Expression of a Subset of Steroid Receptor Cofactors Is Associated with Progesterone Receptor Expression in Meningiomas

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ABSTRACT

The predominance of meningiomas in females, their accelerated growth during the luteal phase of the menstrual cycle and during pregnancy, and the association between meningiomas and breast cancer have led to a number of studies examining the potential role of steroids on the growth of meningiomas. There are numerous discrepancies in the literature about the mitogenic effects of steroids on meningiomas in both in vitro and in vivo models. The aim of this study was to examine the expression of three steroid receptor coactivators, along with progesterone receptor and estrogen receptor in meningiomas. This additional regulatory layer may explain the heterogeneity of hormone responses observed in these tumors. Using Western blot analysis and immunohistochemistry, we demonstrate the expression of the steroid coactivators steroid receptor cofactor (SRC-1), amplified in breast cancer protein (AIB1), and transcriptional intermediary factor 2 (TIF2) in 81, 76, and 76% of meningiomas, respectively. The expression of SRC-1 and TIF2 is significantly related to progesterone but not to estrogen receptor expression. In contrast, seven normal brain specimens were positive for TIF2 and SRC-1 but negative for AIB1. One leptomeningeal specimen was positive for AIB1, SRC-1, and progesterone receptor. The differential expression of steroid receptor coactivators may explain the differential response of these tumors to hormonal therapy.

INTRODUCTION

Meningiomas are thought to arise from the arachnoid cells that form the arachnoid villi, and account for 18% of all primary intracranial tumors and 25% of all intraspinal tumors (1, 2). The incidence of intracranial meningiomas is twice as high in women as men and occurs at nearly a 10:1 female:male ratio in intraspinal meningiomas. In a series of 517 patients with meningiomas seen by the Brain Tumor Group at Brigham and Women’s Hospital, the female:male ratio was 2.4:1 (3). Meningiomas may grow rapidly during periods of relative hormonal excess, during the luteal phase of the menstrual cycle, and during pregnancy (4). Several investigators have reported an increase in the size or symptoms from meningiomas in the latter trimesters of pregnancy, which resolve after childbirth (4, 5). There is also an association between meningiomas and breast cancer. Three separate studies have demonstrated a positive correlation between breast cancer and meningiomas (6–8). Although many studies have attempted to define the role of these hormones in meningioma growth, the specific activities of these hormones in meningioma pathogenesis remain unknown. The potential role of estrogen and progesterone on the growth of meningiomas has been investigated several different ways with in vitro and in vivo models. The first set of studies focused on the expression of the receptors in meningiomas (9, 10). Secondly, studies were conducted to try to assess the mitogenic effects of estrogen, progesterone agonists, and/or progesterone antagonists both on meningioma cell cultures and spheroids and on tumors implanted in vivo (11). Our laboratory demonstrated that 64% of meningiomas express mRNA for the PR and that its expression correlates with nuclear localization of the receptor by immunohistochemistry (12). Using reverse transcription and PCR Southern blot analysis, we have demonstrated the presence of mRNA for ER-α³ and -β in 68 and 44% of meningiomas, respectively (13).

Steroid hormone receptors belong to a large superfamily of nuclear receptors that bind DNA at specific sites to control gene transcription (14, 15). The mechanisms by which steroid receptors modulate the transcription of target genes are under extensive investigation. Once bound to ligand, the receptors undergo conformational changes and dimers of receptors recognize specific regulatory DNA sequences upstream of target genes. Activated receptors, through interactions with coactivator proteins, direct the assembly and the stabilization of a preinitiation complex that will ultimately conduct the transcription of these genes. Recently, several coactivators that associate with these

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³ The abbreviations used are: ER, estrogen receptor; PR progesterone receptor; SRC-1, steroid receptor cofactor; TIF2, transcriptional intermediary factor 2; AIB1, amplified in breast cancer protein; GST, glutathione S-transferase; RT, room temperature; TBST, Tris-buffered saline [10 mM Tris (pH 8) and 0.9% NaCl] containing 0.1% Tween 20.
receptors in a ligand-dependent manner and enhance their ability to trans-activate target genes have been identified and characterized (16). Therefore, to obtain a clear picture of the role of estrogen and progesterone in meningioma growth, it is likely to be important to know not only the status of ER or PR expression in these tumors but also whether the coregulators of steroid hormone receptors are present. These additional regulatory layers may explain the heterogeneity of hormone responses that are observed in normal and malignant tissue. In this report, we studied the expression of three related 160-kDa proteins (17), SRC-1, TIF2, and AIB1 (18–22). Although the role of these coactivating proteins is being intensely studied in other hormonally influenced tumors such as breast cancer, the expression of these coactivators has yet to be examined in any type of brain tumors. The differential expression of steroid receptor cofactors may explain the differential response of these tumors to hormonal therapy.

