Differential Expression of Galectin-3 in Pituitary Tumors

Dominik Riss, Long Jin, Xiang Qian, Jill Bayliss, Bernd W. Scheithauer, William F. Young, Jr., Sergio Vidal, Kalman Kovacs, Avraham Raz, and Ricardo V. Lloyd

Department of Pathology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905 [D. R., L. J., X. Q., B. W. S., W. F. Y., R. V. L.]; Department of Pathology, St. Michaels Hospital, Toronto, Canada [S. V., K. K.]; and Department of Pathology and Tumor Progression and Metastasis Program, Karmanos Cancer Institute Wayne State University School of Medicine, Detroit, Michigan [A. R.]

ABSTRACT

Galectin-3 (Gal-3), a β-galactoside-binding protein, has been implicated in a variety of biological functions, including cell proliferation and differentiation, tumor cell adhesion, angiogenesis, apoptosis, tumor progression, and metastasis. We investigated the role of Gal-3 in the development and progression of pituitary tumors. Immunohistochemical and Western blot analysis of normal and neoplastic human pituitaries showed that only lactotroph (PRL) and corticotroph (ACTH) hormone-producing cells and tumors expressed Gal-3. Gal-3 was present in 24 of 38 (63.2%) PRL adenomas, 5 of 6 (83.3%) PRL carcinomas, 19 of 41 (46.3%) ACTH adenomas, and 7 of 8 (87.5%) ACTH carcinomas, but not in 112 other pituitary adenomas and carcinomas. Pituitary folliculo-stellate cells, which have macrophage-type functions in the anterior pituitary, also expressed Gal-3. Hyperplastic and neoplastic pituitary tumors from p27kip1 (p27)-null mice, which produce mainly ACTH, showed increased Gal-3 expression levels compared with control mice. Treatment with transforming growth factor β1, which regulates pituitary cell proliferation, reduced Gal-3 as well as p27 expression levels in cultured HP75 pituitary cells and Gal-3 in cultured pituitary cells from p27-null mice, suggesting that p27 is not necessary for the inhibitory effects of transforming growth factor β1 on the cell cycle in the pituitary. The role of Gal-3 in pituitary cell function was examined by RNA interference experiments. Inhibition of Gal-3 gene expression by RNA interference decreased HP75 cell proliferation and increased apoptosis. These results indicate that Gal-3 has an important role in pituitary cell proliferation and tumor progression.

INTRODUCTION

Galectins are a family of carbohydrate-binding proteins with a high affinity for β-galactoside (1). Members of the family have specific sequence homologies of specific carbohydrate-binding motifs (1). Although at least 13 different members of the family are involved with β1,3-(4)-galactosylation (2, 3), Gal-3, the only chimeric gene product, is one of the most frequently investigated members of the group. Human Gal-3 is a 31-kDa protein with three distinct structural domains, including a 12-amino acid NH2-terminal domain that controls cellular targeting; a collagen-like sequence rich in glycine, tyrosine, and proline, which functions as a substrate for matrix metalloproteinases; and a COOH-terminal domain similar to Gal-1 with a globular structure that contains a single carbohydrate-binding site (4). Gal-3 is involved in many biological processes, including cell growth and differentiation, cell adhesion, angiogenesis, tumor progression, apoptosis, and metastasis (5, 6).

Gal-3 is highly expressed in many human tumors and cell lines, including thyroid, colon, and breast (6–8). Gal-3 expression has not been previously examined in neuroendocrine cells and tumors such as pituitary adenomas and carcinomas; therefore, the possible functions of Gal-3 in neuroendocrine tumor development and progression are unknown.

