [22, 51, 52].

In the last decade, the alteration of claudins expression has been studied in relation to different malignant tumor processes. Decreased immunoexpression of Claudin 7 has been described in breast, head, and neck tumors, and increased immunoexpression has been reported in stomach malignancies [51, 53, 54]. Claudin 7 appears to have predictive potential, with increased immunoexpression associated with low survival rate and poor prognosis [51, 53, 54]. However, there are relatively few studies that have addressed to the expression of Claudin 7 in an integrated way with the HP prognostic parameters of gastric adenocarcinomas.

Most of the studies that analyzed the immunoexpression of Claudin 7 in relation to the gastric adenocarcinomas' HP types, reported an increased immunoexpression in intestinal type, followed by diffuse type, the lowest immunoexpression being described in mixed type [54]. Other studies have observed the decrease of Claudin 7 expression especially in the diffuse type, when compared to the intestinal one [55, 56].

Decreased immunoexpression of Claudin 7 has been reported in poorly differentiated gastric adenocarcinomas [55], an aspect also observed in our study.

The statistical analysis regarding the immunoexpression of Claudin 7 in gastric adenocarcinomas in relation to the tumor stage of the disease remains controversial. Park *et al.* concluded that Claudin 7 immunoexpression was not associated with tumor stage [55], whereas Shinozaki *et al.* reported a significant association [56, 57].

In our study, Claudin 7 expression was associated with stage I, low grade tumors and mainly in case of tubular/papillary/micropapillary architecture.

Conclusions

In this study, we found differences in the expression of E-cadherin, CD44 and Claudin 7 in relation to the type, grade, and stage of gastric adenocarcinomas. While E-cadherin and Claudin 7 were associated with tumors with tubular architecture, of low grade and in early or intermediate stages, CD44 was associated mostly with tumors of high grade and in advanced stages, regardless of if the architecture was a discohesive, hepatoid or tubular one. The negative linear relation with Claudin 7 and positive linear relation with E-cadherin may suggest a dual role in gastric carcinogenesis for CD44. The markers used in this study can characterize the profile of the intercellular adhesion system, respectively they can contribute to the identification of aggressive lesions with evolutionary potential, to improve the patients' stratification criteria for therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Iwasaki K, Suda T, Takano Y, Ohno Y, Yamada E, Okazaki N, Takahashi K, Watanabe T, Makuuchi Y, Ota Y, Osaka Y, Seshimo A, Katsumata K, Tsuchida A. Postoperative outcomes of gastric carcinoma with lymphoid stroma. World J Surg Oncol, 2020, 18(1):102. https://doi.org/10.1186/s12957-020-01878-9 PMID: 32438924 PMCID: PMC7243312

[2] 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(5):E359–E386. https://doi.org/10.1002/ijc.29210 PMID: 25220842

[3] Qiu J, Sun M, Wang Y, Chen B. Identification of hub genes and pathways in gastric adenocarcinoma based on bioinformatics analysis. Med Sci Monit, 2020, 26:e920261. https://doi.org/10.12659/MSM.920261 PMID: 32058995 PMCID: PMC7034404

[4] Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz R. Gastric cancer: epidemiology, risk factors, classification, genomic characteristics and treatment strategies. Int J Mol Sci, 2020, 21(11):4012. https://doi.org/10.3390/ijms21114012 PMID: 32512697 PMCID: PMC7312039

[5] Jin X, Liu Z, Yang D, Yin K, Chang X. Recent progress and future perspectives of immunotherapy in advanced gastric cancer. Front Immunol, 2022, 13:948647. https://doi.org/10.3389/fimmu.2022.948647 PMID: 35844558 PMCID: PMC9284215

[6] Warsinggih, Syarifuddin E, Marhamah, Lusikooy RE, Labeda I, Sampetoding S, Dani MI, Kusuma MI, Uwuratuw JA, Prihantono, Faruk M. Association of clinicopathological features and gastric cancer incidence in a single institution. Asian J Surg, 2022, 45(1):246–249. https://doi.org/10.1016/j.asjsur.2021.05.004 PMID: 34090784

[7] Läubli H, Borsig L. Altered cell adhesion and glycosylation promote cancer immune suppression and metastasis. Front Immunol, 2019, 10:2120. https://doi.org/10.3389/fimmu.2019.02120 PMID: 31552050 PMCID: PMC6743365

