

The Implications of E-cadherin Expression in Pancreatic Cancer

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Pancreatic cancer is an aggressive neoplasia and has a fatal prognosis, with approximately 85% of patients diagnosed in advanced stages of the disease. The purpose of this study was to determine if the loss of e-cadherin expression in pancreatic cancer is a predictor of the prognosis of this disease. This research is based on a retrospective study performed on a group of 81 patients who benefited from pancreatic resection in our Surgical Clinic, between January 2008 and December 2016 and assessing the positivity of e-cadherin. There were no correlations between e-cadherin expression and other clinic-pathological factors, including gender, age, tumor status, lymph node metastasis, microscopic vascular invasion, perineural invasion. Low-differentiated pancreatic cancer was more likely to exhibit e-cadherin expression loss than well-differentiated forms of cancer (p=0.07). The mean survival in e-cadherin positive patients (17.1 months) was significantly worse compared to those with E-cadherin absent (6.8 months). In conclusion, we found that partial loss of e-cadherin in primary pancreatic adenocarcinomas is an independent predictor of a negative outcome among patients with curative surgical resection of pancreatic lesions.

Keywords: Pancreas, Adenocarcinoma, E-cadherin; Prognosis

Pancreatic cancer (PC) is one of the most aggressive tumors and is the third leading cause of cancer-related death, higher than the one in breast cancer [1]. Pancreatic cancer has a fatal prognosis, with a five-year survival rate less than 6%, due to unspecific symptoms causing late diagnosis and the lack of early effective diagnostic strategies or prognostic markers [2,3]. Although the progress was made in oncology field, due to the unique resistance to chemotherapy, gemcitabine remained the standard therapy for advanced pancreatic cancer, without showing a major improvement in survival rate [4-6].

One of the main reasons for the poor prognosis and the reduce survival rate is the high capacity of the tumor cells to metastasize and at the time of the diagnosis more than 50% of the pancreatic cancer patients presented with lymph node metastases [7,8]. The histological and genetic model of the pancreatic carcinogenesis have been described, but the molecular mechanism responsible of the metastatic spread is still unclear. Have been described genetic mutations involved in activation of oncogenes (K-ras) and tumor suppressor genes inactivation p53, p16 [9,10].

One of the mechanism that seems to be involved in metastatic process is the ability to overcome the apoptosis by an early epithelial-to-mesenchymal transition (EMT) [11]. This process cause a lost of components in the epithelial cell junctions and produce instead a mesenchymal cytoskeleton capable of invasive and chemoresistance properties [12].

E-cadherin is a calcium-dependent adhesion transmembrane glycoprotein that plays an important role maintaining the intercellular adhesiveness of the normal epithelial cells [10]. It has a major role in modulating the metastatic ability of different tumors and E-cadherin expression lost or reduced was found in many human cancers types, including nasopharyngeal cancer, lung cancer, colorectal cancer and cervical cancer [13-15] and was positive correlated with histologic grade, lymph node metastasis and poor prognosis [17].

Studies on E-cadherin expression in PDAs have been reported over the past two decades. The implications of e-cadherin expression in progression of pancreatic cancer is not yet completely described and the aim of this study was to evaluate the impact of e-cadherin expression in the prognosis of pancreatic cancer.

Experimental part

We analyzed 81 patients that underwent pancreatic resections for pancreatic cancer at the First Surgical Clinic, Sf. Spiridon Emergency Hospital from Iasi, Romania, over a 8 years period, from January 2008 to December 2016. All specimens were fixed in 10% buffered formalin and embedded in paraffin.

Tissue microarrays were constructed from representative tissue blocks for each case and the immunohistochemical staining against E-cadherin was performed on 2µm sections from paraffin-embedded fragments of the primary tumor (fig. 1).

