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Immunohistochemical Expression of Prostate-specific Membrane Antigen in Uveal Melanoma

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Abstract: The purpose of this study was to characterize the expression of PSMA and evaluate its possible association with clinicopathological features in Uveal Melanoma (UM). Fifty-five UM specimens were subjected to immunohistochemistry with anti-PSMA monoclonal antibody. Prostatic hyperplasia was used as a positive control. The endothelial cells of the tumor-associated neovasculature and UM cells in all of the 55 UM cases demonstrated negative staining to PSMA. Since there is no expression of PSMA in UM, this protein does not represent a new target for antineovasculature-based therapy for UM patients. Therefore, the search for new treatments, especially those focusing on hematogenous metastatic spread, is necessary.

Key words: Expression, prostate-specific membrane antigen, uveal melanoma

Introduction

Uveal Melanoma (UM) is the most common primary intraocular malignancy in adults with an incidence of seven to ten cases per million people per year in Canada (Egan *et al*., 1988), United States (Parkin *et al*., 2001) and Europe (Parkin *et al*., 2001). Despite the high accuracy of clinical diagnosis (Egan *et al*., 1988) and advances in local treatment (Nag *et al*., 2003), more than 40% of the UM patients die due to hematogenous spread of melanoma cells from the primary tumor to the liver (Melia *et al*., 2001). High tumor vascularity, as assessed by microvessel count or vascular pattern analysis (Folberg *et al*., 1997), is one of the histopathological prognostic factors associated with poor outcome in UM. Consequently, the neovasculature associated with UM represents a critical target for antiangiogenic therapy, which is the focus of much emerging research (Folkman, 1990).

Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein located on chromosome 11p, initially characterized by the monoclonal antibody (mAb) 7E11 (Horoszewicz *et al*., 1987; Israeli *et al*., 1993). Although PSMA exhibits *in vitro* neuropeptidase (Carter *et al*., 1996) and folate hydrolase activity (Pinto *et al*., 1996), its function *in vivo* has not been fully elucidated. PSMA was originally thought to be strictly expressed in prostatic tissues including secretory-acinar epithelium, prostatic intraepithelial neoplasia and prostatic adenocarcinoma (Lopes *et al*., 1990; Israeli *et al*., 1993). However, several studies have discovered that anti-PSMA

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mAbs bind to the neovasculature associated with solid tumors, such as lung, colon, breast carcinomas (Liu *et al*., 1997), pancreatic carcinoma, skin melanoma, primary and metastatic renal carcinoma (Chang *et al*., 2001), which suggests a possible role of PSMA in angiogenesis. Therefore, expression of PSMA in endothelial cells of the UM neovasculature could be a suitable target for the selective delivery of new anti-angiogenic therapeutic drugs. To our knowledge, PSMA expression in UM cells and related neovasculature has not yet been studied.

The purpose of this study was to characterize the expression of PSMA and evaluate its possible association with clinicopathological features in UM. Moreover, since anti-PSMA mAb is currently used as a radiodiagnostic marker for prostate cancer (Kahn *et al*., 1998; Petronis *et al*., 1998; Nanus *et al*., 2003; Milowsky *et al*., 2004), PSMA could be a potential target to treat the primary UM and prevent metastatic disease.

Materials and Methods

Fifty-five UM specimens obtained by enucleation between 1980 and 2004 were collected from the archives of the Henry C. Witelson Ocular Pathology Laboratory and Registry, McGill University, Montreal, Canada. The cases were included in the study based on the availability of representative tissue and clinicopathological data. Each specimen was formalin-fixed and paraffin-embedded and contained sufficient material for H&E staining and immunoassaying.

