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Differential Expression of E-Cadherin and P-Cadherin in Breast Cancer Molecular Subtypes

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Abstract. *Background/Aim: E- and P-cadherin (E-cadh, P-cadh) control tumor cell invasion, metastatic or stemness potential and chemotherapy resistance. The study aimed to assess E- and P-cadherin expression in breast cancer molecular subtypes. Materials and Methods: Immunohistochemistry for E-cadh and P-cadh was performed for 97 breast cancer cases. Membrane (M), cytoplasmic (C) or mixed (MC) patterns of E-cadh and P-cadh were considered in our evaluation. Results: E-cadh and P-cadh C pattern was significantly correlated in the HER2 subtype (p=0.031). P-cadh M pattern was highly specific for the HER2 subtype (p=0.002). Only P-cadh C characterized the triple negative breast cancer subtype (p=0.015). For Luminal B/HER2 cases, P-cadh M pattern was strongly coexpressed with the E-cadh MC pattern (p=0.012). Progesterone receptor (PR) expression influenced E-cadh M pattern in the Luminal B/HER2 subtype (p=0.042). Conclusion: E- and P- cadherins define distinct subgroups within breast cancer molecular subtypes. Our findings support the inclusion of E- and P-cadherin into breast cancer molecular classification.*

Breast cancer is still the most frequent neoplastic disease in women, and despite the significant progress done in the last years, morbidity and mortality remain high. In order to improve the therapeutic strategy, two decades ago a new classification of breast cancer was proposed. Based on gene analysis and later certified by immunohistochemistry, five main subtypes and corresponding specific markers were included in the new molecular classification. A long and controversial discussion was conducted about the possible use-case and practical application of E-cadherin, supported by some authors and rejected or neglected by others.

Cadherins are calcium-channel dependent transmembrane proteins involved in intercellular adhesion (1) in prenatal and postnatal life in normal conditions. They are strongly involved in tissue differentiation during embryogenesis, especially in cell migration and epithelial-mesenchymal transition, which are essential processes for tissue and organ development. In the postnatal life, cadherins continue to play an important role in maintaining tissue and cell integrity (1).

E-cadherin and P-cadherin are type I, classical cadherins (2, 3). E-cadherin is the main component of adherens junctions of all normal epithelial cells (4), while P-cadherin is colocalized with E-cadherin, but it is restricted to the basal proliferative layer of various epithelia (5). Both cadherins are expressed during normal development of the human mammary gland, E-cadherin in luminal cells (6), while P-cadherin in basal and stem cells (7).

During breast carcinogenesis, both E- and P-cadherin have a crucial role in tumor cell invasion, metastasis, chemotherapy resistance and stemness.

In breast cancer, E-cadherin is extensively studied as compared to P-cadherin. E-cadherin expression has been studied in normal breast development and in the molecular subtypes of breast cancer, while the role of P-cadherin remains still highly controversial in both normal and tumor breast tissue. P-cadherin expression is associated with undifferentiated cells during the development of the mammary gland and poorly differentiated carcinoma of the mammary gland. P-cadherin is frequently overexpressed in high-grade tumors, being a poor prognostic factor for breast cancer patients (7). Several years ago, a humanized anti-P-cadherin monoclonal antibody was developed and tested on

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breast cancer cell lines (8). It is currently tested in a Phase I clinical trial, and interferes with P-cadherin involvement in the invasion and metastasis processes (8).

Several papers reported in the past that loss of E-cadherin from luminal cells is responsible for cancer invasion and metastasis in breast cancer (9, 10). Recently, the results reported by Padmanaban *et al*. are in contradiction with previous findings. By using three different experimental models of breast cancer, they proved that metastatic cells retain E-cadherin expression, which improves their survival and metastatic potential (11).

Controversial issues about the impact of a heterogeneous expression pattern (membrane, cytoplasmic or mixed) on breast cancer progression and prognosis have been reported in the literature before, most of them being correlated with conventional diagnosis, grade of differentiation and a worse prognosis (12).

The membrane pattern is the most accepted expression pattern for E-cadherin. Cytoplasmic expression, usually known as aberrant expression, seems to have an important impact on tumor progression and metastasis. Usually E- and P-cadherins are separately evaluated and their expression heterogeneity in the different molecular subtypes of breast cancer is not well certified.

In the present study, we have analyzed the expression of E- and P-cadherin related to the different molecular subtypes of breast cancer, in order to search for a potential impact of both cadherins on molecular stratification of breast cancer.

