The immunohistochemical aspects of protein Mena in cervical lesions

SIMONA GURZU1), I. JUNG1), I. PRANTNER2), L. CHIRA1), I. EMBER2)
1) Department of Pathology, University of Medicine and Pharmacy of Targu Mures, Romania
2) Department of Public Health, University of Pecs, Hungary

Abstract
The proteins Ena/VASP (Enabled/Vasodilator-Stimulated Phosphoprotein) family is involved in the regulation of actin cytoskeleton which activity is very important for physiological tissue formation but is sometimes implied in pathological processes. In this study, we analyzed the immunohistochemical expression of Mammalian Ena (Mena), which is a member of this family, in the cervical intraepithelial neoplasia (CIN) and also in the cervical squamous cell carcinomas. We analyzed 30 cases with CIN (1, 2 and 3), and 10 squamous carcinomas. We used the EnVision system by LabVision. The Mena antibody, izotype mouse IgA, clone 21, provided by BD Biosciences. Results: We observed that Mena was not expressed in the normal cervical squamous epithelium but its expression was increased in parallel with the increasing grade of CIN, with up-regulation upon transition to CIN3 and further to invasive carcinoma. This is the first study in the literature about Mena expression in cervical lesions.

Keywords: cervical squamous carcinoma, cervical dysplasia, Mena immunostain.

Introduction
The actin cytoskeleton plays an important role in the embryonic life but also in the adult life in some processes like immune response or hemostasis. The deregulations of this system could contribute to the pathogenesis of thrombosis, arteriosclerosis, cardiomyopathy and nephritis [1]. The actin nucleation and polymerization depends by the following proteins: N-WASP/Arp2/3 (Wiskott Aldrich Syndrome Protein/Actin related protein), DRFs (Diaphanous-Related Formins) and Ena/VASP proteins (Enabled/Vasodilator-Stimulated Phosphoprotein). The Ena/VASP family members are mammalian Ena (Mena), VASP and Ena-VASP-like (EVL) [2]. The role of Mena in cervical carcinogenesis, tumor invasion and its possible prognostic and predictive role in cervical carcinomas was not studied yet. In this paper, we analyzed the Mena immunostain in cervical intraepithelial neoplasia (CIN) and also in cervical carcinomas.

Material and Methods
Biopsic specimens from 40 women with cervical lesions (30 with dysplasia and 10 with squamous carcinomas) diagnosed in the Department of Pathology of Emergency Hospital of Targu Mures, Romania, were used for IHC staining. We made a retrospective study, the biopsic specimens being paraffin-embedded. According with the criteria of World Health Organization (WHO), the lesions were classified in [3]:
• Cervical intraepithelial lesions (CIN) grade 1 (mild dysplasia, low grade CIN) – maturation is present in the upper two-thirds of the epithelium, and the superficial cells contain variable but usually mild atypia. Nuclear abnormalities are slight. Mitotic figures are not numerous and are present in the basal third.
• CIN grade 2 (moderate dysplasia, high grade CIN) – maturation in the upper half of the epithelium and nuclear atypia in the upper and lower epithelial layers. Mitotic figures are present in the basal two-thirds of the epithelium.
• CIN grade 3 (severe dysplasia, high grade CIN) – maturation may be absent or confined to the superficial third of the epithelium. Nuclear abnormality is frequent. Mitotic figures are present in all levels of the epithelium.
• Early invasive squamous cell carcinoma – the extent of stromal invasion is minimal.
• Squamous cell carcinoma (keratinizing and non-keratinizing).

From the cases with dysplasia, 10 were CIN grade 1, 10 CIN grade 2, the other 10 cases being CIN grade 3. The median age was 59 years, range 40–75 years. All carcinomas were invasive non-keratinized squamous cell carcinomas. Three cases presented lymph node involvement.

The biopsic pieces were fixed in 10% formalin solution, included in paraffin, and sectioned at 3–5 µm thickness. For the immunohistochemical staining, we used the murine Mena antibody, izotype mouse IgA, clone 21, provided by BD Biosciences and the EnVision system by LabVision. After dewaxing and dehydration, it was performed the antigen retrieval in citrate solution, pH 6, at 100°C. After that, we performed the endogenous peroxidase blocking through hydrogen peroxide.
incubation. The pieces were incubating with primary antibody, dilution 1:25, for 60 minutes. It followed the incubation with Primary antibody Enhancer Solution for 20 minutes and with Large Volume HRP Polymer Solution for other 30 minutes. The development was performed with substrate-chromogen solution 3,3’-diaminobenzidine dihydrochloride (DAB) for 3–5 minutes. The nuclei were stained with Mayer’s Hematoxylin. We used like external and internal positive control the smooth muscle cells (Figure 1).

