Immunomorphometric Study of E-caderin Expression in Malignant Skin Tumors

Varo Eniko[1], Egyed-Zsigmond I[2]

1 PhD student, University of Medicine and Pharmacy, Tîrgu Mureş, Romania
2 Department of Pathology, University of Medicine and Pharmacy, Tîrgu Mureş, Romania

Objective: Cadherins are transmembrane glycoprotein molecules, which interact with the cytoskeleton and their role is in cell recognition, tissue morphogenesis and tumor suppression. Our aim is to evaluate cell adhesion behavior in the main forms of skin cancer.
Methods: Between 2003–2007, we made a retrospective study on 150 cases of skin biopsies of basal cell, squamous cell carcinomas and malignant melanomas. In the Laboratory of Pathology of the Clinical Emergency County Hospital Tîrgu Mureş, the formalin-fixed paraffin-embedded tumoral tissues were studied with immunohistochemical (for E-cadherin) and morphometrical methods using a digital technique and statistical evaluation.
Results: Average percentages of immunopositive areas (APMIS) were obtained for both basal cell and squamous cell carcinomas, as well as for malignant melanoma. We showed a rising percentage of disorganization in the cell membrane, with decreased APMIS values, with the increasing degree of tumor malignancy evidenced by immunohistochemical reaction of cadherins.
Conclusion: Cell adhesion decreases with increasing malignancy in all three studied malignant tumors, especially in malignant melanomas.

Keywords: cell adhesion, cadherins, malignant skin tumors

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Introduction

Cadherins are calcium-dependent transmembrane glycoprotein molecules, whose interaction with the cytoskeleton (actin, as well as intermediate filaments) is provided by a series of intracellular proteins: alpha/beta/gamma catenin, vinculin, alpha actinin, plakoglobin and desmoplakin, forming a real transcellular network. Through their extracellular domain, they form various patterns of junctional adhesion (occluding, anchoring, communicating junctions) and nonjunctional patterns, with a particular spatial orientation, which explains cell polarization and stratification, i.e. the functional organization [1,2]. Cadherins have a role in cell recognition, tissue morphogenesis and tumor suppression. Lowering the level of cadherins leads to lymphatic and blood dissemination of tumor cells.

The keratinocytic system in the squamous stratified epithelium (from the so-called basal cells to the surface keratinocytes) shows desmosomal cadherins (D) such as desmocollin and desmoglein, and classic cadherins E and P, respectively. P cadherin is specific to basal and suprabasal layers, while cadherin E and catenins are characteristic of all viable keratinocytic cell layers. In squamous carcinomas, there is a proportional reduction of their expression, depending on the decrease of the degree of histological differentiation (E) and increase of invasiveness (D and P catenins) [3]. Reducing their expression involves suppression of a promoter, their destabilization by the lack of binding to catenins or mutations [4]. Decreased expression of the cadherins leads to tumor progression [5].

Our aim is to evaluate cell adhesion behavior in the main forms of skin cancer.

Correspondence to Eniko Varo
Email: varo_eniko@yahoo.com

Material and methods

A retrospective study of 150 skin biopsies performed between 2003–2007 processed in the Laboratory of Pathology of the County Emergency Clinical Hospital of Tîrgu Mureş, including 50 cases of basal cell carcinoma (BCC), 50 cases of squamous cell carcinoma (SCC) and 50 cases of malignant melanoma (MM). Three groups of malignancy in basal cell carcinomas were formed according to histopathological type: nodular forms Grade I, multicentric micronodular Grade II and infiltrative and morpheaforms Grade III. Also, on the basis of the malignancy grade, squamous cells carcinomas and malignant melanomas were divided in four stages.

Sections obtained from paraffin blocks were subjected to immunohistochemical staining with specific anti E-cadherin, E-cadherin Ab4 in 1:10 dilution, NCH-38 clone, producer Thermo Scientific Lab Vision USA, processed by digital morphometry to track the cell adhesion process.

A basic system for morphometry determinations consists of a digital camera for capturing digital images and a computer with software which manages, processes and carries out morphometry. All histological sections were digitized using a Zeiss MiraxScan scanning system for the slides (Carl Zeiss Jena GmbH, Jena Germany).