[8] Jones MC, Zha J, Humphries MJ. Connections between the cell cycle, cell adhesion and the cytoskeleton. Philos Trans R Soc Lond B Biol Sci, 2019, 374(1779):20180227. https://doi.org/10.1098/rstb.2018.0227 PMID: 31431178 PMCID: PMC6627016

[9] Mendonsa AM, Na TY, Gumbiner BM. E-cadherin in contact inhibition and cancer. Oncogene, 2018, 37(35):4769–4780. https://doi.org/10.1038/s41388-018-0304-2 PMID: 29780167 PMCID: PMC6119098

[10] Na TY, Schecterson L, Mendonsa AM, Gumbiner BM. The functional activity of E-cadherin controls tumor cell metastasis at multiple steps. Proc Natl Acad Sci U S A, 2020, 117(11): 5931–5937. https://doi.org/10.1073/pnas.1918167117 PMID: 32127478 PMCID: PMC7084067

[11] Stepan AE, Pirici D, Bălăşoiu M, Novac MB, Drocaş AI, Ciurea RN, Stepan D, Gheonea DI, Simionescu CE. E-cadherin/CD44 immunophenotype in the epithelial–mesenchymal transition of bladder urothelial carcinomas. Rom J Morphol Embryol, 2015, 56(1):85–91. PMID: 25826491

[12] Stepan AE, Mărgăritescu C, Stoica LE, Stepan MD, Simionescu CE. Clear cell renal cell carcinomas – epithelial and mesenchymal immunophenotype. Rom J Morphol Embryol, 2018, 59(4):1189–1194. PMID: 30845300

[13] Petrova YI, Schecterson L, Gumbiner BM. Roles for E-cadherin cell surface regulation in cancer. Mol Biol Cell, 2016, 27(21): 3233–3244. https://doi.org/10.1091/mbc.E16-01-0058 PMID: 27582386 PMCID: PMC5170857

[14] Kaszak I, Witkowska-Piłaszewicz O, Niewiadomska Z, Dworecka-Kaszak B, Ngosa Toka F, Jurka P. Role of cadherins in cancer – a review. Int J Mol Sci, 2020, 21(20):7624. https://doi.org/10.3390/ijms21207624 PMID: 33076339 PMCID: PMC7589192

[15] Wang P, Zhu Z. Prognostic and clinicopathological significance of E-cadherin in pancreatic cancer patients: a meta-analysis. Front Oncol, 2021, 11:627116. https://doi.org/10.3389/fonc.2021.627116 PMID: 33912451 PMCID: PMC8074677

[16] Schizas D, Moris D, Michalinos A, Kanavidis P, Oikonomou D, Papalampros A, Machairas A, Liakakos T. E-cadherin in gastric carcinomas: relations with histological parameters and its prognostic value. J BUON, 2017, 22(2):383–389. PMID: 28534359

[17] Wang HL, Zhao XK, Zhou FY, Song X, Li LY, Huang GR, Bao QD, Lei LL, Yang HJ, Li L, Xu RH, Li AL, Wang XZ, Han WL, Ren JL, Wang LD. Characterization of E-cadherin expression in normal mucosa, dysplasia and adenocarcinoma of gastric cardia and its influence on prognosis. World J Gastrointest Oncol, 2022, 14(1):265–277. https://doi.org/10.4251/wjgo.v14.i1.265 PMID: 35116116 PMCID: PMC8790427

[18] Hassn Mesrati M, Syafruddin SE, Mohtar MA, Syahir A. CD44: a multifunctional mediator of cancer progression. Biomolecules, 2021, 11(12):1850. https://doi.org/10.3390/biom11121850 PMID: 34944493 PMCID: PMC8699317

[19] Zavros Y. Initiation and maintenance of gastric cancer: a focus on CD44 variant isoforms and cancer stem cells. Cell Mol Gastroenterol Hepatol, 2017, 4(1):55–63. https://doi.org/10.1016/j.jcmgh.2017.03.003 PMID: 28560289 PMCID: PMC5439237

[20] Ahadi M, Moradi A, Musavinejad L, Movafagh A, Moradi A. The expression of p53, CD44, Ki-67, and HER-2/neu markers in gastric cancer and its association with histopathological indicators: a retrospective study. Asian Pac J Cancer Prev, 2020, 21(6):1607–1614. https://doi.org/10.31557/APJCP.2020.21.6.1607 PMID: 32592354 PMCID: PMC7568877