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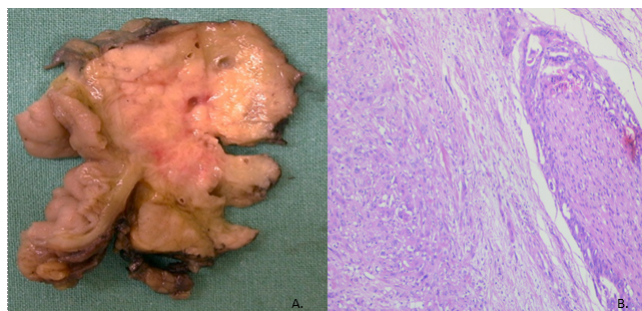


Fig. 1 Pancreatic tumor: A. Macroscopic aspect. B. Hematoxylin - eosin stain x10 pancreatic tumor

All sections were deparaffinized using two bath of xylene, afterwards we rehydrated the sections in decreasing concentrations and finally in distilled water. The antigen retrieval was accomplished with a buffer solution of citrate 10 mM, pH 6.0. Immunohistochemistry was performed using E-cadherin clone EP700Y. The over-night technique was used for antibody incubation and 3,3'-diaminobenzidine (DAB) solution as chromogen prior to final Hematoxylin counterstaining was used for reaction amplification. External positive and negative controls were used. Immunolabeling was considered positive for expressing if the intensity was strong (2+), weak (1+) or absent (0+) and if the extent was more than or equal to 5% of cancer cells.

Results and discussions

Clinicopathologic parameters including patient age, gender, tumor stage, tumor location, histologic grade, lymphovascular and perineural invasion, lymph node metastasis and distant metastasis were evaluated by reviewing the medical records (table 1).

Tumor size and lymph node classification was made using the 8th edition of TNM staging system and the histological confirmation was on surgical specimens and the results establish in all cases pancreatic adenocarcinoma. Neither of the patients included in

this study had histological diagnosis prior to the surgical procedure.

E-cadherin expression was evaluated in all 81 samples of pancreatic tumors (fig. 2, a-e). No statistical significant correlation between E-cadherin expression, age, gender and tumor or lymph node stage.

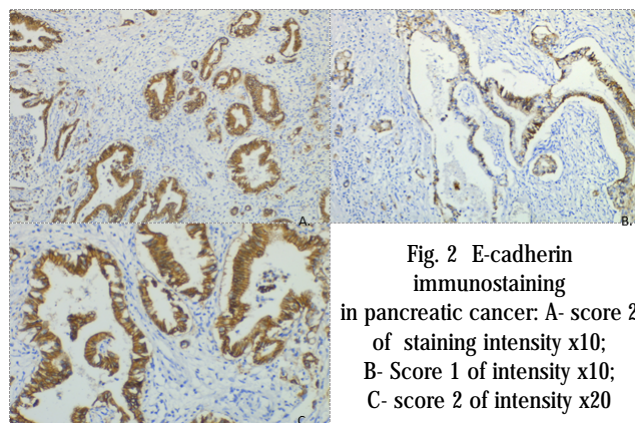


Fig. 2 E-cadherin immunostaining in pancreatic cancer: A- score 2 of staining intensity x10; B- Score 1 of intensity x10; C- score 2 of intensity x20

Moderately and well-differentiated tumors mostly displayed E-cadherin expression (score 2) compared to poorly differentiated tumors that were low expression (score 1) in 14 of 17 cases showing that low-differentiated pancreatic cancer was more likely to have loss of e-cadherin expression than it was in moderately - and well-differentiated forms of cancer (p=0.07). Among the poorly differentiated tumors there were three cases with no E-cadherin expression (score 0). None of the poorly differentiated tumors presented normal E-cadherin expression compared to the well or moderately differentiated tumors (table 2).

Median survival in the patients with highly positive E-cadherin markers was 17.1 months and in those with E-cadherin absent only about 6.8 months and the difference was statistical significant (p = 0.02).

