

Study on the localization of Aminopeptidase A in Invasive Carcinoma of Mammary Gland in Human

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Aminopeptidase A (APA) has a broad tissue distribution in both animals and humans. The enzyme is a part of renin-angiotensin system (RAS) and plays a role in blood pressure regulation. It participates also in other physiological functions such as processing of primary urine in kidneys and protein digestion in small intestine. APA expression has been studied in several types of malignant neoplastic lesions to show that it can serve as a biomarker for certain of them, particularly for active neoangiogenesis. In the present paper APA localization is studied in 5 cases of human invasive carcinoma of mammary gland using enzyme histochemistry. The results show elevated APA activity in the area of newly forming blood vessels as well as in tumor cells. A possible role of APA as a marker enzyme in this type of cancer is suggested.

Key words: Aminopeptidase A, blood vessels, mammary gland, invasive carcinoma.

Introduction

Aminopeptidase A (APA, EC 3.4.11.7) is a membrane-bound zinc metallopeptidase of M1 family [2]. The enzyme cleaves specifically the N-terminal glutamyl or aspartyl residues from peptide substrates, such as angiotensin II, chromogranin A, neurokinin B and cholecystokinin-8 [4]. APA is expressed normally in the brush-border membrane of renal tubules and is overexpressed in clear cell renal cell carcinoma [7]. APA is highly active in neoplastic lesions of the uterine cervix [6], human malignant gliomas and metastatic carcinomas of the brain [3]. Studies show that APA is overexpressed in stromal cells of prostatic carcinoma and show no expression in benign prostatic stroma [1]. Our recent studies indicate that APA is weakly expressed in normal mouse fibroblasts but is active in fibroblasts from mouse fibrosarcoma (unpublished results). Thus, it is possible that APA is involved in the formation of malignant tumors stromae. The enzyme is upregulated and overexpressed in blood vessels of some human tumors and is believed to play a role in pathological angiogenesis [3]. It is also highly expressed in pericytes in vasculature of tumors and wound healing tissues which shows that APA may be in-

volved in neovascularization, as well [5]. No data exist about the enzyme distribution and activity levels in mammary gland carcinomas.

The aim of the present study is to determine the localization of aminopeptidase A in cases of invasive carcinoma of mammary gland in humans. The results are expected to show whether the enzyme deserves to be studied in more details as a possible biological marker for mammary gland carcinoma.

Materials and Methods

Studies were made on anonymous cryostat sections of invasive carcinoma of mammary gland and lymph nodes with metastases, taken from 5 women 53 years middle-aged. The sections were provided by Specialized Hospital for Active Treatment in Oncology, Sofia. Before the enzyme reaction, all the sections were covered by celloidin (1% celloidin in absolute ethanol/diethyl ether/acetone 3:3:4) for a minute at room temperature. The sections were incubated in a substrate medium consisting of 0.5 mM fluorogenic enzyme substrate 4-(α -glutamylhydrazido)-N-hexyl-1,8-naphthalimide (α -Glu-HHNI) and 0.5 mg/ml piperonal in 0.1 M cacodylate/HCl buffer (pH 7.4), supplied with 5 mM of calcium ions, for 20 hours at 37 °C. Then, the sections were post-fixed in neutral formaline for 15 min at room temperature, stained with haematoxyline consistent with classical methods of histology and embedded in glycerol/jelly. All the sections were studied under the fluorescent microscope Leica DM5000B (New York, USA).

Results and Discussion

Many metalloproteinases are known to participate in tumorigenesis and tumor progression and some of them are identified as targets for therapy. APA localization and activity levels have been studied in several types of tumors such as clear cell renal cell carcinoma [7], uterine carcinomas [6], gliomas [3], prostatic carcinoma [1], etc. Most of the studies show elevated enzyme expression although the pathophysiological significance of APA activation in those cases is not fully understood. Recent studies show that APA is involved in both angiogenesis [3] and neovascularization [5] in some types of human tumors. APA distribution and activity levels in human mammary gland carcinomas have not been examined thus far.

In the present work, the APA activity localization was studied in cryostat sections of invasive carcinoma of mammary gland in human and in lymph nodes with metastases using enzyme histochemistry with the novel fluorogenic substrate α -Glu-HHNI, recently developed by us (design and synthesis of the substrate as well as development of the histochemical method will soon be published elsewhere). APA activity was evaluated semi-quantitatively according to the intensity of fluorescent light emitted from the final product of the enzyme reaction.