[21] Gowrikumar S, Singh AB, Dhawan P. Role of claudin proteins in regulating cancer stem cells and chemoresistance – potential implication in disease prognosis and therapy. Int J Mol Sci, 2019, 21(1):53. https://doi.org/10.3390/ijms21010053 PMID: 31861759 PMCID: PMC6982342

[22] Wu Z, Shi J, Song Y, Zhao J, Sun J, Chen X, Gao P, Wang Z. Claudin-7 (CLDN7) is overexpressed in gastric cancer and promotes gastric cancer cell proliferation, invasion and maintains mesenchymal state. Neoplasma, 2018, 65(3):349–359. https://doi.org/10.4149/neo_2018_170320N200 PMID: 29788731

[23] World Health Organization (WHO) Classification of Tumours Editorial Board. Digestive system tumours. 5th edition, vol. 1, International Agency for Research on Cancer (IARC) Press, Lyon, France, 2019. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/Digestive-System-Tumours-2019

[24] Janiszewska M, Primi MC, Izard T. Cell adhesion in cancer: beyond the migration of single cells. J Biol Chem, 2020, 295(8):2495–2505. https://doi.org/10.1074/jbc.REV119.007759 PMID: 31937589 PMCID: PMC7039572

[25] Stepan AE, Ciurea RN, Drăgoescu PO, Florescu MM, Stepan MD. Immunoexpression of transcription factors in urothelial bladder carcinomas. Rom J Morphol Embryol, 2017, 58(3):863–869. PMID: 29250666

[26] 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, 20(18):4452. https://doi.org/10.3390/ijms20184452 PMID: 31509966 PMCID: PMC6769612

[27] Corso G, Figueiredo J, De Angelis SP, Corso F, Girardi A, Pereira J, Seruca R, Bonanni B, Carneiro P, Pravettoni G, Guerini Rocco E, Veronesi P, Montagna G, Sacchini V, Gandini S. E-cadherin deregulation in breast cancer. J Cell Mol Med, 2020, 24(11):5930–5936. https://doi.org/10.1111/jcmm.15140 PMID: 32301282 PMCID: PMC7294130

[28] Karayiannakis AJ, Syrigos KN, Chatzigianni E, Papanikolaou S, Karatzas G. E-cadherin expression as a differentiation marker in gastric cancer. Hepatogastroenterology, 1998, 45(24):2437–2442. PMID: 9951940

[29] Arévalo F, Monge E, Morales D. E-cadherina: expresión molecular en carcinomas de estómago de tipo difuso y tipo intestinal. Estudio realizado en Lima, Perú [E-cadherin molecular expression in the diffuse and intestinal types of gastric adenocarcinoma. A report from Lima, Peru]. Rev Esp Enferm Dig, 2007, 99(6): 325–329. https://doi.org/10.4321/s1130-01082007000600004 PMID: 17883295

[30] Liu F, Sun AJ, Sun LP, Chen LR. [Gastrointestinal adenocarcinomas with a micropapillary pattern: a clinicopathologic and immunohistochemical study]. Zhonghua Bing Li Xue Za Zhi, 2011, 40(5):304–309. PMID: 21756823

[31] Chen HC, Chu RY, Hsu PN, Hsu PI, Lu JY, Lai KH, Tseng HH, Chou NH, Huang MS, Tseng CJ, Hsiao M. Loss of E-cadherin expression correlates with poor differentiation and invasion into adjacent organs in gastric adenocarcinomas. Cancer Lett, 2003, 201(1):97–106. https://doi.org/10.1016/j.canlet.2003.07.007 PMID: 14580691

[32] Zhou Y, Li G, Wu J, Zhang Z, Wu Z, Fan P, Hao T, Zhang X, Li M, Zhang F, Li Q, Lu B, Qiao L. Clinicopathological significance of E-cadherin, VEGF, and MMPs in gastric cancer. Tumour Biol, 2010, 31(6):549–558. https://doi.org/10.1007/s13277-010-0068-y PMID: 20563765

[33] Zhang HK, Zhang QM, Zhao TH, Li YY, Yi YF. Expression of mucins and E-cadherin in gastric carcinoma and their clinical significance. World J Gastroenterol, 2004, 10(20):3044–3047. https://doi.org/10.3748/wjg.v10.i20.3044 PMID: 15378790 PMCID: PMC4576269

[34] Torabizadeh Z, Nosrati A, Sajadi Saravi SN, Yazdani Charati J, Janbabai G. Evaluation of E-cadherin expression in gastric cancer and its correlation with clinicopathologic parameters. Int J Hematol Oncol Stem Cell Res, 2017, 11(2):158–164. PMID: 28875011 PMCID: PMC5575728

