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Original Article

Prognostic value of ezrin expression in common epithelial tumors: An immunohistochemical study

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ABSTRACT

Ezrin is a member of the ezrin–radixin–moesin family of proteins. Overexpression of ezrin protein might play an important role in the process of tumor cell invasion and metastasis. Non-melanoma skin cancer (NMSC) is the most common form of cancer seen in population. The term NMSC can theoretically be applied to all cutaneous cancers excluding melanoma. The aim of the study was to evaluate the prognostic value of ezrin expression in basal cell carcinoma (BCC), cutaneous squamous cell carcinoma (SCC), seborrheic keratosis (SK) and keratoacanthoma (KA). This retrospective study included 76 paraffin blocks classified into: group I (20 paraffin blocks of BCC), group II (20 paraffin blocks of SCC), group III (12 paraffin blocks of SK), group IV (14 paraffin blocks of KA) and group V (10 paraffin blocks of normal healthy skin were used as a control group). All were subjected to the ordinary hematoxylin and eosin stain (H&E) and immunohistochemical staining for ezrin. This study revealed a statistically significant difference between ezrin expression in different tumor groups and controls. The expression of ezrin in SCC was higher than in BCC, SK and KA. There was a statistically significant difference of ezrin expression in different tumor groups regarding both membranous and cytoplasmic expression of ezrin. The current study suggested that dysregulation of ezrin may be important in the development of cutaneous epithelial malignancies and tumor grade. Immunohistochemical localization of ezrin may be useful marker in the differentiation between cutaneous SCC and KA.

1. Introduction

Ezrin is a member of the ezrin–radixin–moesin (ERM) family of proteins, which link the actin-containing cytoskeleton to the plasma membrane. It is functionally involved in signaling events that regulate cell survival, cell proliferation, migration, and cellular morphogenesis. Ezrin is expressed in a variety of normal and neoplastic cells, including many types of epithelial, lymphoid, and glial cells [1]. Overexpression of ezrin protein is correlated with the metastatic potential in several cancers [2–4] through regulating adhesion molecules and signal transduction pathways and affection of cell–cell and cell–matrix interactions [2].

Basal cell carcinoma is the most common skin neoplasm in humans, and is usually characterized by local aggressiveness with a slight metastatic potential. BCC is a slow-growing tumor but, if not treated properly, the invasion of subcutaneous adipose tissue, muscle, cartilage and even bones may occur [5]. During the process of infiltration, neoplastic cells have to pass through various barriers such as the extracellular matrix, interstitial tissue and basement

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membrane [6]. Cutaneous SCC is a malignant tumor that arises from the keratinizing cells of the epidermis or its appendages. It is locally invasive tumor and has the potential to metastasize to other organs of the body [7]. SKs are non-cancerous (benign) skin growth that some people develop as they age. They often appear on the back or chest, but they can occur on any part of the body. SK grows slowly, in groups or singly [8]. KA is a rapidly growing skin tumor which is characterized by a distinct keratin-filled cup-shaped appearance, and spontaneous regression is a unique clinical feature. Most KA cause only minimal skin destruction, but a few behave more aggressively and can spread to lymph nodes. In addition, the histopathological findings of KA are occasionally indistinguishable from those of well-differentiated SCC, leading some authors to believe that KA is a subtype of SCC [9,10].

Little is known about the distribution of ezrin in normal epidermis and NMSC; therefore there is a need to study the expression of ezrin in normal skin and epithelial skin tumors and to explore its value.

2. Materials and methods

The current retrospective case controlled study was conducted on 76 cases with available paraffin blocks and clinical data collected from the archives of the Pathology Department, Faculty of Medicine, Tanta University Hospitals. The blocks were divided into: group I (20 paraffin blocks of BCC), group II (20 paraffin blocks of SCC), group III (12 paraffin blocks of SK), group IV (14 paraffin blocks of KA) and group V (10 paraffin blocks of normal healthy skin were used as a control group). The control group was age and sex matched to the cases whose paraffin blocks were the subjects of the current study. Specimens of the control group were obtained from the normal skin specimens received during plastic operations. All blocks were sectioned and stained with H&E for detection of general histopathological criteria and immunohistochemistry for ezrin, by the following steps.