From each immunohistochemical scanned section, 5 digital photos of the so-called hot spot areas were taken and saved in JPG format (Joint Photographic Experts Group) using 3DHistech Panoramic Viewer program. This resulted in a total of 250 digital images. These underwent examination by digital morphometry.

The purpose of morphometry measurements was to assess the positive territory for a marker relative to the background tissue. In the measurements we used a colorimetric method, which is based on the color differences between the immunohistochemical marker, DAB (brown in color) com-

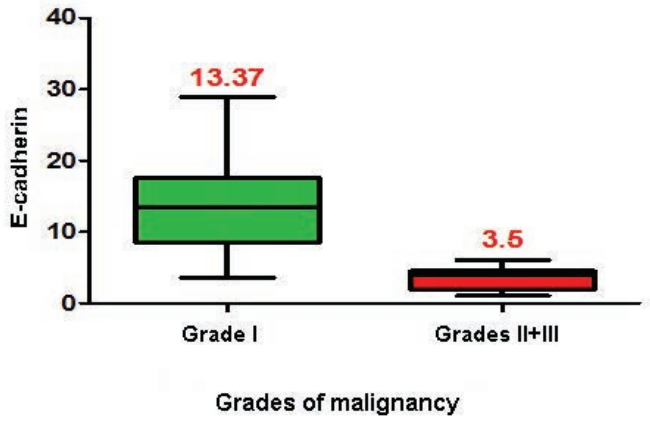

Fig. 1. Box-plot distribution into groups of malignancy of Average Percentage of Immunopositive Surface of the E-cadherin in basal cell carcinomas

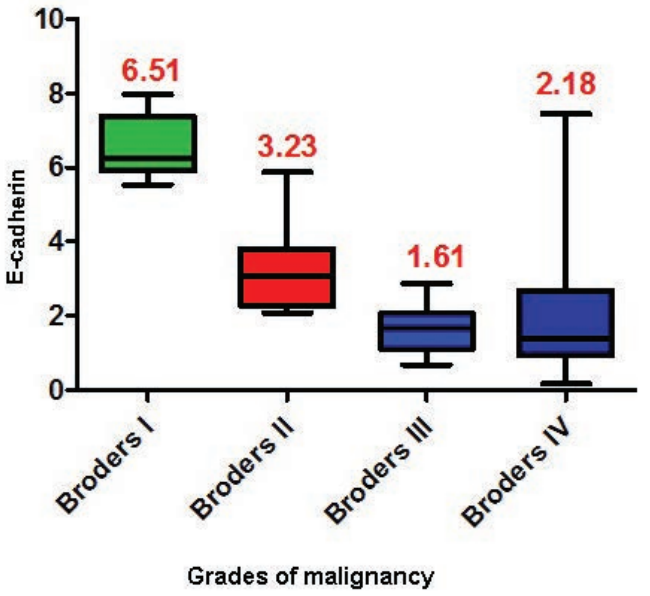

Fig. 3. Box-plot distribution of Average Percentage of Immunopositive Surface of the E-cadherin squamous cell carcinoma according to histopathological grade of malignancy

pared to the rest of the tissue, unstained by the marker used, which was stained with hematoxylin (blue). Thus, using ImageJ software we determined a ratio for each digital image, between the brown and blue (positive/ negative) areas, indicating the immunopositive surface (IS). This determination is based on the separation of brown areas by colorimetric filtering. Filtering is done by specifying a limit (a "threshold") for the brown color in terms of saturation, brightness, time/ image, total time, shade. This step is done manually. We used the same parameters for different markers, attached to the tables, to produce reproducible results. We obtained imunopositive surfaces values in five microscopic fields of the same section, from which we calculated the average of percentage of median imunopositive surfaces (APMIS).

