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Expression of the Focal Adhesion Protein Paxillin in Normal and Breast Cancer Tissues

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Abbreviations: BSA, bovine serum albumin; ECL, enhanced chemiluminescence; EDTA, ethylenediaminetetraacetic acid; IgG, immunoglobulin G; SDS, sodium dodecyl sulphate

INTRODUCTION

Cell–matrix interactions regulate various cellular processes including survival, cell growth and differentiation (Boudreau and Bissel, 1998; Giancotti, 2000). Integrins are the major surface receptors that mediate such interactions (Hynes, 1992). They are composed of α and β chain heterocomplexes. Engagement of integrin receptors and their clustering leads to the formation of 'focal adhesions' where integrins link to intracellular cytoskeletal complexes and bundles of actin filaments. However, these complexes not only serve to connect the cytoskeleton to the matrix but also serve as a framework for the association of signalling proteins that regulate signal transduction pathways leading to integrin-induced changes in cell behaviour (Burridge *et al*., 1988). Focal adhesions contain multiple proteins such as vinculin, talin and tensin. Other proteins localized in focal adhesions include kinases, phospholipases and proteases. The actin cytoskeleton needs to be dynamic for cell shape changes in cell contacts and cell motility. The deregulation of cytoskeletal function contributes to malignant transformation. In normal cells, the cytoskeleton is very stable and there is little movement of cells. In cells that have become cancerous, the cytoskeleton is disrupted in such a way as to increase cell motility.

Paxillin is a focal adhesion-associated adaptor protein that plays a key role in cell spreading and motility (Schaller, 2001). This protein was originally identified as a substrate for the non-receptor tyrosine kinase oncogene $pp^{60v\text{-}src}$ in Rous sarcoma virus-transformed fibroblasts (Glenney and Zokas, 1989). Paxillin is ubiquitously expressed, albeit in variable amounts, with low levels in platelets and neuronal cells. It is highly conserved among various species.

Although the role of paxillin as a regulatory protein in focal adhesion dynamics and cell movement, has been well studied in cultured cells, its specific role in signalling in the complex organization of individual tissues has not yet been established. The

aim of this work was to evaluate the expression levels of paxillin and other focal adhesion-associated proteins (talin, vinculin, α-actinin) in mammary tumour tissues from cats and dogs.

MATERIALS AND METHODS

Tissue preparation

Mammary tumour and normal tissues were obtained from the dogs and cats undergoing surgical resection at the Department of Veterinary Clinical Sciences of the University of Napoli Federico II (Italy). Tissue samples were divided into two parts: the first was used for routine histological diagnosis using fixation in formalin. The second part, consisting of fresh material, was used for analysis of protein profiles using the Western blotting technique. Tissue samples were perfused thoroughly with 0.9% NaCl and homogenized using an Ultraturrax L-407 at 4°C with 5 ml of buffer (10 mmol/L Tris-HCl, pH 7.4, 10 mmol/L EDTA, 5 mmol/L dithiothreitol, 10% glycerol, 0.1 U trypsin/ml aprotinin, 10 μg/ml leupeptin and 4 μmol/L pepstatin) per 1.5 g of tissue. Homogenates were divided into small aliquots and stored at −80°C until use. The protein content of each sample was determined by Bio-Rad DC protein assay.

Electrophoresis and Western blot

Samples containing equal amounts of protein were run on an electrophoresis apparatus using 7.5% SDS/polyacrylamide gels. Following electrophoresis, the samples were transferred onto nitrocellulose filter. The blots, after blocking (for 1 h at 42°C in Tris/NaCl/P_i containing 5% BSA) and washing, were incubated for 2 h with either anti-paxillin IgG or anti-vinculin or anti α-actinin or anti-talin IgG. After incubation, the filters were washed and incubated for 2 h with peroxidase anti-mouse IgG. The proteins were visualized using a chemiluminescence method (ECL). Quantification of proteins was performed using densitometry.

RESULTS AND DISCUSSION

Mammary tissue samples obtained from cats and dogs were homogenized, loaded onto a SDS-polyacrylamide gel and immunoblotted with paxillin, talin, vinculin and α-actinin antibodies. No difference in the amount of paxillin was detected for normal and tumour tisses from cats affected by breast adenoma or from dogs affected by breast chondroma. Conversely, a decrease of the amount of paxillin, as compared to

normal tissues of the same animal, was observed for tumour tissues from cats affected by breast solid carcinoma or from dogs affected by adenocarcinoma. The decrease in the paxillin level correlated with the grading of breast adenocarcinomas in dogs (Scibelli *et al*., 2003).

No difference in the expression levels of the focal adhesion-associated proteins vinculin, talin and α-actinin was detected in tumour tissues as compared to normal tissues from the same species.

The amount of paxillin has been shown to be significantly reduced during mitosis of the cell cycle (Yamaguchi *et al*., 1997). Furthermore, low levels or absence of paxillin expression have been demonstrated in some human lung cancers and liver metastases (Ayaki *et al*., 2001). Cell motility has been shown to be reduced by paxillin overexpression in lung cancer cell lines (Salgia *et al*., 1999). Metastatizing cells do not tend to form clusters and exhibit cell-to-cell contacts which are the opposite of those occurring in normally functioning cells. Low levels of paxillin may confer an advantage in migration to cancer cells by interfering with the attachment to the extracellular matrix, and this may involve blocking or sequestering signalling molecules, or interfering with signal transduction from focal adhesions. Thus, the observation that paxillin levels are reduced in malignant mammary tumours at a high grade of progression, and which are therefore more aggressive, may lead to further insights into the role of paxillin in normal and transformed cells.

The low level of paxillin expression in invasive feline and canine mammary tumours suggests that paxillin may represent a useful prognosticator of feline and canine breast cancer malignancy. This finding may provide the basis for the development of new diagnostic and therapeutic strategies in veterinary oncology.

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