3–5 μm sections were deparaffinized in xylene, and then rehydrated in descending concentration of alcohols [ethanol]. Sections were incubated in 0.3% H_2O_2 for 30 min to block endogenous peroxidases. Slides were then washed with phosphate-buffered saline (PBS) and heated in an 830-W microwave oven for at least 15 min in 10 mM sodium citrate buffer (pH 6.0) for antigen retrieval. Sections were incubated with the primary antibody (Mouse, anti-ezrin Monoclonal antibody dilution 1:100, Lab Vision Corporation Fremont, CA, USA; Catalog #MS-661-P0) overnight at 4 °C. For the negative control, the primary antibody was replaced with PBS. Biotinylated Goat anti-polyvalent, ready to use available form (Lab Vision Corporation, USA) secondary antibody was added followed by incubation for 10 min at room temperature. The color was developed using diaminobenzidine (DAB) as a chromogen. Slides were extensively washed with PBS after each step. Finally they were counter-stained with Meyer's hematoxylin. The internal positive control was the sweat glands in each section. The ezrin showed cytoplasmic expression. The stained sections were scanned using the 4× objective lens to allocate the areas of highest positivity was evaluated in the lesional squamous epithelial cells, basaloid cells when appropriate as well as the adjacent epidermis (when available) and the underlying inflammatory infiltrate. For each of these 3 components; the intensity was evaluated using the positivity of the sweat glands epithelium. The percentage of positive cells was determined in 10 high power fields and the mean percentage for each type of cells was recorded separately in each section. The overall intensity of the stain in each section was assessed subjectively, in relation to the positivity of the sweat glands. Then the extent of positivity for both components were scored as follow: 0: no immunoreactivity; 1: <10% of the cells positive; 2: 10–25% of cells stained; 3: 25–50% of cells stained; 4: more than 50% of cells stained. In healthy controls the immunoreactivity limited to sweat glands which considered non-specific reaction which was used as a reference where the investigated cells were considered positive if the intensity of their staining is higher than the intensity of the sweat glands [11]. The intensity of the stain was classified into negative (score zero = the intensity of the sweat gland staining), mild (score 1), moderate (score 2) and intense (score 3). Score 1, 2 and 3 were higher than the sweat gland staining intensity. An immunoreactivity index was obtained by multiplying the extent by the intensity of staining and the range of score was; negative: 0–3, mild >3–5, moderate >5–9, strong >9 [11].

2.1. Statistical analysis

All data obtained were transferred to the statistical package for the social sciences version 15 (IBM Co., New York, USA) for analysis. Data were summarized using mean, standard deviation (mean ± SD) for quantitative variables. Comparison between non-parametric quantitative variables were made by Kruskal–Wallis ANOVA test. Statistical significance was determined at a level of $P < 0.05^*$ and highly significance at a level of $P \leq 0.001^{**}$.

3. Results

The demographic data of the studied groups were represented in Table 1.

3.1. Histopathological results

Histopathological data of the tumors were represented in Table 1 and from Figs. 1a–4c.

3.2. Immunohistochemical results

In healthy controls, the ezrin immunoreactivity limited to sweat glands epithelium which considered non-specific negative reaction (Fig. 5). The ezrin lesional score expression in SCC ranged from 2 to 9 with mean ± SD 6.40 ± 2.91, in BCC, it ranged from 3 to 9 with mean ± SD 6.30 ± 1.70, in SKs it ranged from 1 to 6 with mean ± SD 3.00 ± 2, in KA it ranged from 4 to 9 with mean ± SD 6.29 ± 2.06 and in control group it ranged from 2 to 3 with mean ± SD 2.40 ± 0.55 there were statistically highly significant differences when comparing its expression between different tumor groups and controls ($P = 0.001$). The highest mean value of lesion