Results

Our immunomorphometric study of cell adhesion in basal cell carcinomas presents membrane characteristic disor-

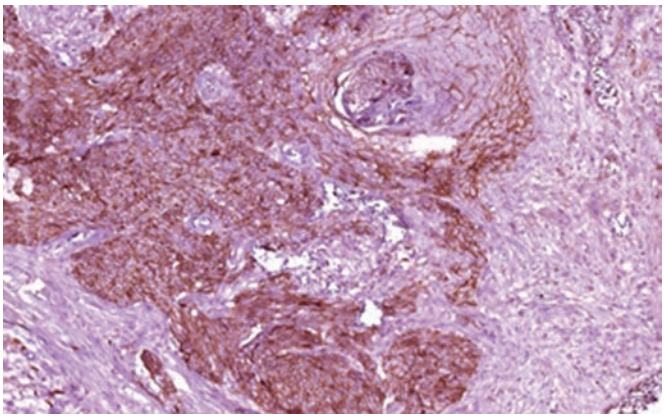

Fig. 2. Characteristic membrane aspects of E-cadherins. S140879 – Solid adenoid basal cell carcinoma gr. I. Ecad, ob 10x

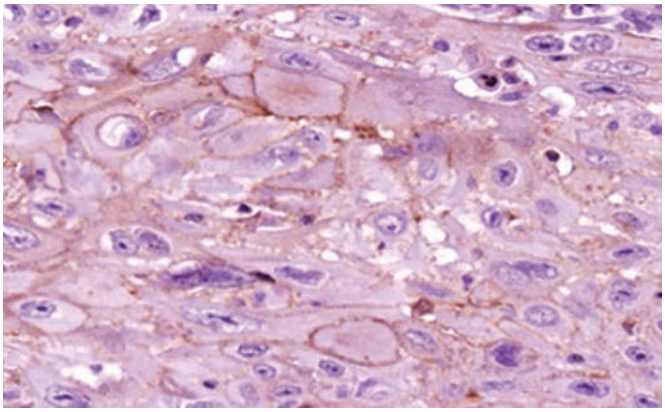

Fig. 4. Poor membrane positivity. S154212 – Squamous cell carcinoma Broders III. Ecad, ob 10x

ganization of E-cadherins. The APMIS values of E-cadherins present in the tumor parenchyma decreased from the less invasive histopathological forms to the most aggressive ones (Table I), and by applying Student statistical test: p less than 0.0001, i.e. significant statistical differences of the APMIS values in the three degrees of malignancy (Figure 1).We found characteristic membrane aspects of E-cadherin (Figure 2) and sometimes reduced expression, especially in infiltrative or morpheaform types.

Table I. Mean PMSI values in the antigen used to study cell adhesion in basal cell carcinomas

Mean /grade of malignity	I	II	III
Ecad	13.37	4.14	2.71

Table II. Values of PMSI means in antigen cellular adhesion of squamous cell carcinomas

Mean /grade of malignity	I	II	III	IV
Ecad	6.51	3.23	1.61	2.18

Table III. Mean values of PMSI in the antigen used to study cell adhesion in malignant melanomas

Mean /grade of malignity	I	II	III	IV
Ecad	10.55	5.76	1.78	0.23

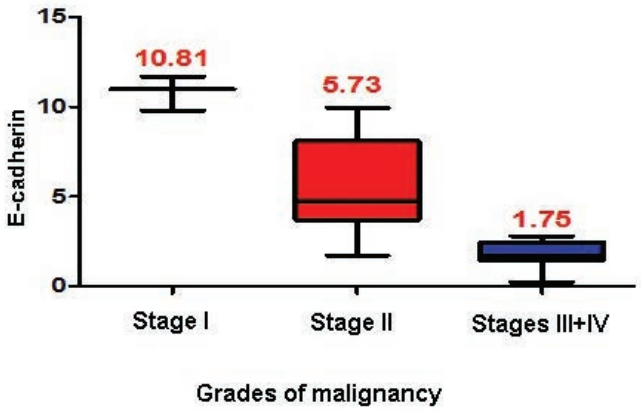

Fig. 5. Box-plot distribution of Average Percentage of Immunopositive Surface of the E-cadherin in malignant melanomas according to the groups of malignancy

