Original Paper

Ezrin Immunoexpression in Prostate Adenocarcinomas

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ABSTRACT: Ezrin is a component of cell surface structures, the most important member of the Ezrin/radixin/moesin family. In this study, we aimed to evaluate the expression of ezrin in 50 cases of prostate carcinoma (PA) in relation to the ISUP (International Society of Urological Pathology) groups. Ezrin expression analysis was identified in 78% of the PA cases investigated, with predominantly cytoplasmic staining pattern and variable intensity. Overall, we observed an increase in the intensity of the immunostaining progressively with the decrease in cell differentiation. Statistical analysis indicated the predominance of high FSS in the ISUP 4-5 groups and low FSS in the ISUP 1-2 groups, aspects that were statistically significant. Ezrin was expressed in the majority of PAs analyzed and its expression was associated with ISUP grades, an aspect that suggests involvement in PA progression.

KEYWORDS: *Prostate adenocarcinoma, ISUP groups, ezrin.*

Introduction

Ezrin (villin2), the product of the Vil2 gene, is the most important member of the Ezrin/radixin/moesin (ERM) family, phosphorylated by threonine and tyrosine kinases [1].

It has a role in mediating signal transduction, coordinating dynamic cellular processes and acts by reorganizing the cytoskeleton [2].

It also plays a role in connecting membrane proteins and actin filaments [2,3], being considered a membrane-cytoskeleton linker protein, involved in the organization of the cytoskeleton [4].

Ezrin is present in the cytoplasm in an inactive form, but after threonine and tyrosine phosphorylation, ezrin acquires an active form, moving to the cell membrane and binding F-actin to the cell membrane [2].

The oncogenic role of Ezrin has been intensively studied in cancers with different locations, several studies indicating Ezrin as an important signaling molecule, with a vital role in the tumorigenesis of some epithelial and non-epithelial neoplasias [5-7].

It is well documented the association of ezrin with processes such as cell proliferation and adhesion, cell motility and signal transduction [1,8], through interaction with adhesion molecules and various growth factor receptors [9,10].

Ezrin has also been shown to intervene in multiple signaling pathways [11], in certain cancers mediating cell growth and survival through Akt signaling but not the mitogen-activated protein kinase (MAPK) pathway [12,13].

In breast and prostate cancers (PA) it participates in the activation of MAPK and PI3K [14].

It has been suggested that the increased expression of ezrin in PA is the result of increased expression of the Myc oncogene [15], as Ezrin can regulate the level of Myc through the PI3K/Akt pathway, creating a positive feedback.

In this study we aimed to evaluate the expression of ezrin in PA in relation to the ISUP groups, as well as the possible correlations between them.

Materials and Methods

The study included a number of 50 cases with the diagnosis of PA.

We analyzed the tissue specimens obtained through transurethral prostatic tumor resection, from patients hospitalized in the Urology Department of the Emergency County Clinical Hospital Craiova.

The tumor fragments were fixed in 10% buffered formalin, processed by the usual paraffin embedding technique and stained with Hematoxylin-Eosin.

Serial sections were made from paraffin blocks and processed immunohistochemically with an amplification polymer detection system (EnVision™ FLEX+System, code K8002, Dako).

10.12865/CHSJ.48.04.08

The reactions were visualized with DAB chromogen (3,3′-diaminobenzidine, cod 3467, Dako), and to validate the reactions we performed positive and negative external controls, omitting the primary antibody.

We followed the quantification of Ezrin expression (clone 3C12/Invitrogen, dilution 1:150) in relation to ISUP groups, assessed according to WHO/ISUP recommendations [16].

The analysis of Ezrin semi-quantitative expression was carried out by two specialists (CES, AES), based on a system adapted from specialist literature [4].

We graded the intensity of the score with 1 (mild), 2 (moderate) and 3 (high), with a cut-off value for reaction positivity of 5%, as well as the percentage of stained cells that was scored as 1 (6-25% positive cells), 2 (26-50% positive cells), 3 (51-75% positive cells) and 4 (>75% positive cells).

Subsequently, by multiplying the two scores, we calculated the final staining scores (FSS), which we rated as low for values 1-4 and high for values 6-12.

The mean final immunostaining score (FSSm) was then calculated for each category.

We performed the statistical analysis using comparison tests ($\chi 2$ test) within SPSS10 (Statistical Package for the Social Sciences) automatic software.

The study was approved by the Local Ethics Committee, the written informed consent being obtained from patients.