Materials and Methods

Specimens. A total of 97 formalin-fixed parraffin embedded (FFPE) biopsies from patients diagnosed with breast cancer between 2011-2017 were selected from the archive of the Department of Pathology. Inclusion criteria referred to the quality of the FFPE specimens (tested by their positiviy to vimentin, clone V9 and also by the presence of enough material in order to be processed for immunohistochemistry). Only cases with a previous molecular classification, tested for a minimum of four markers, were included in the study. Based on these data, patients were classified as Luminal A, Luminal B, mixed Luminal B/HER2, HER2 and triple negative breast cancers (TNBC) subtypes. All biopsies were previously collected by open surgery and processed following steps of a routine pathology protocol by the FFPE method. We selected from each case the haematoxylin and eosin stained slide and paraffin block.

Tissue microarray. From each FFPE specimen, we performed an automated tissue microarray method by using automated TMA Grand Master microarrayer (provided by 3DHistech, Budapest, Hungary). We created TMA parrafin blocks by selecting four areas (2 from the middle and two from the periphery of the tumor); we collected 2 mm tissue cores from each of the previously selected areas and transfered them to the recipient parraffin block. By using this method we created the final parraffin block, which included five different cases per block, each case having 4 cores.

Immunohistochemistry (IHC). A three micrometer thick section from each TMA paraffin block was stained with haematoxylin and eosin, and based on microscopic analysis they were selected for immunhistochemistry. Because of the external origin of the FFPE, Vimentin (clone V9) was performed first to select the tissues proper for IHC. On the selected specimens, we performed immunohistochemistry for E-cadherin and P-cadherin by using monoclonal mouse anti-human E-cadherin antibody (clone 36B5) and monoclonal mouse anti-human P-cadherin antibody (clone 56C1, Labvision, Fremont, CA, USA). All IHC steps were performed following a protocol selected on MaxBond Autostainer (Leica, Microsystems, Cambridge, UK).

Microscopic analysis and data interpetation. Immunostained slides were scanned with Pannoramic DESK Scanner (3DHistech, Budapest, Hungary) and stored in the Web Slide Library (Case Center, 3DHistech, Budapest, Hungary). Three pathologists analysed the scanned slides by using Pannoramic Viewer Software (3DHistech, Budapest, Hungary) and had high accuracy of microscopic images. They were also able to take pictures from areas of interest. Membrane (M), cytoplasmic (C) or mixed (MC) patterns were assessed and correlated with molecular subtypes of breast cancers. Statistical analysis included data processing with SPSS software version 20 and correlations were considered significant when p was less than 0.05.

Results

A positive reaction for E-cadherin was found in 72% of the cases, and P-cadherin was positive in 56% of the cases included in the study. In 24% of the cases, both cadherins were negative. Subsequently, we investigated separately the cytoplasmic, membrane or mixed expression of E- and P-cadherin.

In consequence, 36% of the cases showed a mixed, membrane and cytoplasmic E-cadherin expression (Figure 1a), whereas 28% had membrane restricted expression (Figure 1b). Cytoplasmic expression alone was found in 8% of cases (Figure 1c). The expression pattern was membrane restricted in 40% of the cases (Figure 1e), exclusively cytoplasmic in 8% of cases (Figure 1f), while membrane and cytoplasmic coexpression was found in only 8% of the cases (Figure 1d).

A 56% of the cases was characterized by an increased intensity of E-cadherin expression noted as +3 regardless of the expression pattern, 8% having moderate expression and another 8% low expression.

P-cadherin expression was observed in a smaller number of cases compared to E-cadherin expression and was also quantified at the membranous and cytoplasmic levels. The maximum intensity of expression was observed in 20% of cases (Figure 2a), 24% having poor expression (Figure 2c). The remaining positive cases (2%) were moderately positive (+2) (Figure 2b).

The heterogeneous expression of these three patterns had a spatial distribution such that, at the periphery of the tumors or the invasion front, tumor cells expressed cytoplasmic/