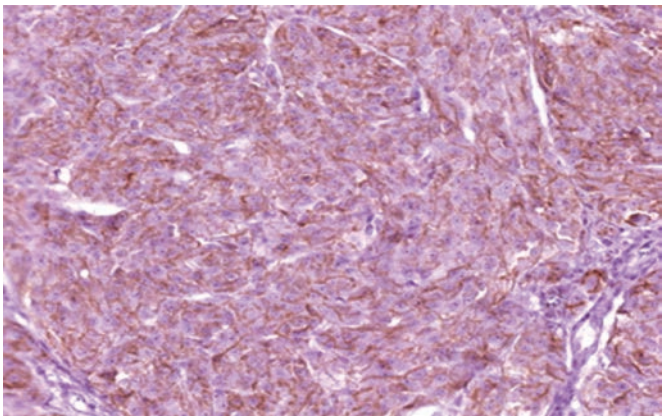

Fig. 6. Discontinuous membrane positivity. S15261 – Malignant melanoma gr. II. Ecad, ob 10x

Immunomorphometric study of cell adhesion presents poor membrane positivity in squamous cell carcinomas. By applying the ANOVA test, we identified a statistically significant difference between the E-cadherin APMIS values according to the four grades ($p<0.0001$, Figure 3). We recorded a decrease in the value of E-cadherin APMIS levels from lower to higher grades of squamous cell carcinomas. The decrease of E-cadherin APMIS values, parallel with the increase of malignancy, is associated with disintegration of membrane structure (Table II). Our imunohistochemical study showed decreased membrane positivity, parallel with increased malignancy (Figure 4).

Immunomorphometric study of cell adhesion presents weak membrane positivity in malignant melanomas. Table III shows the net decrease of E-cadherin APMIS values simultaneously with the transition to higher grades of malignancy. The ANOVA test reveals a statistically significant difference between the E-cadherin APMIS values according to grades of malignancy ($p<0.0001$, Figure 5). The decrease of E-cadherin levels is more evident in malignant melanomas (Figure 6).

Discussions

The expression of E-cadherins decreases in infiltrating, morpheaforms of basal cell carcinomas [6]. The loss of E-cadherin expression was more pronounced in poorly differentiated carcinomas [7] and sometimes totally absent [8]. Loss of expression of E-cadherin leads to the commencement of the invasion of squamous cell carcinomas [9]. Losing E-cadherin expression is a critical point in tumor progression [10]. E-cadherins are found in melanocytes, nevic cells, show low values in primary tumors, and are absent in the invasive and metastasizing forms of malignant melanomas [11]. With the loss of E-cadherin expression, malignant transformation occurs not only in skin tumors, but also pharyngeal, gastric, colon, and breast carcinomas. Melanomas with low levels of E-cadherin indicate the appearance of metastases and a poor survival rate [12,13]. Malignant transformation of melanocytes coincides with the loss of E-cadherin expression [14,15]. Similar to the E-cadherin expression in normal epidermis, E-cadherin was strongly expressed in all samples of BCC on the cell borders, whereas marked decrease or loss of E-cadherin expression was found in the tumor cells of SCC, Paget's disease, and Bowen's disease (invasive type). On the other hand, E-cadherin expression of trichilemmal carcinoma was slightly reduced. Considering the clinical and histological features of these skin carcinomas, the reduction of E-cadherin expression is considered to be associated with the invasion and metastasis of human skin carcinoma [16]. Melanoma cells at initial stages of the disease show reduction or loss of E-cadherin expression, but the recovery of its expression is frequently found at advanced phases [17]. Many studies showed that epithelial E-cadherin expression is reduced in squamous cell carcinoma [18]. Loss of E-cadherin is an important marker for tumoral progression and shortened survival [19]. Decreased E-cadherin expression activates an invasive, aggressive tumoral phenotype [20]. The loss of expression of E-cadherin in basal cell carcinoma is a marker of malignant behavior [21]. Loss of E-cadherin expression show malignant transformation of melanocytes [22]. The most important event in the development in the malignant melanoma is the loss of the tumor-supression protein E-cadherin [23].

Conclusions

Compared to other immunohistochemical reactions, the study of cell adhesion does not contribute to assessing the prognosis of the disease, because cellular adhesion decreases along with the increase in malignancy in all three skin tumors studied.

The decrease of E-cadherin APMIS levels is more obvious in malignant melanomas where, in the higher stages of the disease, the expression is almost lost.

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