E-cadherin expression in invasive ductal carcinoma associates ultrastructural changes in desmosomes structure

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Abstract
The relationship between E-cadherin presence/absence and the integrity of the desmosomes at the same level becomes important regarding the discrepancies between E-cadherin lack of expression in severe invasive carcinomas and the desmosome activity. Purpose: In the present study, we have evaluated the presence of E-cadherin (EC) in 34 cases of metastatic or non-metastatic invasive ductal breast carcinomas (IDC) by immunohistochemistry followed by transmission electron microscopy (TEM) evaluation on samples prepared from paraffin sections. Materials and Methods: Our study has analyzed 34 paraffin blocks incoming from 20 cases with documented presence of metastatic invasion and 14 cases without metastases. All samples were processed and stained by classic Hematoxylin–Eosin, immunohistochemistry to detect EC presence and transmission electron microscopy. Results: Our results, even on a small pilot group, emphasize that EC presence is associated with the complete desmosomal integrity in non-metastatic cases, at least for the investigated areas. From the metastatic IDC cases, we have observed reduced EC expression in only three cases and important loss of desmosomal arrangement, mainly at the desmosomal plate level. Conclusions: This observation can issue the hypothesis that even in IDC cells that express EC, the invasive potential depend not only on EC-dependent junctions but also on the desmosome integrity. Also, correlated relationship between EC expression, potentially explored desmogleins, desmocollin, desmoplakin and plakoglobin expression and TEM ultrastructure can lead to a conclusion about the invasive potential of these malignant cells.

Keywords: desmosomes, invasive ductal carcinoma, E-cadherin.

Introduction
Compared to normal cells, malignant cells from breast tumors may show various phenotypic changes. Part of the main changes in the phenotypic membrane and submembrane protein expression refer to junctional complexes, here including adhesion belts and desmosomes. Molecular structure and ultrastructural appearance in the junctional complexes are changing once with malignant transformation and they usually represent markers for the malignant invasion and/or tumor aggression [1].

In mammary epithelial structures, the tissue integrity, cell organization in three-dimensional scaffolds and functional polarization is provided by accurate organization of various adhesion intercellular junctions [2, 3]. Adhesive intercellular junctions are always mediated by transmembrane glycoproteins and are classified in adhesion belts and spot desmosomes. Both adhesive junctions type consist of transmembrane glycoproteins called cadherins which are being connected either to an intracellular submembrane bundle of actin filaments – for the adhesion belts – or to the intermediate filaments – for the desmosomes. Adhesion belts are mainly important during embryonic stages for normal tissue development, function, and organization. Desmosomes, by their spotty structure, participate to reinforce intercellular contacts and are able to distribute shear and stress forces that may appear among the cells in an epithelium. This function is due not only to the particular structure of the desmosome, with a submembrane plate, connected at the same time to the transmembrane cadherins and to intermediate filaments on the cytoplasmic side, but also to the interconnected cytoskeleton fibers that are performing dynamic links between desmosomes found between two cells. Cell adhesion molecules that participate to these two types of junctions are definitely cadherins, divided into classic cadherins (E-cadherin being found in epithelial adhesion belts) and non-classic cadherins [4]. Among non-classic cadherins found in the desmosomes, desmogleins (1 to 4) and desmocollins (1 to 3) form the transmembrane contact area while proteins as plakoglobin and desmoplakin are forming the desmosomal plates, in which intermediate filaments are anchored [5, 6]. The desmosome presence between cells of the mammary epithelium was demonstrated during normal gland morphogenesis [7] while other authors mention that despite membrane presence of E-cadherin and associated proteins, a defined zonula adherens connecting ductal cells is lacking [1].

If we admit that polarity and adhesion during breast cancer progression may partially reproduce the normal gland developmental program, the relationship between E-cadherin presence/absence and the integrity of the desmosomes at the same level becomes important regarding the discrepancies between E-cadherin lack of expression in severe invasive carcinomas and the desmosome activity.
In the present study, we have evaluated the presence of E-cadherin (EC) in 34 cases of metastatic or non-metastatic invasive ductal breast carcinomas (IDC) by immunohistochemistry followed by transmission electron microscopy (TEM) evaluation on samples prepared from paraffin sections.

Materials and Methods

Our study has analyzed 34 paraffin blocks incoming from 20 cases with documented presence of metastatic invasion and 14 cases without metastases. Samples were processed and cut at 5 μm thick by a Microm HM 325 vertical microtome, then stained by classic Hematoxylin–Eosin. Examination and diagnosis was performed independently by two pathologists.

Immunohistochemical analysis was performed using an indirect Streptavidin-peroxidase. Five-μm sections were disposed on FLEX IHC Microscope Slides (Dako) slides, deparaffinized in two xylene baths for one hour in a thermostat at 56°C then rehydrated in graded alcohol baths. Primary antibodies were applied following blocking endogenous peroxidases by hydrogen peroxide 3% for 5 minutes and also following antigen retrieval by Target Retrieval Solution, pH 6.1 (Dako), in a coplin jar in a microwave oven for one minute. Primary antibodies (Mouse anti-human E-cadherin, Invitrogen, clone 4A2C7) were applied at 1:200 dilution, overnight, in humid chamber, at 4°C. Reaction development was performed by a LSAB+ kit (Dako, K0689) that includes a secondary antibody universally targeted on mouse/rabbit/goat. Following PBS washing, chromogenic substrate is applied (1 mL DAB in 60 μL hydrogen peroxide) and sections are incubated in the humid chamber for 30 minutes at room temperature. Mounting step was performed in balm of Canada. Examination was performed by an Olympus BX40 microscope.