Histopathological evaluation of the specimens was performed with regard to prognostic factors including cell type (modified Callender's classification) (McLean *et al*., 1983), largest (linear) tumor dimension (LTD) (Diener-West *et al*., 1992), number of mitotic figures in 40 high power fields (HPF) (McLean *et al*., 1977), scleral invasion and tumor infiltrating lymphocytes (TIL) in 20 HPF (de la Cruz *et al*., 1990). For the purpose of statistical analysis, tumors were classified as having a low mitotic rate (0-1 mitotic figures in 40 HPF) or a high mitotic rate (two or more mitotic figures in 40 HPF). These parameters have previously been used (McLean *et al*., 1977). The presence of TIL was classified as low ($\leq$ 200 lymphocytes in 20 HPF) or high ($>$ 200 lymphocytes in 20 HPF) according to a previous publication (de la Cruz *et al*., 1990). All of the slides were analysed by two independent pathologists. Controversial findings were discussed to reach a consensus.

Immunohistochemical analysis was performed according to the avidin-biotin complex (ABC) technique. Briefly, sections were deparaffinized in xylene and rehydrated through graded ethanol washes. A 10 min incubation in boiling citrate buffer (pH = 6.0) was used for antigen retrieval. To block endogenous peroxidase, incubation with 3% hydrogen peroxidase in methanol for 5 min was performed. Non-specific binding was blocked with a 30 min wash with 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS, pH 7.6). The anti-PSMA mouse mAb (Novocastra, Newcastle, United Kingdom) in a 1:50 dilution was applied and the slides were incubated overnight at 4°C. Next, rabbit anti-mouse secondary antibody E0354 (1:500 dilution; DAKO, Mississauga, Ontario, Canada) was applied for 30 min at 37°C. Sections were then incubated with horseradish peroxidase-conjugated ABC complex (DAKO) for 30 min at 37°C. Immunostaining was visualized using 3-amino-9-ethylcarbazole (AEC) chromogen (DAKO). Finally, the slides were counterstained with Giu-II haematoxylin and cover-slipped. Prostatic hyperplasia was used as a positive control. Negative control sections were incubated with non-immune serum (0.1% BSA in TRIS) instead of the primary antibody.

Samples were classified into two categories: negative (if none of the endothelial or UM cells displayed immunostaining) and positive (if any endothelial or UM cell displayed distinctive immunostaining, irrespective of the staining intensity). Tumor cells were distinguished from macrophages based on morphology.

Results

To evaluate the PSMA expression in UM, immunohistochemistry with monoclonal anti-PSMA antibody was performed. The 55 UM specimens studied presented the following cell types: 48 mixed, five epithelioid and two spindle. Scleral invasion was observed in 5.5% of the cases studied (n = 3). Tumor infiltrating lymphocyte index was low in 83.3% of the cases (n = 45) and high in 16.7% (n = 9). Approximately 58% of cases presented two or more mitotic figures in 40 HPF (n = 32) and the remaining had one or less (n = 23). The average of the LTD was 10.2 mm.

Both UM cells (Fig. 1A) and endothelial cells of the tumor-associated neovasculature (TAN) (Fig. 1B, arrow) in all of the 55 cases of UM demonstrated negative staining to PSMA. Seventy-five percent of the prostatic secretory-acinar epithelium of the prostatic hyperplasia specimen, used as positive control, stained positive (Fig. 2). This confirmed that the immunohistochemical technique was correctly performed.

Discussion

PSMA expression is not confined exclusively to the prostate. It has also been reported in select benign nonprostatic tissues such as salivary gland, brain, duodenal columnar epithelium, proximal renal tubular epithelium, colonic ganglion cells and benign breast epithelium (Chang *et al.*, 1999a).

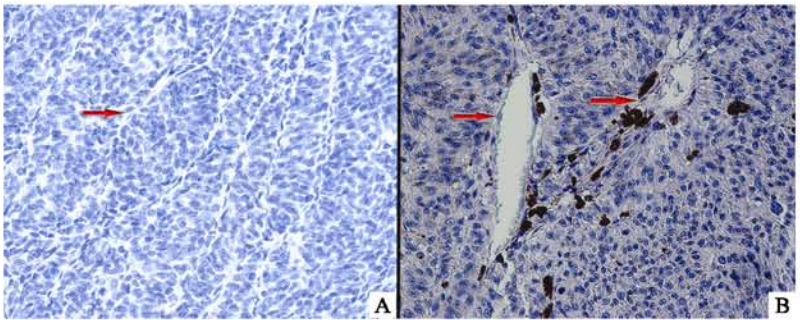

Fig. 1: (A) UM cells stained negative to PSMA, 400x. (B) Endothelial cells of UM-associated neovasculture (arrows) stained negative to PSMA, 200x. The brown pigments are melanin granules