To date, only a few molecular events leading to the development of anterior pituitary tumors have been identified (9). Recent studies have implicated dysregulation of cell cycle genes, including p27kip1 (p27) and p16INK4A (p16) in the pathogenesis of pituitary tumors (10, 11). The levels of p27 protein are decreased in many human cancers compared with normal tissues, and these changes have prognostic significance, suggesting that this cyclin-dependent kinase inhibitor may be a tumor suppressor (12–14). However, there are few mutations in the p27 gene, and p27 mRNA levels are relatively unchanged compared with the decreased levels of p27 proteins in tumors (12). Thus, proteins regulating posttranslational processing of p27, including the ubiquitin-proteasome system, Jak1, and other cell cycle proteins that interact with p27 in G1, such as cyclin E and cyclin D1, are suggested as possible regulators of p27 activity (13). Recent studies have reported interactions between Gal-3 and p27 because Gal-3 induced p27 as well as p21WAF1/CIP1 (p21) in monolayer cell culture and p27 was further up-regulated in suspension cultures of human breast carcinoma cells, suggesting that Gal-3 regulates cyclin-E-associated kinase activity (6).

We examined Gal-3 expression in pituitary tumors and the regulation of Gal-3 and p27 by TGFβ1, a known regulator of pituitary cell growth, to determine the roles of Gal-3 and p27 in pituitary tumor development and progression. Our results show that Gal-3 has an important role in pituitary cell proliferation and tumor progression.

MATERIALS AND METHODS

Tissues. Formalin-fixed, paraffin-embedded tissues from 148 surgically removed human pituitary adenomas and 14 pituitary carcinomas were used for immunohistochemical analyses. Normal pituitary tissues, obtained within 8 h postmortem from patients without endocrine disease (n = 6), were also analyzed. Eight adenomas and 3 normal pituitary tissues were also used for ISH analyses. Five-μm sections were cut, deparaffinized, and used for immunohistochemical and ISH analyses.

Cell Culture. The HP75 cell line (15) was maintained in DMEM supplemented with 15% horse serum, 2.5% fetal bovine serum, 1 μg/ml insulin, 1% Antibiotic-Antimycotic (100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, and 250 ng/ml amphotericin B; Life Technologies, Inc., Grand Island, NY) in a humidified incubator at 37°C and 5% CO2. For experiments with TGFβ1 treatment, cells were cultured in 2% fetal bovine serum for 4 days.

p27-null Mice. p27-null mice were obtained from Dr. J. L. Roberts, (Fred Hutchinson Cancer Center, Seattle, WA) and maintained as described previously (16). Tail snips from neonatal mice were used for genotyping as reported previously (16). Animals were sacrificed at 2–3 months of age, and the pituitaries were excised, weighed, and used for Western blot analysis or dissociated and cultured in the presence of 10-6 M TGFβ1 as described previously (16). These cells were harvested and used for Western blot analysis.

Immunohistochemistry. Immunohistochemical analysis was performed as reported previously (15, 16). Gal-3 monoclonal antibody (Vector Laboratories, Burlingame, CA) was used at a 1:500 dilution with the avidin-biotin-peroxidase system. Antibodies to pituitary hormones (National Pituitary Agency, 2251

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2 To whom requests for reprints should be addressed, at Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905. Phone: (507) 284-4022; Fax: (507) 284-1875; E-mail: lloyd.ricardo@mayo.edu.

3 The abbreviations used are: Gal-3, galectin-3; TGFβ1, transforming growth factor β1; ISH, in situ hybridization; siRNA, small interference RNA; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated nick end labeling; FS, folliculo-stellate; PRL, lactotroph; ACTH, corticotroph; GH, growth hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

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Bethesda, MD) were used to characterize the pituitary tumors as reported previously (15). Combined staining to localize two antigens was done sequentially with a monoclonal anti-Gal-3 antibody and polyclonal antibodies to pituitary hormones as reported previously (14). Positive controls for Gal-3 included a thyroid tumor shown to produce Gal-3 by immunostaining and Western blotting. Negative controls consisted of substituting normal mouse serum of the same isotype (IgG1) for the monoclonal antibody.

**ISH.** A 456-bp DNA fragment of human Gal-3 corresponding to the coding region of nucleotides 95–553 (GenBank accession no AB006780) was generated by reverse transcription-PCR, cloned into the pCR II-TOPO cloning vector, and used as a probe. Single-strand riboprobes were prepared as described previously (14), labeled with digoxigenin, and used for ISH. ISH and IHC combined with immunohistochemistry were done as reported previously on three normal pituitaries and eight pituitary tumors.