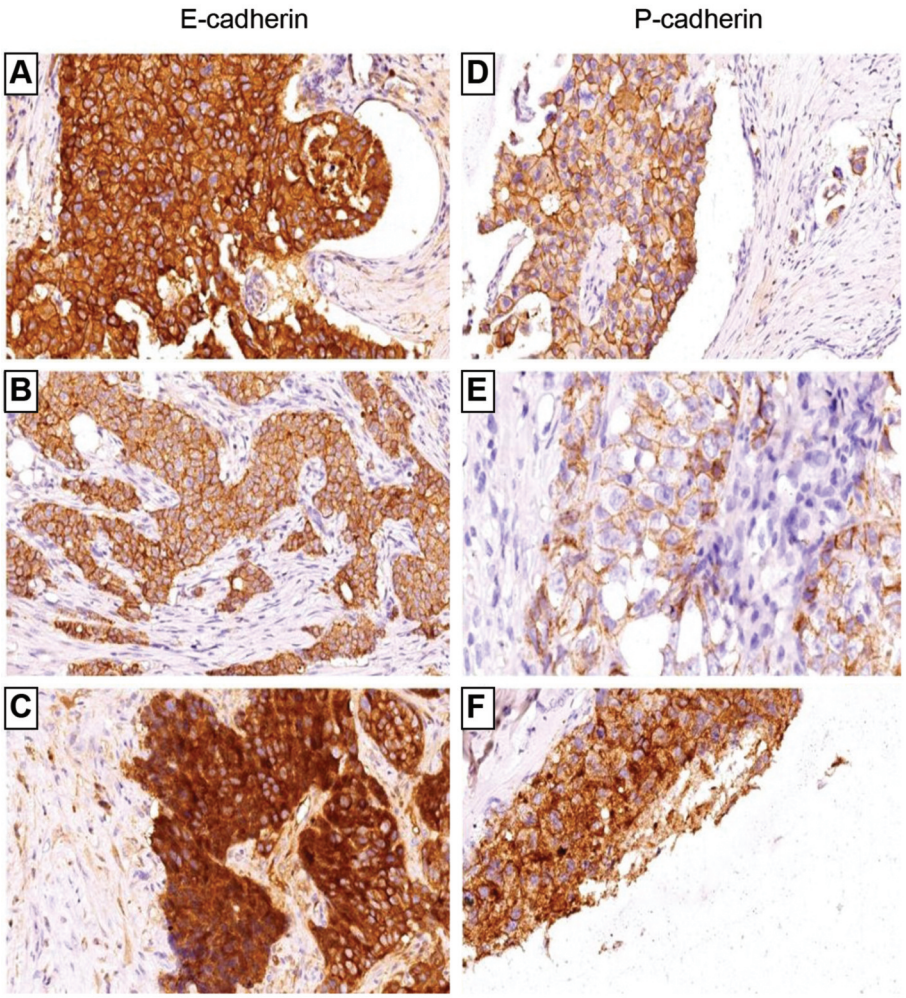

Figure 1. *E- and P-cadherin expression patterns in breast cancer. Three types of expression have been identified for both types of cadherins. Mixed membrane and cytoplasmic expression (a, d) was much more intense for E-cadherin. Membrane expression was also much better outlined for E-cadherin (b) compared to P-cadherin (e). In contrast, cytoplasmic expression had relatively the same distribution and intensity (c, f) for both cadherins.*

membrane mixed patterns. The pure cytoplasmic pattern was the least common in evaluating E- and P-cadherin in malignant breast tumors.

After assessing the patterns of expression and the intensity of E- and P-cadherin immunoexpression, the expression of cadherins related to the different breast cancer molecular subtypes was subsequently evaluated.

Thus, for Luminal A breast carcinoma, E-cadherin was positive in 58.33% of cases, all cases having a maximum intensity of expression of +3. Within these, the pattern of expression was extremely heterogeneous, 71.42% having a mixed membrane (M) and cytoplasmic (C) pattern, while only 28.58% had an expression pattern restricted to the membrane. Of the cases with mixed M+C expression, 60% were G2, the others being G3. In contrast, negative E-cadherin cases were G2 in 80% of cases. Type M expression was present in 50% of G3 cases and in 50% of G1 cases.

P-cadherin had also a heterogeneous expression and intensity in Luminal A. The 58.33% of cases were negative for P-cadherin, the remaining cases (41.67%) being positive. The intensity of expression was much weaker than that of E-cadherin; 33.34% of cases showed an intensity of +1 and 8.33% were strongly positive for P-cadherin. M+C coexpression was not found in Luminal A breast cancer cases, and M restricted expression was present in 60% of the cases, 40% being C-type. Regarding the grade of differentiation, it was heterogeneously distributed between G1, G2, and G3. Consequently, 60% were associated with G3, 20% with G2 and 20% with G1. The comparative summary of the results obtained is shown in Figures 3, 4 and 5.