[35] Xia M, Xie Y, Zan L, Reddy S, Tan C, Li J, Zhou D, Tan D. Membranous staining of β-catenin and E-cadherin expression in patients with gastric cancer. Int J Clin Exp Pathol, 2017, 10(8):8980–8990. PMID: 31966768 PMCID: PMC6965369

[36] Jang BI, Li Y, Graham DY, Cen P. The role of CD44 in the pathogenesis, diagnosis, and therapy of gastric cancer. Gut Liver, 2011, 5(4):397–405. https://doi.org/10.5009/gnl.2011.5.4.397 PMID: 22195236 PMCID: PMC3240781

[37] Ibrahim HM, AbdElbary AM, Mohamed SY, Elwan A, Abdelhamid MI, Ibrahim A. Prognostic value of cyclin D1 and CD44 expression in gastric adenocarcinoma. J Gastrointest Cancer, 2019, 50(3):370–379. https://doi.org/10.1007/s12029-018-0079-2 PMID: 29497929

[38] Jung WY, Kang Y, Lee H, Mok YJ, Kim HK, Kim A, Kim BH. Expression of moesin and CD44 is associated with poor prognosis in gastric adenocarcinoma. Histopathology, 2013, 63(4):474–481. https://doi.org/10.1111/his.12202 PMID: 23889297

[39] Setälä L, Lipponen P, Tammi R, Tammi M, Eskelinen M, Alhava E, Kosma VM. Expression of CD44 and its variant isoform v3 has no prognostic value in gastric cancer. Histopathology, 2001, 38(1):13–20. https://doi.org/10.1046/j.1365-2559.2001.01038.x PMID: 11135041

[40] Wang T, Ong CW, Shi J, Srivastava S, Yan B, Cheng CL, Yong WP, Chan SL, Yeoh KG, Iacopetta B, Salto-Tellez M. Sequential expression of putative stem cell markers in gastric

