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Distribution of thrombospondin and integrin $\alpha_v$ in DCIS, invasive ductal and lobular human breast carcinomas.

Analysis by electron microscopy

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Abstract The ultrastructural distribution of thrombospondin (TSP) and its cell surface receptor, integrin $\alpha_v$, was studied in two cases of human breast carcinoma: one of ductal carcinoma in situ (DCIS) with an invasive component, and one of invasive lobular carcinoma. In DCIS, moderate immunolabelling for TSP and integrin $\alpha_v$ was observed in the rough endoplasmic reticulum and at the plasma membrane of intraductal carcinoma cells. TSP was also associated with extracellular matrix collagen fibrils surrounding in situ carcinoma cells. In the invasive part of this ductal carcinoma, most of the malignant cells were negative for TSP, while integrin $\alpha_v$ was moderately expressed in these cells. In sharp contrast, typical strands of invasive lobular carcinoma cells in "Indian file" showed moderate TSP immunostaining in the rough endoplasmic reticulum and strong immunoreactivity for TSP at the plasma membrane and in the extracellular matrix. Moderate to strong immunoreactivity for integrin $\alpha_v$ was also observed in invasive lobular carcinoma cells. Because of the role of TSP during cancer cell invasion, the different expression patterns of TSP in invasive ductal versus lobular carcinoma may well reflect biological differences between these two types of breast carcinoma and could account for the peculiar diffuse invasive behaviour of breast lobular carcinoma cells.

Key words Thrombospondin · Integrin $\alpha_v$ · Breast carcinoma

Introduction

Thrombospondin (TSP) is a 450-kDa extracellular matrix glycoprotein synthesized and secreted by a wide range of cultured cells [6]. TSP has been found to be involved in a variety of physiopathological contexts, such as development, wound healing, atherosclerosis, angiogenesis, tumorigenesis and cancer cell metastasis [15]. Recently, five distinct genes have been described that encode for four structurally different TSPs (TSP1, TSP2, TSP3 and TSP4) and cartilage oligomeric matrix protein (COMP) [2]. However, most functional studies have been performed with TSP1. The functions of TSP2, TSP3, TSP4 and COMP are unknown. During tumorigenesis, TSP1 functions by modulating the adhesion, migration and proliferation of malignant cells [1, 15], and it mediates platelet–tumor cell interactions during metastasis formation [8, 13, 25].

TSP is present in normal breast secretions, and its concentration is markedly increased in malignant breast secretions [10, 20]. In situ, excessive TSP deposits are observed in the basement membrane surrounding intraductal breast carcinoma (potential precursor of invasive cancer), and in desmoplastic areas of invasive breast carcinomas [6, 26]. Moreover, using both immunohistochemistry and in situ hybridization, we found that the expression of TSP varies greatly depending on the type of invasive breast carcinoma studied [9]. Few invasive malignant cells (10%) express TSP in breast ductal carcinoma, whereas it is expressed by most cells in invasive breast lobular carcinoma (40–80%). Alteration of integrin expression in human breast carcinoma has been reported by several groups [14, 19, 27], and a conspicuously elevated expression of $\alpha_1\beta_1$ and $\alpha_5\beta_1$ integrins is also observed in invasive lobular carcinoma cells compared with their ductal counterpart [14]. It is well established that altered integrin expression contributes to the invasive and metastatic behaviour of malignant cells [3]. Among these integrins, integrin $\alpha_v\beta_3$ serves as a cellular receptor for TSP [16], and its expression is directly related to the tumorigenicity of malignant cells [11]. In order
to gain further insights into the differences between these two types of breast carcinoma, the present study was performed to examine the ultrastructural immunolocalization of both TSP and integrin αv in one case of DCIS with some invasive components and in one case of invasive lobular carcinoma.

Materials and methods

Breast tissues

Tissue from two neoplastic breast lesions was obtained following systematized resections. The case of DCIS with invasive components was observed in a 51-year-old woman and that of invasive lobular carcinoma, in a 48-year-old woman. These samples were immediately processed for transmission electron microscopy and for immunoelectron microscopy.

Transmission electron microscopy

Tissues from the central part of the tumor were minced into 1 mm-thick pieces, fixed for 2 h with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, and washed overnight with 0.2 M saccharose (in cacodylate buffer). The material was then postfixed for 1 h with 1% osmium tetroxide in 0.15 M sodium cacodylate buffer, dehydrated in graded ethanol and embedded in Epon. Semi-thin sections (1 μm-thick) were stained with Azur-Methylene Blue. Ultrathin sections were obtained with an Ultracut E (Leica), counterstained with uranyl acetate and lead citrate, and then observed under a JEOL 1200 EX transmission electron microscope.