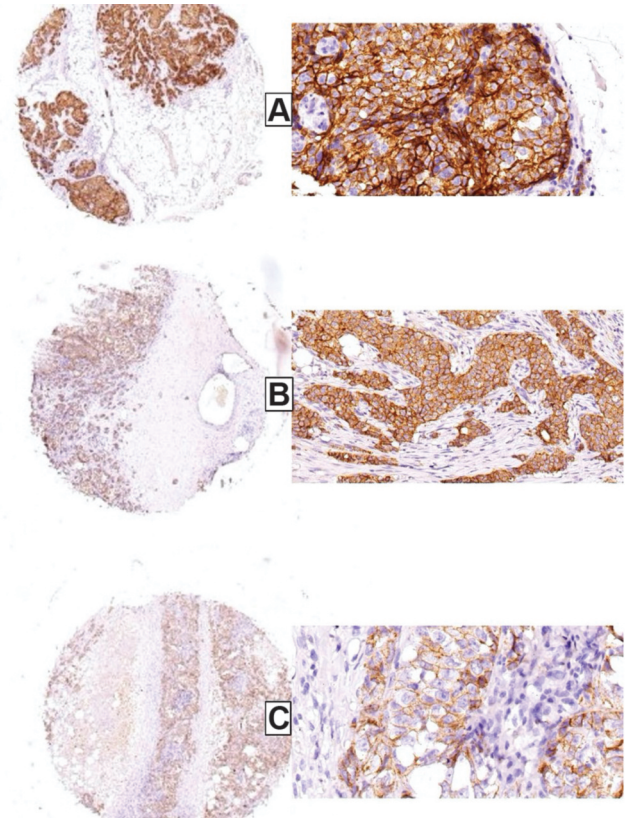

Figure 2. *Evaluation of expression intensity of the two cadherins in relation to immunostaining. Three intensities marked as 3(a), 2(b) and 1(c) for both types of cadherins were indentified, these intensities being present regardless of the expression pattern of E- and P-cadherin.*

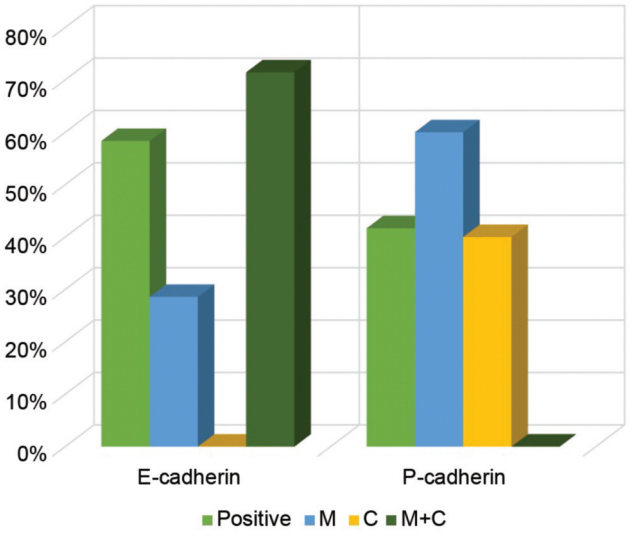

Figure 3. *Comparison of E- and P-cadherin in Luminal A type.*

Global analysis of the included cases concluded with the identification of statistically significant correlations between the grade of differentiation, E- and P-cadherin expression, as well as between the expression of estrogen and progesterone receptors and those of E- and P-cadherin. The molecular type had a statistically significant correlation with E-cadherin expression (p=0.005) with both M (p=0.001) and C (p=0.005) patterns. Also, the molecular type was correlated with cytoplasmic expression of P-cadherin (p=0.05), but not with membrane expression. Therefore, the degree of differentiation was statistically correlated with the cytoplasmic expression of P-cadherin (p=0.022), but not with the other parameters.

The expression of estrogen receptor (ER) had a poor (p=0.07) correlation with the C pattern of E-cadherin expression. In contrast, progesterone receptor (PR) had a statistically significant inverse correlation with both M (p=-0.04) and C (p=-0.006) E- cadherin patterns. Also, PR expression is statistically significantly influenced by the membrane expression of P-cadherin. HER2 expression correlated with the membrane expression of E-cadherin (p=0.04), but not with P-cadherin expression. The androgen receptors (AR) that were included in the evaluation of our cases correlated with E- cadherin, both patterns (p=0.04), but also had an inverse correlation with the C pattern of P-cadherin (p=0.05).

Thus, for Luminal A-type, the degree of differentiation had a correlation coefficient p=0.07 with the M-type expression of E-cadherin, which suggested a weak correlation between the two parameters. A statistically significant inverse correlation was recorded between the global expression of E- and P- cadherin, suggesting that E-cadherin expression excludes P-cadherin expression.

For Luminal B/HER2 mixed cases, E- and P-cadherin expression was extremely heterogeneous and revealed specific aspects. Accordingly, in the mixed form, none of the studied parameters correlated with G. Instead, a significant correlation was recorded between the E- and P-cadherin expression on the one hand, as well as for the differentiated expression of E-cadherin. The statistical data are summarized in Table I.

In the case of triple-negative breast cancers, cytoplasmic expression of P-cadherin predominated, and a statistically significant correlation with the total expression of P-cadherin was found for a correlation coefficient p=0.015. Also, the membrane expression of E-cadherin showed a statistically significant correlation with G (p=0.001). This statistically significant correlations are summarized in Table II.