carcinogenesis. Br J Cancer, 2011, 105(5):658–665. https://doi.org/10.1038/bjc.2011.287 PMID: 21829201 PMCID: PMC3188930
[41] Lu L, Wu M, Sun L, Li W, Fu W, Zhang X, Liu T. Clinicopathological and prognostic significance of cancer stem cell markers CD44 and CD133 in patients with gastric cancer: a comprehensive meta-analysis with 4729 patients involved. Medicine (Baltimore), 2016, 95(42):e5163. https://doi.org/10.1097/MD.0000000000005163 PMID: 27759647 PMCID: PMC5079331
[42] Ghaffarzadehgan K, Jafarzadeh M, Raziee HR, Sima HR, Esmaili-Shandiz E, Hosseinnezhad H, Taghizadeh-Kermani A, Moaven O, Bahrani M. Expression of cell adhesion molecule CD44 in gastric adenocarcinoma and its prognostic importance. World J Gastroenterol, 2008, 14(41):6376–6381. https://doi.org/10.3748/wjg.14.6376 PMID: 19009655 PMCID: PMC2766121
[43] Sanaat Z, Halimi M, Ghojezadeh M, Pirovi AH, Gharamaleki JV, Ziae AE, Kermani IA. Immunohistochemical analysis of p53, Ki-67, CD44, HER-2/neu expression patterns in gastric cancer, and their association with one year survival in north-west of Iran. Int J Hematol Oncol Stem Cell Res, 2013, 7(3):15–20. PMID: 24505530 PMCID: PMC3913147
[44] Yan Y, Zuo X, Wei D. Concise Review: Emerging role of CD44 in cancer stem cells: a promising biomarker and therapeutic target. Stem Cells Transl Med, 2015, 4(9):1033–1043. https://10.5966/sctm.2015-0048 PMID: 26136504 PMCID: PMC4542874
[45] Kodama H, Murata S, Ishida M, Yamamoto H, Yamaguchi T, Kaida S, Miyake T, Takebayashi K, Kushima R, Tani M. Prognostic impact of CD44-positive cancer stem-like cells at the invasive front of gastric cancer. Br J Cancer, 2017, 116(2):186–194. https://10.1038/bjc.2016.401 PMID: 27931044 PMCID: PMC5243989
[46] Witschen PM, Chaffee TS, Brady NJ, Huggins DN, Knutson TP, LaRue RS, Munro SA, Tiegs L, McCarthy JB, Nelson AC, Schwertfeger KL. Tumor cell associated hyaluronan–CD44 signaling promotes pro-tumor inflammation in breast cancer. Cancers (Basel), 2020, 12(5):1325. https://10.3390/cancers12051325 PMID: 32455980 PMCID: PMC7281239
[47] Dhingra S, Feng W, Brown RE, Zhou Z, Khoury T, Zhang R, Tan D. Clinicopathologic significance of putative stem cell markers, CD44 and nestin, in gastric adenocarcinoma. Int J Clin Exp Pathol, 2011, 4(8):733–741. PMID: 22135720 PMCID: PMC3225784
[48] Lin JX, Yoon C, Li P, Yu Q, Qiu SL, Zheng CH, Yoon SS, Huang CM. Increased CD44 expression and MEK activity predict worse prognosis in gastric adenocarcinoma patients undergoing gastrectomy. J Gastrointest Surg, 2021, 25(5):1147–1155. https://doi.org/10.1007/s11605-020-04616-4 PMID: 32410176
[49] Tongtawee T, Wattanawongdon W, Simawaranon T, Kaewpitoon S, Kaengpenkae S, Jintabanditwong N, Tangjanyatham P, Ratchapol W, Kangwantas K, Dechsukhum C, Leeanansaksiri W, Kaewpitoon N, Matrakool L, Panpimanmas S. Expression of cancer stem cell marker CD44 and its polymorphisms in patients with chronic gastritis, precancerous gastric lesion, and gastric cancer: a cross-sectional multicenter study in Thailand. Biomed Res Int, 2017, 2017:4384823. https://doi.org/10.1155/2017/4384823 PMID: 29445738 PMCID: PMC5763069
[50] Jian-Hui C, Er-Tao Z, Si-Le C, Hui W, Kai-Ming W, Xin-Hua Z, Chuang-Qi C, Shi-Rong C, Yu-Long H. CD44, sonic hedgehog, and Gli1 expression are prognostic biomarkers in gastric cancer patients after radical resection. Gastroenterol Res Pract, 2016, 2016:1013045. https://doi.org/10.1155/2016/1013045 PMID: 26839535 PMCID: PMC4709647
[51] Hashimoto I, Oshima T. Claudins and gastric cancer: an overview. Cancers (Basel), 2022, 14(2):290. https://doi.org/10.3390/cancers14020290 PMID: 35053454 PMCID: PMC8773541
[52] Ding L, Lu Z, Lu Q, Chen YH. The claudin family of proteins in human malignancy: a clinical perspective. Cancer Manag Res, 2013, 5:367–375. https://doi.org/10.2147/CMAR.S38294 PMID: 24232410 PMCID: PMC3825674
[53] Hewitt KJ, Agarwal R, Morin PJ. The claudin gene family: expression in normal and neoplastic tissues. BMC Cancer, 2006, 6:186. https://doi.org/10.1186/1471-2407-6-186 PMID: 16836752 PMCID: PMC1538620
[54] Jun KH, Kim JH, Jung JH, Choi HJ, Chin HM. Expression of claudin-7 and loss of claudin-18 correlate with poor prognosis in gastric cancer. Int J Surg, 2014, 12(2):156–162. https://doi.org/10.1016/j.ijsu.2013.11.022 PMID: 24333468
[55] Park JY, Park KH, Oh TY, Hong SP, Jeon TJ, Kim CH, Park SW, Chung JB, Song SY, Bang S. Up-regulated claudin 7 expression in intestinal-type gastric carcinoma. Oncol Rep, 2007, 18(2):377–382. PMID: 17611659
[56] Rendón-Huerta E, Teresa F, Teresa GM, Xochitl GS, Georgina AF, Veronica ZZ, Montaño LF. Distribution and expression pattern of claudins 6, 7, and 9 in diffuse- and intestinal-type gastric adenocarcinomas. J Gastrointest Cancer, 2010, 41(1):52–59. https://doi.org/10.1007/s12029-009-9110-y PMID: 19960275
[57] Shinozaki A, Ushiku T, Morikawa T, Hino R, Sakatani T, Uozaki H, Fukayama M. Epstein–Barr virus-associated gastric carcinoma: a distinct carcinoma of gastric phenotype by claudin expression profiling. J Histochem Cytochem, 2009, 57(8):775–785. https://doi.org/10.1369/jhc.2009.953810 PMID: 19398608 PMCID: PMC2713077

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