Antibodies

The characterization and specificity of anti-TSP mouse monoclonal antibody P10 has been described earlier [6]. The epitope for P10 is located within the type 2 repeats of human platelet TSP1. Because of the high homology between TSP1 and TSP2 in type 2 repeats [2], the antigen immunodetected by P10 has been named TSP rather than TSP1. Mouse monoclonal antibody LM 142 directed against the subunit integrin αv was a gift from Dr. David Cheresh (Scripps Clinic and Research Foundation, La Jolla, California). Rabbit anti-mouse (RAM) IgG conjugated with horseradish peroxidase was obtained from Nordic Immunology (Tebu, France).

Immunoelectron microscopy

Immunolabelling of breast carcinoma samples was performed prior to embedding using an indirect immunoperoxidase technique previously described [4]. Briefly, specimens (1–2 mm-thick) were fixed for 18 h with 2% paraformaldehyde-lysole-phosphate (PLP), washed with phosphate buffer, incubated in glycerol/saccharose and subsequently frozen in liquid-nitrogen-cooled isopentane. After treatment with 0.2% hyaluronidase to allow permeabilization of the extracellular matrix, floated cryostat sections (15–20 μm-thick) were treated with 100 mM glycine to allow saturation of aldehyde groups. Following incubation with a blocking buffer (10 mM sodium azide, 1% H2O2, 1% BSA and 1% RAM IgG) to inhibit endogenous peroxidases and to avoid non-specific adsorptions, floated sections were then incubated overnight at 4°C with specific antibodies (1 μg/ml). Finally, a rabbit anti-mouse IgG conjugated with horseradish peroxidase was added for 90 min and the reaction revealed with a solution of 3-3' diaminobenzidine (1 mg/ml) in 10 ml of 50 mM Tris buffer, pH 7.6, containing 0.1 ml of 1% H2O2. Sections were then washed, postfixed in 1% osmium tetroxide, dehydrated in graded ethanol and embedded "à plat" in Epon. Ultrathin sections from representative areas were observed without any lead citrate counterstaining. Such counterstaining would have masked any specific immunostaining resulting from use of the diaminobenzidine solution.

Results

Expression of TSP and αv in DCIS associated with invasive components

The peripheral myoepithelial layer usually observed in normal ducts was generally absent in carcinoma in situ. A few cells with peripheral areas of high density and abundant microfilaments were observed and were indicative of remnants of myoepithelium (Fig. 1A). The lumen of these ducts was filled by a layer of loosely cohesive malignant cells showing a high nucleus-to-cytoplasm ratio (Fig. 1A). Most of these cells were necrotic, and calcifications were seen on cellular fragments (Fig. 1A, inset). A basement membrane surrounding ducts filled with malignant cells was present in all cases. Its thickness was irregular, reaching 1–2 μm in places. Microtubules were localized inside this basement membrane or associated at the periphery of the ducts with type I collagen fibrils. In some ducts filled with malignant cells, cells surrounded by a basement membrane were invading the underlying extracellular matrix (Fig. 1B).

In invasive ductal areas (IDC), mononuclear stromal infiltrates (including inflammatory cells and malignant cells) were surrounded by collagen fibrils (Fig. 1C). Blood vessels were found adjacent to these clusters. Invasive malignant cells had a high nucleus-to-cytoplasm ratio, a well-developed Golgi apparatus and an abundant rough endoplasmic reticulum. A basement membrane material could be observed surrounding malignant cells located at the periphery of the infiltrates in a discontinuous pattern. The observation of hemidesmosomes demonstrated the epithelial origin of these invasive cells (Fig. 1E).

Moderate immunolabelling for TSP was observed in the rough endoplasmic reticulum, at the plasma membrane and in the basement membrane of DCIS cells (Fig. 2A). In addition, strong immunostaining was associated with type I collagen fibrils present in the extracellular matrix adjacent to in situ areas (Fig. 2B). The αv subunit integrin was moderately expressed in the rough endoplasmic reticulum, in the perinuclear space and at the plasma membrane level of intraductal malignant cells (Figs. 2C, D).

The invasive ductal area contained malignant infiltrating cells surrounded by collagen fibrils. Most of these invasive ductal carcinoma (IDC) cells were negative for TSP. In only a few malignant cells TSP was expressed faintly in the rough endoplasmic reticulum. These observations were in agreement with the previous report [9] showing the presence of TSP in only 10% of IDC cells. Moderate staining for TSP was also observed at the plasma membrane level and in the extracellular matrix close