For HER2 positive cases, a statistically significant correlation was noted between the cytoplasmic expression of E- and P-cadherin and the fact that for HER2 positive cases the membrane expression of P-cadherin was predominant (Table III).

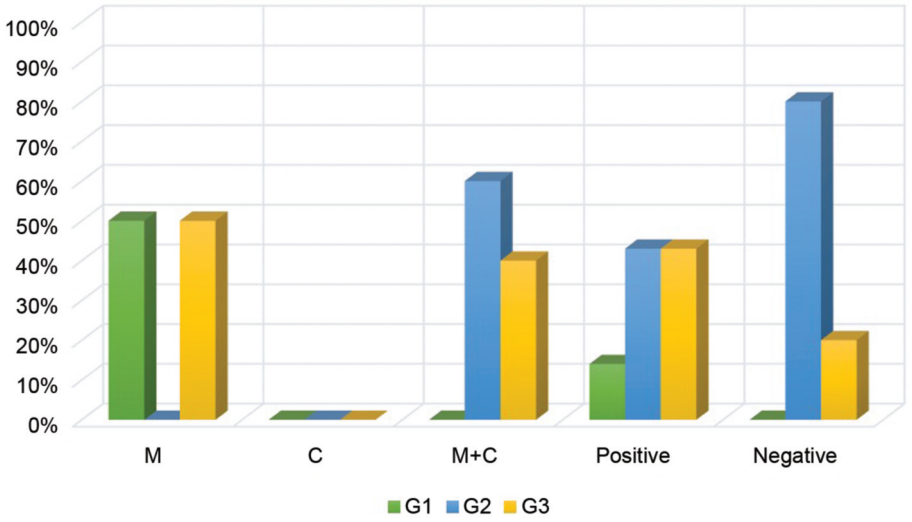

Figure 4. *E-cadherin expression according to Grade in Luminal A molecular type.*

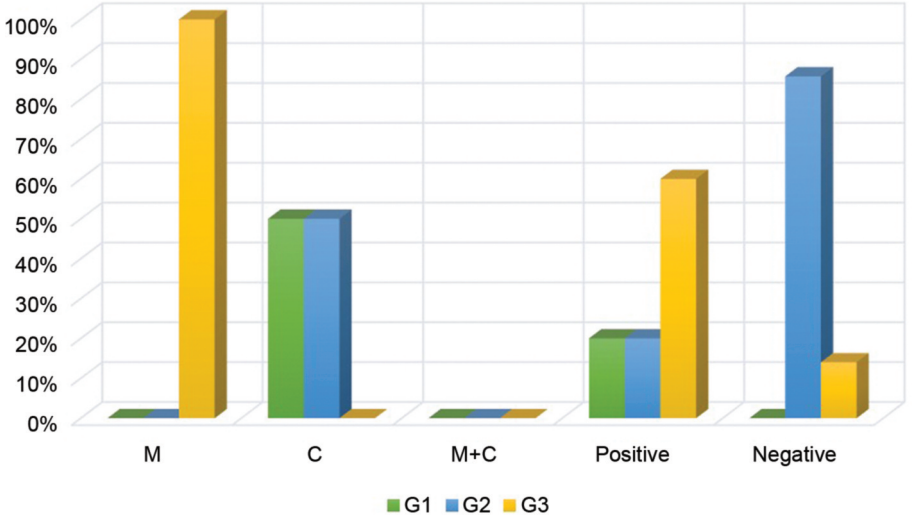

Figure 5. *The heterogeneity of P-cadherin expression. There is a lack of membrane and cytoplasmic coexpression of P-cadherin in Luminal type A.*

Discussion

Cadherins are known as adhesion molecules involved in the formation of adherence-type junctions within the transmembrane interrelations between cells. Cadherins behave both as receptors and as ligands for other molecules. During development, their behavior helps to correctly position the cells: they are responsible for separating the different tissue layers and for cell migration (13). Many cadherins are specified for specific functions in the cell and are differentially expressed in a developing embryo.

E-cadherin is also known as Cadherin 1 and is encoded by the *CDH1* gene. Cadherin-1 is a classic member of the cadherin superfamily. The encoded protein is a calcium-dependent cellular adhesion molecule composed of five extracellular units, a transmembrane region, and a well conserved cytoplasmic tail. The mutations in this gene are correlated with gastric, breast, colorectal, thyroid and ovarian cancers. Loss of function is thought to contribute to cancer progression by increasing proliferation, invasion and/or metastasis. The ectodomain of this protein mediates bacterial adhesion, while the cytoplasmic domain is required for internalization.