In tissue sections of invasive carcinoma of mammary gland, tumor foci consisting of cells with different shape, size and degree of staining were observed (**Fig. 1**). The enzyme histochemistry revealed APA overexpression mainly in the region of blood vessels (**Fig. 1**).

A much lower enzyme reaction was visible also in the tumor foci. In the metastatic lymph nodes a considerable APA activity was detected in tumor cells (**Fig. 2**). Thus, our results show increased enzyme activity in the blood vessels region to suggest a possible role of APA in neoangiogenesis in this type of tumors, as well.

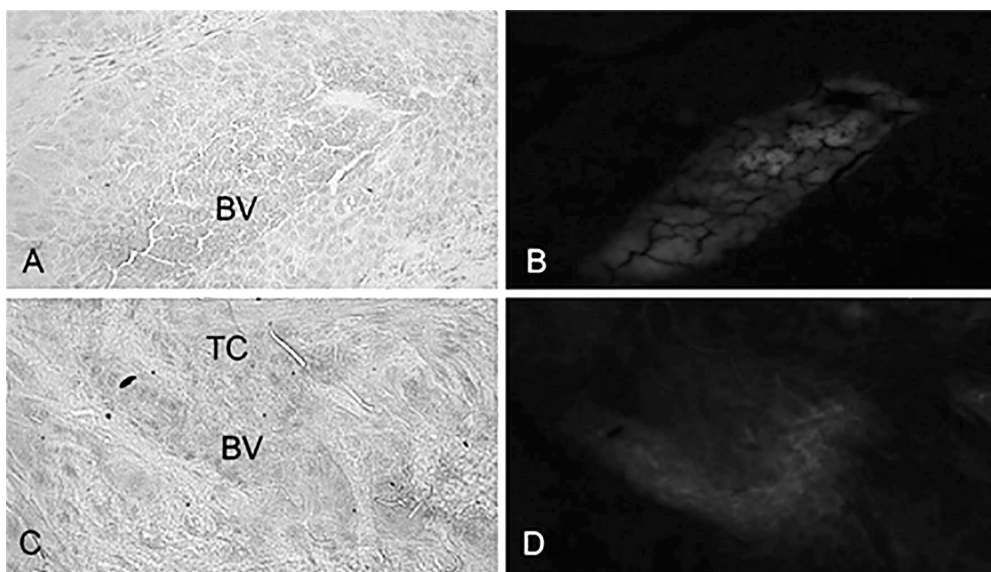


Fig. 1. APA activity in mammary gland carcinoma. Enzyme reaction in the area of blood vessels (BV). Lower reaction in tumor cells (TC). A, C – light microscopy; B, D – fluorescent microscopy. Magn. 200×

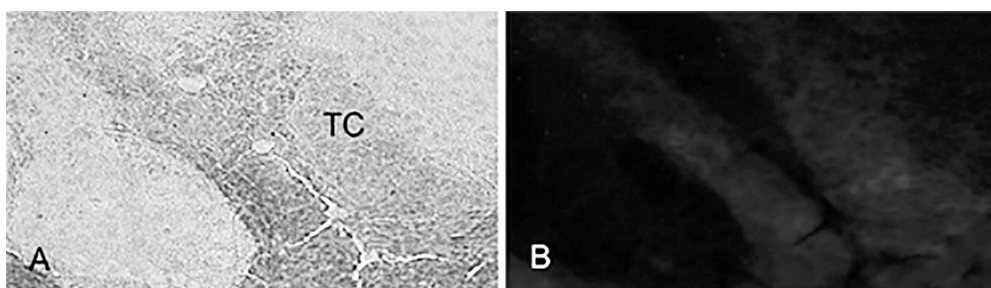


Fig. 2. APA activity in metastatic lymph node. Enzyme reaction in tumor cells (TC). A – light microscopy; B – fluorescent microscopy. Magn. 200×

In conclusion, our results show high expression of APA in invasive carcinoma of mammary gland in human as well as in lymph nodes with metastases. It is possible that the degree of APA activation can serve to predict the degree of aggression of the tumors. This possibility remains to be explored in our future studies.

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