Results

The histopathological analysis of the 50 PAs indicated: in 16 cases tumors corresponding to ISUP grade 1, in 7 cases tumors corresponding to ISUP grade 2, in 4 cases to ISUP grade 3, in 10 cases to ISUP grade 4 and in 13 cases to ISUP grade 5.

The immunoreaction for Ezrin was identified in 39 of the PA cases investigated (78%), with a predominantly cytoplasmic and focal basolateral staining pattern at the level of the cytoplasmic membrane, in the epithelial component of the tumors, with variable intensity (Table 1).

Table 1. Ezrin expression according to ISUP groups.

ISUP group	Total cases / Positive cases	Mean value (%) of labelled cells	Intensity	FSSm
1	16 / 12	35±9.2	1, 2, 3	3.6
2	7 / 5	46±11.9	1, 2, 3	5
3	4 / 2	47.5	2, 3	5
4	10 / 9	61.6±10	1, 2, 3	6.4
5	13 / 11	67.2±10	2, 3	7.4

We observed a general increase in the intensity of immunostaining in PA, progressively with the decrease in cellular differentiation, respectively with the increase in the ISUP degree groups.

Regarding the percentage of positive cells, we found for ISUP 1 tumors an average percentage of positive cells of 35±9.2, with variations between 15-50% of the tumor cells (Figure 1A).

In PA corresponding to ISUP 2 we identified 46±11.9 positive cells, with variations between 30-60% of neoplastic cells (Figure 1B).

ISUP 3 tumors had an average percentage of positive cells of 47.5%, with variation between 45-50% of neoplastic cells (Figure 1C).

ISUP 4 tumors had an average percentage of positive cells of 61.6±10, with variations between 45-75% of tumor cells (Figure 1D).

In the case of ISUP 5 tumors, they presented 67.2±10 positive cells, with variations between 50-80% of tumor cells (Figure 1E).

The analysis of Ezrin expression depending on the number of labeled cells and the intensity of the reactions for the selected tumors, highlighted different FSSm values according to the ISUP groups.

We observed FSSm values of 3.6 for PAs corresponding to ISUP group 1, for tumors classified in ISUP groups 2 and 3 it was 5, for those corresponding to ISUP 4 it was 6.4, and 7.4 for PAs included in ISUP group 5.

Statistical analysis indicated the predominance of high FSS in the ISUP 4-5 groups and low FSS in the ISUP 1-2 groups, while for the ISUP 3 group the FSS values were balanced, aspects that were statistically significant ($p=0.004$, $\chi 2$ test) (Figure1F).