Table I. *Statistical correlations for E- and P-cadherin expressions in Luminal B/HER2 mixed cases.*

		G	ECAD M	ECAD C	ECAD	PCAD	PCAD M	PCAD C	PR	HER2
G	Pearson correlation	1	–0.029	0.161	–0.175	0.031	0.000	0.343	–0.012	–0.039
	Sig. (2-tailed)		0.909	0.523	0.486	0.903	1.000	0.163	0.962	0.874
	N	19	18	18	18	18	18	18	19	19
ECAD	Pearson correlation	–0.029	1	0.794**	0.630**	0.589*	0.579*	0.318	0.476*	0.186
	Sig. (2-tailed)	0.909		0.000	0.005	0.010	0.012	0.199	0.046	0.460
	N	18	18	18	18	18	18	18	18	18
ECAD M	Pearson correlation	0.161	0.794**	1	0.269	0.394	0.403	0.304	0.484*	0.152
	Sig. (2-tailed)	0.523	0.000		0.281	0.105	0.097	0.220	0.042	0.546
	N	18	18	18	18	18	18	18	18	18
ECAD C	Pearson correlation	–0.175	0.630**	0.269	1	0.516*	0.351	0.391	0.305	0.331
	Sig. (2-tailed)	0.486	0.005	0.281		0.028	0.153	0.109	0.218	0.179
	N	18	18	18	18	18	18	18	18	18
PCAD	Pearson correlation	0.031	0.589*	0.394	0.516*	1	0.867**	0.202	0.137	0.082
	Sig. (2-tailed)	0.903	0.010	0.105	0.028		0.000	0.422	0.587	0.746
	N	18	18	18	18	18	18	18	18	18
PCAD M	Pearson correlation	0.000	0.579*	0.403	0.351	0.867**	1	–0.171	0.158	0.094
	Sig. (2-tailed)	1.000	0.012	0.097	0.153	0.000		0.496	0.531	0.709
	N	18	18	18	18	18	18	18	18	18
PCAD C	Pearson correlation	0.343	0.318	0.304	0.391	0.202	–0.171	1	0.217	0.130
	Sig. (2-tailed)	0.163	0.199	0.220	0.109	0.422	0.496		0.387	0.608
	N	18	18	18	18	18	18	18	18	18
PR	Pearson correlation	–0.012	0.476*	0.484*	0.305	0.137	0.158	0.217	1	0.217
	Sig. (2-tailed)	0.962	0.046	0.042	0.218	0.587	0.531	0.387		0.373
	N	19	18	18	18	18	18	18	19	19
HER2	Pearson correlation	–0.039	0.186	0.152	0.331	0.082	0.094	0.130	0.217	1
	Sig. (2-tailed)	0.874	0.460	0.546	0.179	0.746	0.709	0.608	0.373	
	N	19	18	18	18	18	18	18	19	19

*Significant correlation with a value less than 0.05. **Strong significant correlation with a value less than 0.005.

E-cadherin is the best studied member of the cadherins family. The intracellular domain contains a highly phosphorylated vital region for beta-catenin binding and, therefore, for the E-cadherin function. Beta-catenin can also bind to alpha-catenin. Alpha-catenin participates in the regulation of cytoskeleton filaments that contain actin. In epithelial cells, cell-to-cell junctions that contain E-cadherin are often adjacent to cytoskeletal filaments that contain actin.

E-cadherin is primarily expressed in the mammalian 2-cell stage and becomes phosphorylated in the 8-cell stage. In adult tissues, E-cadherin is expressed in epithelial tissues, and is constantly regenerated with a half-life of 5 hours on the cell surface. Cell-cell interactions mediated by E-cadherin are essential for the formation of blasts in many animals (13). Cadherins is certified as having an essential role in the progression and metastasis of carcinomas. This type of adhesion molecule induces and supports the phenomenon of epithelial-mesenchymal transition, which increases the aggressiveness of carcinomas and promotes metastasis. Numerous recent studies have as the main subject of study E-cadherin interrelation with the prognosis and long-term survival of patients with oncological diseases.

A slightly less studied aspect in the literature is the polymorphic heterogeneous expression of E-cadherin, *i.e.*, membrane, cytoplasmic or combined dependent of the molecular type of breast cancer. It is well known that the decrease in E-cadherin membrane expression is accompanied by its cytoplasmic and/or nuclear overexpression, these latter two aspects being suggested as a negative prognostic factor associated with reduced survival in the various types of cancer (14, 15). Differentiated, membranous or cytoplasmic expression in breast cancer molecular subtypes has not been reported so far.