The Mena antibody marked the cytoplasm cell. In the cervical lesions, the Mena immunostaining was observed in the cytoplasm of the tumoral and dysplastic cells.

The intensity of Mena was scored according to the following criteria: score 0, no staining; score 1+, weak diffuse cytoplasmic staining <10% of cells; score 2+, moderate cytoplasmic staining in 10–70% of cells; score 3+, strong cytoplasmic staining >70% of cells.

The pictures were realized in JPEG format with Nikon 800E microscope.

The small number of cases did not allowed to made statistical correlations.

Results

Mena immunostain was not observed in the normal cervical squamous epithelium, but its intensity was increased in parallel with the grade of CIN. In the cases with CIN grade 1, Mena expression was observed only in the lower third layers of squamous epithelium, but in CIN grade 3 its expression was observed in all layers of this epithelium. All cervical squamous cell carcinomas presented overexpression for Mena protein (Figures 2–6).

Figure 1 – The Mena expression in the smooth muscle cells like external positive control (ob. ×4).

Figure 2 – The absence of Mena expression in the normal squamous cervical epithelium (ob. ×10).

Figure 3 – The Mena expression in the lower third layers of squamous epithelium in CIN grade 1 (ob. ×10).

Figure 4 – The Mena expression in CIN grade 2 (ob. ×10).

Figure 5 – The overexpression of Mena in the entired squamous epithelium in CIN grade 3 (ob. ×10).
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We should mention that the Mena expression was observed in the cytoplasm of tumoral and dysplastic cells, but it did not mark the stromal cells. The intensity was homogenous and did not presented differences between the microscopic fields. In the carcinomas, the intensity was 2/3+ in seven cases, and 3+ in the other three cases. From CIN, the intensity was 1+ in CIN grade 1, 1/2+ in CIN grade 2, respectively 2+ in CIN grade 3. The number of cases is too small to elaborate a definite conclusion.

Regarding the correlation with clinico-pathological features, we observed that the intensity of Mena was 3+ in the carcinomas with lymph node involvement and 2/3+ in the other carcinomas. To have a statistical correlation is necessary to enlarge the number of cases.

Discussion

The actin cycle deregulation could be determined by actin mutation or Ena/VASP alterations. The consequence could be the changing of the mobility cells and the carcinogenesis but the exact mechanism is not known yet. The Mena activity, a member of Ena/VASP family, could be observed through immunohistochemical reactions with Mena antibody.

Recent studies revealed that the Mena expression could immunohistochemically observe in different premalignant and malignant lesions. Di Modungo F et al. [4] revealed that Mena play a role in breast carcinogenesis. In our previous study, we observed that Mena was not expressed in normal mucosa of colon neither in the adenomatous polyps without dysplasia, but was overexpressed in the glands with severe dysplasia, and also in 80% of colorectal carcinomas [5]. In the most recent study, Pino MS et al. [6] revealed the overexpression of Mena in primary and metastatic pancreatic carcinomas and suggested that the expression of Mena could be a predictive factor of EGFR inhibitors.

Based on these previous studies [4–7], we tried to find if the Mena immunostain is observed in the premalignant and malignant cervical lesions. We found that Mena was not expressed in the normal cervical epithelium but its intensity was correlated with the grade of CIN.

As in the breast lesions and tumors [4], and also in the colorectal lesions [5], the Mena overexpression could be an early event in cervical dysplasia and cancer development.

The Mena immunoexpression could help the pathologist to evaluate the malignant potential of cervical lesions. It could complete the results obtained by cytology, histology and colposcopy to define high grade of cervical intraepithelial neoplasia lesions. In the malignant lesions, its expression could be a prognostic factor. The further studies in a high number of cases are necessary to define exactly its role in some premalignant and malignant lesions.

Conclusions

Our study proved that the Mena overexpression in CIN could help to determine the risk for malignant transformation.

The small number of cases does not allow yet to state definite conclusions but we believe that it is necessary to enlarge the researches about Mena to demonstrating its role in the development of premalignant and malignant lesions.

In correlations with the classical prognostic factors, and also with the expression with other immunohistochemical markers, Mena could be a prognostic factor in many premalignant and malignant lesions.

This is the first study in the literature about Mena expression in cervical lesions.

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References

Corresponding author
Simona Gurzu, Assistant Professor, MD, PhD, Department of Pathology, University of Medicine and Pharmacy, 38 Gheorghe Marinescu Street, 540139 Târgu Mureș, Romania; Phone +40745–673 550, Fax +40265–210 933, e-mail: simonagurzu@yahoo.com

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