10.12865/CHSJ.48.04.08

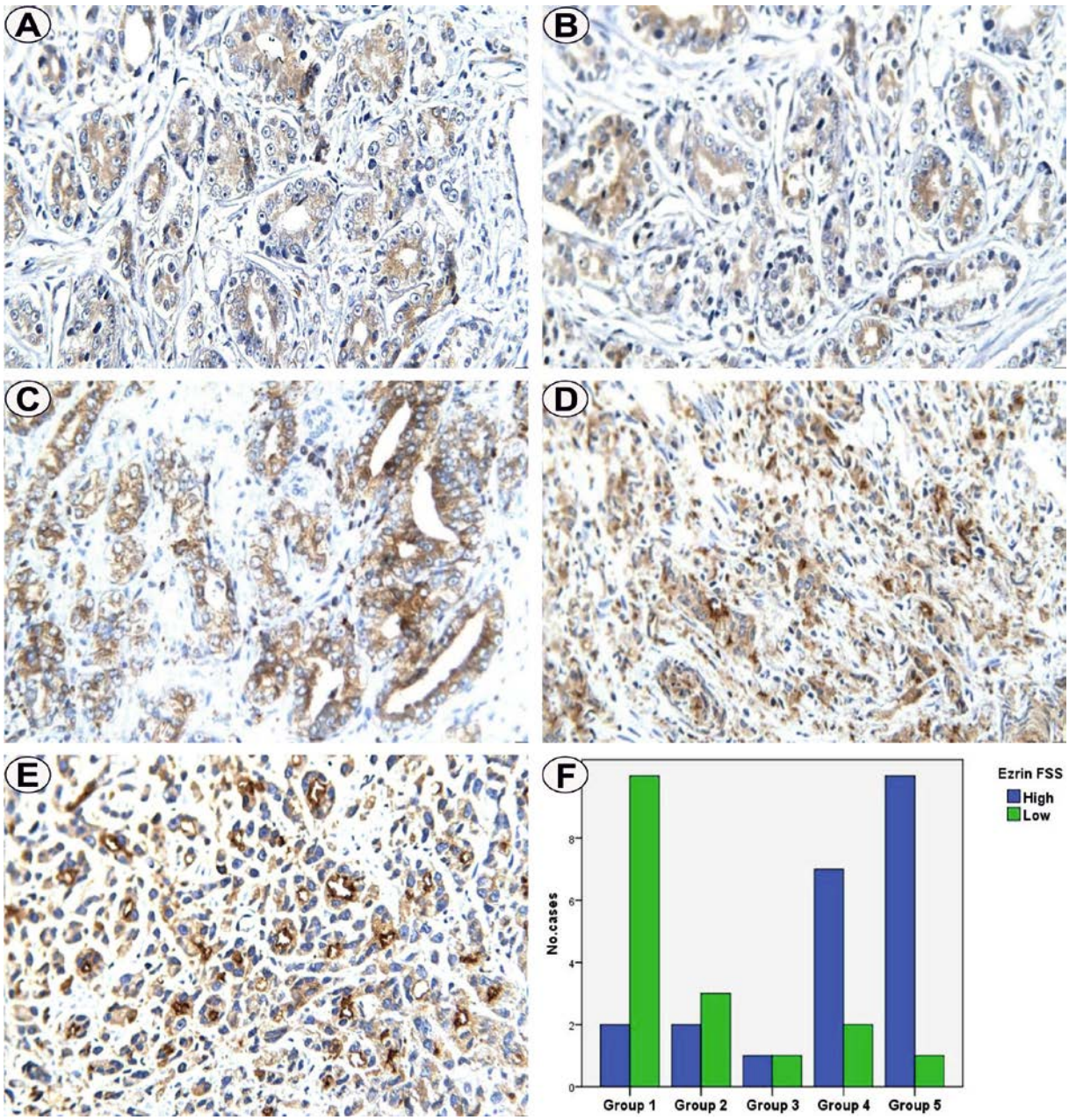

Figure 1. Ezrin immunoexpression in PA, x400: A. ISUP 1 (3+3) group; B. ISUP 2 (3+4) group; C. ISUP 3 (4+3) group; D. ISUP 4 (3+5) group; E. ISUP 5 (5+4) group; F. Distribution of cases in relation to Ezrin FSS and ISUP grading groups.

Discussion

Several studies have demonstrated that Ezrin is an oncogenic protein, as high Ezrin expression is associated with metastatic behavior in various types of cancer [4].

In pathological conditions, Ezrin proteins are regulated and activated so that they can promote cancer progression and metastasis, including in PA [4,17].

Many immunohistochemical studies have reported the overexpression of Ezrin in high-grade prostatic intraepithelial neoplasia (HGPIN) and advanced PA, the authors concluding that Ezrin may be important in the tumorigenesis of precursor lesions, and may play a role in triggering invasiveness [18,19].

In a large study that included more than 5000 human cancers, including PA, the immunohistochemical expression of Ezrin compared to normal tissues was reported, with the authors noting that PA revealed the highest expression of Ezrin among all epithelial cancers analyzed [20].

However, due to the uniform staining of Ezrin and the relatively high Gleason score of the PA analyzed, there were no associations

between Ezrin expression and Gleason score [20].

Valdman A et al. reported Ezrin expression in PA as moderate or intense in 70% of specimens, and negative or only weakly positive in benign epithelium, correlated with Gleason score and seminal vesicle invasion, but not with extraprostatic extension or resection margin status [19].

In our study, we identified positivity in 78% of selected PAs, with predominantly cytoplasmic staining pattern and variable intensity.

We also found the predominance of high FSS values in advanced ISUP groups, aspects that were statistically significant.

Several studies on the cytoplasmic expression of Ezrin in carcinomas with various locations have reported that it was associated with a more aggressive behaviour and unfavorable evolution [6,21,22].

Also, a number of studies have reported that Ezrin may be a potential prognostic marker, overexpression being associated with poor prognosis in a variety of solid, epithelial and non-epithelial tumors, and a key modulator of metastasis [23-26].

In PA its overexpression was associated with shorter survival and disease progression [18].

Conclusions

Ezrin was expressed in most analyzed PAs and its expression was significantly correlated with ISUP grades.

As a result, Ezrin has a role in PA biology and requires further studies to be validated as a marker of tumor progression and as a potential therapeutic target.

Conflict of interests

None to declare.

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