The involvement of E-cadherin in breast cancer is not a novelty, being extensively studied in the past (16-18). The interfering E-cadherin with other metastasizing factors such as EGFR or the Akt/STAT mediated pathway, has been reported as the main cause of induction of the epithelial-mesenchymal transition in triple-negative cancers and has been tested *in vitro* as a potential therapeutic target (19). Data on differentiated E-cadherin involvement in molecular subtypes of breast cancer are very few, most of the existing articles referring to breast cancer's classical histopathologic classification and not the molecular one.

Table II. *Correlation of E- and P-cadherin expression with G and the particularity of cytoplasmic expression of P-cadherin in triple negative breast cancer.*

	G	ECAD	ECADM	ECAD C	PCAD	PCADC
G						
Pearson correlation	1	–0.250	1.000**	–0.408	–0.559	–0.612
Sig. (1-tailed)		0.343	0.000	0.248	0.164	0.136
N	5	5	5	5	5	5
ECAD						
Pearson correlation	–0.250	1	–0.250	0.612	0.000	0.408
Sig. (1-tailed)	0.343		0.343	0.136	0.500	0.248
N	5	5	5	5	5	5
ECAD M						
Pearson correlation	1.000**	–0.250	1	–0.408	–0.559	–0.612
Sig. (1-tailed)	0.000	0.343		0.248	0.164	0.136
N	5	5	5	5	5	5
ECAD C						
Pearson correlation	–0.408	0.612	–0.408	1	0.456	0.667
Sig. (1-tailed)	0.248	0.136	0.248		0.220	0.110
N	5	5	5	5	5	5
PCAD						
Pearson correlation	–0.559	0.000	–0.559	0.456	1	0.913*
Sig. (1-tailed)	0.164	0.500	0.164	0.220		0.015
N	5	5	5	5	5	5
PCAD C						
Pearson correlation	–0.612	0.408	–0.612	0.667	0.913*	1
Sig. (1-tailed)	0.136	0.248	0.136	0.110	0.015	
N	5	5	5	5	5	5

*Significant correlation with a value less than 0.05. **Strong significant correlation with a value less than 0.005.

Table III. *E- and P-cadherin expression in HER2 positive cases. Note the particularity of the membrane expression of P-cadherin.*

	ECADC	PCAD	PCADMM	PCADC
ECAD C				
Pearson correlation	1	0.198	0.471	0.730*
Sig. (1-tailed)		0.335	0.143	0.031
N	7	7	7	7
PCAD				
Pearson correlation	0.198	1	0.910**	0.271
Sig. (1-tailed)	0.335		0.002	0.278
N	7	7	7	7
PCAD M				
Pearson correlation	0.471	0.910**	1	0.645
Sig. (1-tailed)	0.143	0.002		0.059
N	7	7	7	7
PCAD C				
Pearson correlation	0.730*	0.271	0.645	1
Sig. (1-tailed)	0.031	0.278	0.059	
N	7	7	7	7

*Significant correlation with a value less than 0.05 but higher than 0.005. **Strong significant correlation with a value less than 0.005.

P-cadherin or cadherin 3 encoded by the *CDH3* gene is less studied in breast cancer. In contrast, in other types of neoplasia, P-cadherin is recognized as a marker of cancer stem cells and, moreover, as a stimulating factor for local migration and the distance of neoplastic cells also favoring metastasis.

P-cadherin is a calcium-dependent cell adhesion glycoprotein, which plays a crucial role in preserving the structural integrity of epithelial tissues. Similar to other cadherin family members, P-cadherin regulates several cellular homeostatic processes that participate in embryonic development and maintain adult tissue architecture, being important for cell differentiation, cell form, cellular polarity, growth and migration (20-22). By distributing approximately 67% of homology with the E-cadherin protein, P-cadherin differs mainly in the extracellular portion and is much less characterized (23, 24).

Despite being expressed in human fetal structures (23, 25), P-cadherin is present in several adult tissues, usually co-expressed with E-cadherin, such as mammary gland and prostate, as well as mesothelium, ovary, cervix, hair follicle and corneal endothelium (26, 27).

Recent studies have clarified that P-cadherin expression is crucial to maintain a normal mammary gland epithelial architecture. LaBarge group used an antibody that specifically marks the mediated intercellular P-cadherin interactions in an *in vitro* human breast bone self-organizing test to show that the migration of mammary myoepithelial cells that occurred during the normal differentiation of both layers, was compromised (28). Furthermore, the use of P-cadherin-knockout isolated mammary cells by Andrew Ewald's group has recently shown that P-cadherin loss results in prematurely branched morphogenesis in matrigel and sustained enhanced dissemination in Type I collagen, indicating the importance of this adhesion molecule in maintaining normal mammary gland epithelial architecture (29).