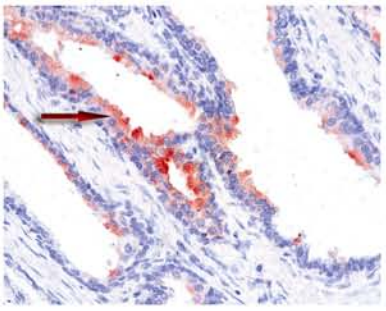

Fig. 2: Prostatic secretory-acinar epithelium of the prostatic hyperplasia specimen (arrow) stained positive to PSMA

PSMA expression has been reported in endothelial cells of several nonprostatic primary and metastatic TAN. Silver *et al.* (1997) demonstrated the expression of PSMA in endothelial cells in a subset of tumors, including renal cell carcinoma, transitional cell carcinoma of the urinary bladder and colonic adenocarcinoma. More recently, Chang *et al.* (1999b) examined multiple anti-PSMA antibodies and showed that each antibody consistently bound the TAN in cases of testicular embryonal carcinoma, neuroendocrine carcinoma, malignant skin melanoma, pancreatic duct carcinoma, non-small cell lung carcinoma, soft tissue sarcoma and breast carcinoma. RT-PCR and *in situ* hybridization analysis demonstrated that these endothelial cells, but not the tumor cells, contain PSMA mRNA transcripts. These findings explain the lack of PSMA expression in nonprostatic tumor cells and support the hypothesis that endothelial cells of TAN synthesize PSMA protein instead of sequestering it from the serum or from the surrounding stromal cells (Chang *et al.*, 1999c).

In contrast to TAN, the endothelium of benign vascular tumors does not express PSMA. This is not surprising given that in these tumors the endothelium is altered itself and presumably not stimulated by angiogenic factors. Furthermore, the fact that vascular endothelial cells of benign tissues do not express PSMA (Chang *et al.*, 1999b) strongly suggests that endothelial cell-PSMA expression is restricted to TAN and may be stimulated by one or more tumor-secreted angiogenic factors (Chang *et al.*, 1999c).

With the purpose of characterizing the PSMA expression in endothelial cells of TAN and in neoplastic melanocytes, 55 cases of UM were submitted to immunohistochemistry. Every malignant melanocyte demonstrated negative staining to PSMA. This result supports a trend observed in other studies that also demonstrated a negative expression of PSMA in tumor cells of various nonprostatic neoplasms (Silver *et al.*, 1997; Chang *et al.*, 1999b; Chang *et al.*, 1999c).

Angiogenesis, essential for the growth and development of both primary and metastatic tumors, is a complex process involving a dynamic interrelationship between stimulators and inhibitors (Folkman and Cotran, 1976; Folkman, 1995; Wang *et al.*, 1998). PSMA is a unique antiangiogenic target because it is selectively and consistently expressed in nonprostatic TAN but not in normal vessels in benign tissue. This is in contrast to other endothelial cell targets such as vascular endothelial growth factor receptors, integrin a_vb_3, CD34 and Tie receptors, which are typically expressed in normal vasculature and are up-regulated in TAN (Skobe *et al.*, 1997; Brown *et al.*, 1998; Gasparini *et al.*, 1998). In addition, PSMA has folate hydrolase activity that has been used in a pro-drug strategy with cytotoxic agents (Heston, 1997). However, in the present study, the endothelial cells of TAN in all the 55 cases of UM expressed negative staining to PSMA.

Antiangiogenic strategies have been successful in the laboratory setting and a single angiogenic target can be useful in treating a variety of diverse tumors (O'Reilly *et al.*, 1994; Saleh *et al.*, 1996; Arora *et al.*, 1999). Since both the endothelial cells of the TAN and neoplastic cells in 55 cases of UM do not express PSMA, this protein does not represent a new target for antineovasculature-based therapy in cases of UM.

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