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Visualization of Tissue Kallikrein in Human Breast Carcinoma by Two-Dimensional Western Blotting and Immunohistochemistry

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Tissue kallikrein is well known to liberate the vasoactive peptide kallidin from L-kininogen. Recently it was reported to activate matrix degrading metalloproteinases *in vitro* and to be present in gastric carcinoma cells.

By immunohistochemistry we localized tissue kallikrein in the cytoplasm of ductal breast cancer cells. In addition, two-dimensional Western blotting was used to further characterize its biochemical properties. By this method immunoreactive tissue kallikrein was found to have a molecular mass of 25 kDa and an isoelectric point close to pH 6. Furthermore its presence in human milk could be demonstrated.

Key words: Breast tumours / Human milk / Proteases.

Introduction

Tumour proliferation, invasion, and metastasis have been proposed to be facilitated by proteinases degrading the components of the extracellular matrix. The so-called metastatic cascade consists of a sequence of steps in which several proteinases may play a crucial role, for example, urokinase-type plasminogen activator, collagenase and other matrix metalloproteinases and their inhibitors (TIMP), and the lysosomal proteinases Cathepsin D, B and L. All these enzymes are able to degrade basement membranes. Some of them, such as cathepsin D and urokinase-type plasminogen activator, have been shown to be of prognostic relevance in breast cancer (Khokka *et al.*, 1991; Rochefort, 1992; Schmitt *et al.*, 1990). More recent studies were also performed on the involvement of tissue kallikrein in malignant diseases, showing that tissue kallikrein is secreted by gastric carcinoma cells (Koshikawa *et al.*, 1992) and that specific inhibitors of tissue kallikrein can influence metastasis of Lewis lung tumour cells (Uetsuji *et al.*, 1992).

In healthy man, tissue kallikrein is present in salivary glands, saliva, pancreas, kidney, urine, small and large intestine, endometrium, and in low concentrations in blood (Bhoola *et al.*, 1992; Clements and Mukhtar, 1994; Witzgall *et al.*, 1992). Functionally this kallikrein is involved in the release of the bioactive peptide kallidin from L-kininogen (Geiger and Fritz, 1979; Haberland *et al.*, 1983; Schachter, 1980) and thus in vasodilation, increase of vascular permeability and upregulation of glucose utilisation, effects that are important for tumour growth.

Tissue kallikrein might participate also in the generation of other biologically active substances like, for example, insulin, renin and growth factors (Jones *et al.*, 1992). It was shown to be able to convert the precursor of epidermal growth factor into its active form (Isackson *et al.*, 1987).

Another important function of tissue kallikrein could be the activation of matrix degrading metalloproteinases (collagenase I, IV, V and gelatinase) as it activates collagenase IV even more effectively than plasmin (Desrivières *et al.*, 1993; Tschesche *et al.*, 1989). Collagenase IV activation results in degradation of the basement membrane, a process essential for tumour growth and metastasis.

The aim of our study was to localize tissue kallikrein by immunohistochemistry in breast cancer cells and to characterize this enzyme further by its biochemical properties in two-dimensional Western blotting.

Results

By immunohistochemistry 27 malignant breast tumours were examined for the presence of tissue kallikrein. The specificity of the immunohistochemical method was proven by positive and negative controls. Salivary gland sections, used as positive control, showed a granular staining in the apical portions of salivary duct cells (Figure 1A). Preincubation of the primary antibody with purified tissue kallikrein or substitution of the first antibody by non-immune sera supplied the negative controls, which showed no detectable staining (Figure 1B).

Among 18 breast carcinomas of ductal origin, four were positive for tissue kallikrein. The number of stained tumour cells and the intensity of fine granular staining within the cell varied. Immunoreactivity for tissue kallikrein was localized in the perinuclear area of single tumour cells. Stained cells formed cluster-like groups within the tumour (Figure 1C). None of the nine lobular carcinomas examined showed specific staining for tissue kallikrein.

Results obtained by immunohistochemistry correlated well with the immunoblot results of the same tissues. In extracts of the four ductal breast carcinomas, which were positive in immunohistochemistry, tissue kallikrein was also detected by two-dimensional Western blotting.