It would be interesting to clarify the mechanisms behind P-cadherin-mediated homeostatic function in the normal breast, because the loss of this adhesion molecule can cause rupture of the myoepithelial cell layer and may lead to pre-

neoplastic lesions. Future cellular studies should provide information on the influence of P-cadherin on tissue architecture and cell form, and the mediation with cellular determinants and other junctional proteins. In breast cancer, P-cadherin has received more attention and the mechanisms that lead to tumour progression have been characterized on a large scale. Aberrant expression of P-cadherin is associated with high histological grade carcinomas as well as expression of well-established markers associated with poor patient prognosis such as Ki-67, EGFR, CK5, vimentin, p53 and HER-2, and negatively associated with hormone receptor expression (ER and PgR) (30-32). In fact, overexpression of P-cadherin is mainly found in the triple-negative and basal-like subgroup of breast cancer (32, 33) and is strongly correlated with the presence of *BRCA1* mutations (34). Interestingly, none of these reports showed a significant association with tumor size and metastasis in lymph nodes.

None of the aforementioned studies reported differentiated membrane, cytoplasmic or mixed expression of E- and P-cadherin in mammalian breast cancer forms, nor did they establish a correlation between E- and P-cadherin expression, much less a correlation between cytoplasmic/membranous or mixed expression.

Our results support a differentiated expression and coexpression of the two types of cadherins in distinct molecular subtypes of breast cancer. Triple-negative cases are characterized by the correlation between membrane E-cadherin and G. Furthermore, cytoplasmic expression of P-cadherin in triple-negative cases supports their mediated aggressiveness and cytoplasmic translocation of P-cadherin. Cytoplasmic expression of P-cadherin has been statistically significantly correlated with decreased survival in urinary bladder cancers (35). Also, Ribeiro and his collaborators demonstrated that overexpression of P-cadherin increases tumor cell motility and the number of cancer stem cells in triple-negative mammary tumors, and furthermore, by interacting with the SRC family of kinases potentiates these undesired effects. Inhibition of the P-cadherin/SRC pathway with dasatinib reduced tumor cell aggression *in vitro*, suggesting that P-cadherin represents a potential therapeutic target in triple-negative mammary tumors. The validation of these observations on human specimens is only at the beginning (36).

The interaction between HER2 and cadherins has been extensively studied in gastric cancers (37). The interaction of HER2 with E-cadherin, especially with its cytoplasmic domain, causes a destabilization of the interaction between E-cadherin and β catenin, an induction of the epithelium-mesenchymal transition and implicitly with resistance to trastuzumab therapy. For this reason, we can assume that the presence of co-expression of HER2 with cytoplasmic E-cadherin is a negative prognostic factor for HER2-positive breast cancer in terms of development of resistance to anti-HER2 therapy.

Moreover, the interaction between E-cadherin and HER2 causes increased metalloproteinase activity and stimulates tumour angiogenesis as well as tumour cell dissemination. E-cadherin/P-cadherin cytoplasmic expression in HER2 positive cells identifies a stem cell population responsible for the development of resistance to trastuzumab therapy. Our data correlate with those previously reported by Ribeiro and collaborators, on an *in vitro* breast cancer model, where P-cadherin function is essentially influenced by the presence and function of E-cadherin in tumour cells, and affects cancer progression and metastasis (38).

Conclusion

The study of E- and P-cadherin expression in the different molecular subtypes of breast cancer revealed significant variations. Differences in expression intensity and distribution have demonstrated the distinct involvement of the two cadherins in every breast cancer molecular subtype, but also within the same molecular type. The cytoplasmic expression of cadherin was considered to be an unfavorable prognostic factor, favoring mesenchymal-epithelial transition, progression, metastasis and development of resistance to therapy. The heterogeneity of E- and P-cadherin expression was observed within the same tumor, an aspect that suggests the existence of unstable tumor areas, including a particular and potentially increased invasion capacity with the risk of dissemination and metastasis phenotype. In the case of P-cadherin, the positive areas should be identified, being potential sources of stem cells and an adaptive mechanism to conventional and targeted therapy. The expression of E- and P-cadherin correlated with the tumor grade for Luminal type A, an aspect that has not been encountered before in mixed cases. In the HER2 type, the cytoplasmic expression of E-cadherin correlated statistically significantly with that of P-cadherin. The type of TNBC was characterized by the expression of P-cadherin, as well as by the statistically significant correlation between its expression and G. Luminal type B cases showed the highest expression variability of the two cadherins.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

Margan MM designed the study, collected FFPE specimens, evaluated the cases, performed statistical analysis and wrote the paper; Ceausu AR performed immunohistochemistry; Cimpean AM and Raica M evaluated cases and classified them into molecular subtypes, made immunohistochemical specimens' interpretation, performed statistical analysis and supervised the manuscript draft.

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