MATERIALS AND METHODS

Tissue Samples/Cell Culture. Tissues were collected at the time of craniotomy for tumor resection, immediately snap frozen in liquid nitrogen, and subsequently stored in liquid nitrogen. Normal brain specimens were taken from patients undergoing temporal lobectomy or an area of normal tissue adjacent to a tumor. One leptomeninges (arachnoid and pia mater) was obtained from an autopsy case at the time of brain removal. Each sample was taken from a specimen, which was used by the neuropathologist (Dr. Matthew Frosch) for diagnosis (1, 2). All meningiomas were classified according to the WHO criteria. MCF-7 breast cancer cells were obtained from the American Type Culture Collection and maintained in DMEM-10% FBS to be used as a positive control for expression of SRC-1, TIF2, and AIB1 steroid cofactors. Breast carcinoma tissue known to be positive for PR and ER was used as a positive control of immunohistochemical analysis.

Antibodies. The AIB1 antibody is a polyclonal antibody generated against GST-AIB1 (amino acids 695–933). The rabbit serum was affinity purified with the use of GST-AIB1 which has been cleaved by thrombin and covalently immobilized to Affi-Gel 15 (Bio-Rad). The specificity of the antibody was confirmed by Western blot analysis (22). The TIF2 antibody is a monoclonal antibody against GST-TIF2 (amino acids 885–1122). The specificity of the antibody was confirmed by immunoprecipitation with in vitro translated TIF2 followed by Western blot analysis. The AIB1 antibody was a monoclonal antibody against GST-AIB1 which has been cleaved by thrombin and covalently immobilized to Affi-Gel 15 (Bio-Rad). The specificity of the antibody was confirmed by Western blot analysis (22). The TIF2 antibody is a monoclonal antibody against GST-TIF2 (amino acids 885–1122). The specificity of the antibody was confirmed by immunoprecipitation with in vitro translated TIF2 followed by Western blot analysis.

Western Blot Analysis. Each frozen tissue specimen was crushed in a porcelain mortar and then transferred to a Dounce tissue grinder containing 3 ml of cold NP40 lysis buffer [20 mM HEPES (pH 8.0), 1% NP40, 10% glycerol, 2.5 mM EGTA, 2.5 mM EDTA, 10 μg/ml leupeptin, 2 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 0.5 mM vanadate]. The protein lysates were centrifuged at 10,000 × g for 30 min at 4°C to remove any cellular debris. The amount of protein was quantified (Bio-Rad protein analysis), and stored at −70°C.

For all Western blots 100 μg of protein lysate from each sample were reconstituted in Laemmli sample buffer (23), boiled for 5 min, and analyzed by SDS-polyacrylamide gel (7.5% acrylamide) electrophoresis.

After PAGE, the gels were transblotted to Immobilon-P (Millipore, Bedford MA) and blocked at RT with 5% nonfat milk in TBST for 1 h at RT. The first antibody was then diluted in TBST, and blots were incubated for 1 h at RT. The first antibodies included (a) the mouse monoclonal SRC-1 (1:100 in TBST) (24), (b) the mouse monoclonal TIF2 (1:100 in TBST), and (c) the rabbit affinity-purified AIB1 (1:100 in TBST) (22). After a rinsing in TBST, the blots were incubated with the secondary antirabbit or antihorseradish peroxidase-conjugated antibody (1:1500 in 5% nonfat milk-TBST) for 1 h at RT. The blots were again rinsed in TBST, and detection was conducted with the ECL-Plus Western detection system (Amersham Life Science, Arlington, IL) per manufacturer’s instructions. The blots were then exposed to Kodak XAR film for 1–60 min.

PR, ER, SRC-1, AIB1, and TIF2 Immunohistochemistry. Sections of 6 μm were cut from formalin-fixed, paraffin-embedded tissue blocks and mounted on polylysine-coated glass slides. The sections were deparaffinized in a xylene bath and hydrated in graded ethanol washes. To improve the staining pattern, antigen retrieval with 10 mM sodium citrate (pH 6.0) at 100°C was used for 30 min. Briefly, slides were incubated with the peroxidase-blocking agent (0.3% hydrogen peroxide) for 30 min. They were then incubated with the primary PR antibody (Biogenex PR 88; diluted 1:40 in PBS containing horse serum), ER antibody (diluted 1:400 in PBS containing horse serum), SRC-1 antibody (diluted 1:3 in PBS containing horse serum), TIF2 antibody (diluted 1:3 in PBS containing horse serum), and AIB1 antibody (diluted 1:50 in PBS containing goat serum) overnight at 4°C. The slides were then incubated with a mouse or rabbit biotinylated secondary antibody provided in the Vectastain ABC kit (Vector Laboratories, Burlingon, CA) and ABC reagent for 30 min. Lastly, they were incubated with the substrate-chromagen 3,3′-diaminobenzidine

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**Fig. 1** Western immunoblots of protein lysates (100 μg) of seven representative meningioma specimens, MCF-7 cells which served as a positive control, and two normal brain specimens probed with three different 160-kDa steroid receptor cofactors, TIF2, AIB1, and SRC-1, detected with the ECL-Plus detection system. M, meningioma; NB, normal brain.
for 2–5 min. Sections were counterstained with methyl green for 5 min. All incubations except primary antibody were conducted at RT. For each sample, an adjacent section was incubated with horse serum as a negative control.