Pancreatic cancer remains one of the most fatal neoplastic diseases with only one potential curable

Patient characteristics	
Mean age (SD), years	60.2
Males/ Female, n (%)	49 /32(60.49/39.51)
Tumor stage	
> T1	7 (8.65)
> T2	24 (29.62)
> T3	45 (55.55)
> T4	5 (6.18)
Tumor location, n (%)	
> Head	57(70.38)
> Body	5(6.17)
> Tail	19(23.45)
Surgical procedure	
> Pancreaticoduodenectomy Whipple	61(75.30)
> Splenopancreatectomy	
> Subtotal pancreaticoduodenectomy	19 (23.46)
	1(1.24)
Lymph node stage, n (%)	
> N0	28(34.56)
> N1	46(56.80)
> N2	7(8.64)
Tumor grade	
> G1	31(38.28)
> G2	33(40.74)
> G3	17(20.98)
Tumor invasion	
> Lymphovascular	39 (48.14)
> Perineural	64(79.01)

Table 1
PATIENT CHARACTERISTICS

Tumor grade	No. of cases	E-cadherin score			p-value
		0	1	2	
Well differentiated	31	0	8	23	0.07
Moderately differentiated	33	0	18	15	
Poorly differentiated	17	3	14	0	

Table 2
CORRELATION BETWEEN E-CADHERIN
EXPRESSION AND TUMOR GRADING

treatment, surgical resection. At the time of the first diagnosis, less than 20% of patients are eligibles for surgical treatment but the median survival rate is under 20 months with a 5-year survival of approximately 20%, due to the high rate of recurrence and the lack effective medical therapy [18, 19].

E-cadherin is a calcium-dependent adhesion protein with a well-known role as a tumor suppressor. Recent research revealed that the lost of e-cadherin in tumor cells associated with epithelial-mesenchymal transition is encountered in tumor progression and metastasis process [20-22]. The dissociated cell due to the lost of cellular adhesion mediated by lateral dimerization between e-cadherin molecules creating a homodimer capable to interact with another adjacent homodimer may be responsible of the surrounding tissues or the distant migration [23,24]. Also the e-cadherin molecules seems to be involved in collective cell behavior responsible of invasion and metastasis process and therefore can serve an an targeting molecule or an adhesion molecule [25,26].

Reduction or loss of E-cadherin expression has been reported in 30.0% to 61.8 % of pancreatic cancer [27,28]. In our study, 53% of pancreatic malignant tumors showed mild, moderate or severe loss of E-cadherin expression. The difference encountered in different studies vcan be explained by the different types of antibody used or the antigen retrieval methods also the criteria used for assessing positivity and the different types of samples fixation can cause this heterogeneity of results.

Studies revealed also relations between e-cadherin loss and tumor histologic grade [29]. In this study, poorly differentiated pancreatic tumors had higher loss of E-cadherin expression than well or moderately differentiated tumors but this difference was not statistical significant.

The lost of e-cadherin causes interferences with β -catenin signaling in the Wnt pathway, and this leads to uncontrolled proliferation due to the decreased in the growth suppression and promotes cancer progression. In pancreatic cancer, the e-cadherin loss has been associated with lymph node metastasis and advanced stage [29,30]. Other study showed that loss of E-cadherin expression has no correlation with age or gender of patients, tumor size, invasion, lymph node involvement, distant metastasis, and survival of patients [27].

In our study, E-cadherin expression lost had no significant correlation with age and gender of patients, location, tumor size, lymphovascular or perineural invasion, lymph node metastasis, pT classification and stage but was significant correlated with median survival rate.

In the research of Hong et al., they described the loss of E-cadherin expression as an independent predictor of poor prognosis in patients with pancreatic adenocarcinoma [29].

In order to understand the pathogenesis of pancreatic cancer sthe studies of e-cadherin extended also to PanIN lesions and in this area Al-Aynati et al. reported that the loss of E-cadherin membranous staining was significantly more common in adenocarcinoma than in normal ductal epithelium and PanIN lesions[31].

To establish the role of E-cadherin expression as a predictor in patients with pancreatic cancer are needed further research because mutations of e-cadherin genes

have been identified in cancer cells but the the molecular events of the E-cadherin gene implicated in the development of pancreatic cancer are not yet completely disclosed [25,32].

Conclusions

Loss of E-cadherin expression is usually encountered in high-grade PanIN and pancreatic cancer and this molecule may be a key factor in the judgment of the malignant potential of pancreatic tumors. Further studies might help to understand the onset and underlying effects of E-cadherin in pancreatic cancer carcinogenesis.

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