For TEM samples, paraffin serial sections (consecutive to those used for immunohistochemistry) mounted on slides were dried overnight and then at 50°C for one hour. Sections were deparaffinized and rehydrated as previously in methods. A re-fixation step is performed by 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M PBS for one hour and was followed by 8% sucrose incubation overnight. Post-fixation was performed the next day by 1% osmium tetroxide for one hour. Dehydration was performed by graded ethanol and completed by two baths of propylene oxide for 1–2 hours. Following overnight drying in a dessicator, slides were placed on a flat surface and one drop of Epon 812 was applied over the section. On the top of this drop, a flat pre-hardened Epon block was applied. Following 60°C incubation overnight, the slide and the Epon block were briefly immersed in liquid nitrogen, and the block together with the section from the slide was detached. Blocks were trimmed by a Reichert ultramicrotome. Following control semi-thin Toluidin Blue stained sections on the light microscope, corresponding ultrathin sections (90 nm thick) were examined by TEM Philips CM100.

Results

From the investigated number of paraffin embedded breast tumors, we have chosen mainly invasive ductal breast carcinomas (Figures 1 and 2).

Thus, we have analyzed 34 paraffin blocks from 20 cases with known presence of metastatic invasion and 14 cases without metastases. All cases with metastases showed lymph node invasion while from the 14 cases without metastases, in eight cases staging procedure denoted T2–T3 tumor size and N1–2 grades lymph node involvement; for six cases, the tumor size was in T1–T2 range with N0 – no lymph node involvement.

Results from immunohistochemical analysis were evaluated independently by light microscopy.

Membrane staining was the only index to be considered and pattern was evaluated on a scale of 0 to 3, in which the scores of 0–1 were considered negative (Figure 3) while scores of 2–3 were considered positive (Figure 4). Cytoplasmic staining was inconstant and was excluded from assessment. Eventual positive control was considered for normal ducts and acini staining.

EC presence was observed in all cases of IDC without metastases, with no significant differences according to tumor size, node involvement or Nottingham grading. From the 20 cases with documented metastatic invasion, EC positivity included various degrees: three cases showed limited or no immunoreactivity, 17 cases were positive (score 2–3) for EC.
From the metastatic IDC cases, we have observed reduced EC expression in only three cases and important loss of desmosomal arrangement, mainly at the desmosomal plate level (Figure 5). The other 17 cases showed EC positivity (score 2) but with partial impairment of desmosomal integrity, showed by electron microscopy (Figure 6). Desmosomal plates in this partially impaired desmosomes were scarcely represented while the density of intermediate filaments was decreased.

Discussion

As demonstrated previously [8] tumor variables (tumor size, nodal status, PR status, and HER-2/neu status) are not significantly associated with EC loose expression, regarding all types of invasive carcinomas. Our observations were consistent with the observation that EC expression in almost all IDCs is slightly to moderate reduced, mainly associated with poor differentiation and high tumor grade [9–11]. We have observed poor correlation between EC presence and the lymph node status, despite literature data [12, 13].

As considered by Qureshi HS et al. [8] and Nurismah MI et al. [14], reduced staining in IDCs may be due to tumor degeneration. However, most studies [8, 9, 15–19] found poor correlation between EC presence and invasion degree or survival rate.

Our results, even on a small pilot group, emphasize that EC presence is associated with the complete desmosomal integrity in non-metastatic cases (Figure 4), at least for the investigated areas. However, in order to allege that cadherin-based junctions undergo correlated changes in the same way in the IDC cells, a further exploration of the focused molecular profile of the junctional complexes is required.

While desmosomes structure was impaired, as observed in electron microscopy, we issue the hypothesis that even in IDC cells that express EC, the invasive potential depend not only on EC-dependent junctions but also on the desmosome integrity. Also, correlated relationship between EC expression, potentially explored desmogleins, desmocollin, desmoplakin and plakoglobin expression and TEM ultrastructure can lead to a conclusion about the invasive potential of these malignant cells.

Thus, immunohistochemical and electron microscopy correlative studies indicate that reduction in the number of desmosomes is correlated with adhesive proteins expression in EC-dependent junctions only in IDCs with invasive and metastatic behavior. EC by itself represent an uncertain marker for adhesion loss while literature data indicated that loss of staining for main desmosomal
elements are corresponding to all cases of poorly differentiated carcinoma, which is related with invasion and lymph node metastasis.

Conclusions

We consider that there is a connection between EC staining and ultrastructural aspects of desmosomes showing the alteration of desmosomal structures and promoting metastatic and invasive behavior; these changes can be considered as prognostic markers. It is then required to extend correlated IHC and TEM studies to lymph nodes and metastases in order to emphasize potential relationship between EC expression and desmosomal integrity or desmosomal proteins expression (mainly desmogleins, desmocollin, desmoplakin and plakoglobin).

References


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