RESULTS

Steroid Receptor Coactivators. Twenty-one meningioma specimens were examined for their expression of the steroid receptor coactivators AIB1, TIF2, and SRC-1 by Western blot analysis (Fig. 1; Table 1). For a subset of these specimens, immunohistochemistry was done with the same antibodies used for Western blot analysis (Fig. 2). For SRC-1, 81% (17 of 21) of the meningiomas had a detectable band by Western blot analysis. For AIB1 and TIF2, 76% (16 of 21) of the tumors were positive by Western blot analysis (Table 1). Two meningioma tumors, one anaplastic and one syncytial, were negative for all three coactivators. There was a tendency for more malignant tumors to express lower levels of all three coactivators.

All normal brain specimens (n = 7) were positive for SRC-1 and TIF2, but not AIB1, by Western blot analysis and immunohistochemistry. In contrast, leptomeningeal tissue obtained from an autopsy patient was positive for SRC-1 and AIB1 but negative for TIF2 (Fig. 3; Table 1).

Steroid Hormone Receptors. Fifty-seven percent of the meningiomas were positive for PR expression by immunohistochemistry (Fig. 1; Table 1). Of the specimens that were positive, 67% were from female patients and 33% were from male patients. These percentages are similar to those we previously reported on a distinct set of tumors (12).

Forty-three percent of the meningiomas were positive for ER expression by immunohistochemistry (Fig. 1; Table 1). Of the specimens, which were positive, sixty-seven percent were from female patients and thirty-three percent were from male patients. These percentages are similar to those we previously reported on a distinct set of tumors (13). Normal brain tissue was negative for ER and PR expression by immunohistochemistry. In contrast, the leptomeningeal specimen was positive for PR.

The expression of SRC-1 and TIF2 are significantly related to the PR but not ER expression in the meningiomas examined (P < 0.05, Fisher’s exact test). Among those tumors, which are PR positive, 100% are SRC-1 positive and 92% are TIF2 positive. In contrast, among the PR-negative tumors, 56% are SRC-1 positive and TIF2 positive. No significant relationship was observed between AIB1 and PR status.

DISCUSSION

The role of steroid hormones and their receptor expression in meningioma initiation and proliferation remains undefined. We and others have suggested that meningioma heterogeneity may be due to some type of genetic variability. Therefore, phenotypic subsets could be defined according to patterns of genetic alterations carried by the tumors. A number of coactivators and corepressors that interact with nuclear receptors have
been identified and characterized (16). From in vitro binding studies, it appears that both can bind simultaneously to nuclear receptors under appropriate conditions. Thus, one can hypothesize that the complex process of transcriptional activation of the steroid hormone receptors is influenced by the relative amounts of these components. The ratio may be modulated by the type of ligand (agonist, antagonist), the structure of the target gene promoter, and tissue-specific expression of endogenous coactivators and corepressors. Different cell types may have different relative compositions of these coregulators associated with the receptors. In this study, we investigated the expression of three coactivators in a number of different histological types of meningiomas.

Our studies show that the expression of patterns of these genes varies among the meningiomas examined, suggesting their expression may be tissue dependent. The marked variation observed in the expression of the coactivators examined prompted us to study the expression of the steroid hormone receptors in the meningiomas. Our results show that the expression of SRC-1 and TIF2 are significantly related to the PR expression in the meningiomas examined. The majority of the meningiomas, which were positive for PR, were also positive for SRC-1 and TIF2. In mammary epithelium, the expression of ER-α and SRC-1 was segregated into distinct subsets of cells (24). By immunohistochemistry, we were able to show that the steroid cofactors and receptors are both expressed in meningioepithelial cells of the tumor; it remains unclear whether they are in the same cell or distinct subpopulations. Normal brain specimens

Fig. 2 Immunohistochemical detection of TIF2, AIB1, SRC-1, PR, and ER in one representative meningioma specimen. × 40. Nuclear staining is present with all five antibodies. Right column, negative control omitting the primary antibody.
were all positive for SRC-1 and TIF2 and negative for ER, PR, and AIB1. In contrast, one leptomeningeal specimen obtained from an autopsy was positive for SRC-1, PR, and AIB1 but negative for TIF2 and ER. This raises the possibility that TIF2 expression may be involved in meningioma initiation. This is different from previous reports for breast cancer. In breast tumors, AIB1 amplification was correlated with ER and PR positively, as well as tumor size (20), suggesting that AIB1 amplification may be related not only to ER activation but also to PR activation.

In conclusion, we show tumor to tumor variation in the expression of SRC-1, AIB1, and TIF2 genes. These findings are consistent with our hypothesis that the relative expression of coactivators in meningiomas may contribute to the heterogeneity of hormonal responses observed in vitro and in vivo. Ultimately, an understanding of the function of these factors and their role in the pathways of steroid-mediated trans-activation will contribute to the development of new approaches in the prevention and treatment of meningiomas.

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REFERENCES


Fig. 3 Immunohistochemical detection of SRC-1 (A) and AIB1 (B) in one representative leptomeningeal specimen × 40. C and D, negative control omitting the primary antibody. Nuclear staining is present with both antibodies.