**RNA Interference Experiments.** RNA interference experiments were performed with cultured HP75 cells according to the manufacturer’s protocol (Dharmacon Research, Lafayette, CO; Ref. 17). siRNA duplexes targeting Gal-3 were prepared by annealing two pairs of 21-ribonucleotide oligonucleotides synthesized by Dharmacon Research. The coding strand was 5’-AGC GGA UGG CAG ACA AAA-3’ (GenBank Accession No. BC001120).

HP75 cells were transfected with Trans It-TKO transfection reagent (Dharmacon) in a 24-well plate with 1 × 10^5 cells. Control dishes received a scrambled I duplex RNA (Dharmacon), whereas the parental cell line was treated with Trans IT-TKO only. Sixteen h after transfection, the 2% serum was replaced with complete medium, and cells were cultured for another 3 days. The cells were then harvested and used for Western blotting, proliferation, and apoptosis assays.

**Transfection with Antisense Plasmid.** HP75 cells were transfected in a serum-free medium with either the pCNC10 vector or pCNC10Gal3AS (pCNC10 containing a human Gal-3 insert in the antisense orientation; Ref. 6), with use of the GenePorter transfection reagent (Gene Therapy Systems, San Diego, CA). Three days after transfection, 50 µg/ml Geneticin (Life Technologies, Inc.) was added for 1 week; the cells were then maintained in 2 µg/ml Geneticin for 1 week and 1 µg/ml Geneticin thereafter.

**Cell Proliferation.** After 4 days of treatment, the medium was removed, and 0.5 ml of fresh medium containing 2.5 µCi/ml [3 H]thymidine (specific activity, 15.0 Ci/mmol; DuPont/NEN, Boston, MA) was added for 6 h. The cells were harvested by trypsin treatment and washed three times with PBS.

**Fig. 1. Localization of Gal-3 in human pituitary tissues, as detected by light microscopy.** A, normal anterior pituitary tissue (NP) showing a small percentage of cells expressing Gal-3, indicated by brown immunostaining (magnification, ×250). B, combined staining with anti-ACTH antibody (brown) and anti-Gal-3 antibody (blue) showing expression of Gal-3 in ACTH cells (arrow; magnification, ×300). C, ISH with an antisense riboprobe showing expression of Gal-3 mRNA in normal anterior pituitary cells (NP) indicated by blue staining (magnification, ×200). D, the sense control probe is negative in normal anterior pituitary cells (NP; magnification, ×200). E, lactotroph (PRL) adenoma showing strong (3+) staining for Gal-3 (magnification, ×250). F, thyrotroph (TSH) adenoma with negative staining for Gal-3 (magnification, ×250). G, corticotroph (ACTH) adenoma showing strong (3+) staining for Gal-3 (magnification, ×250). H, corticotroph carcinoma (ACTH CA) metastatic to the liver, showing diffuse positive staining for Gal-3 (magnification, ×300).
Cell aliquots were used for examination of [3H]thymidine incorporation by scintillation counting as described previously (15).

Apoptosis. For analysis of apoptosis, an aliquot of HP75 cells was harvested, placed on slides by cytocentrifugation, and fixed in 4% paraformaldehyde for 20 min. Apoptosis was analyzed by TUNEL (Boehringer Mannheim, Indianapolis, IN) as reported previously (18).

Western Blotting. Western blot analysis was performed as described previously (14). The anti-Gal-3 antibody was used at a 1:1000 dilution. Anti-p27 antibody (Transduction Labs, Lexington, KY) was used at 1:1000 dilution. Anti-p21 (Transduction Labs) and anti-cyclin D1 (Novocastra, Newcastle, U.K.) were used at a 1:1000 dilution. Bands were visualized by chemiluminescence (Amersham, Arlington, IL).

Quantitation. Experiments were repeated three to six times except where indicated. Immunohistochemical and ISH staining were evaluated as follows: 0, no staining; 1+, weak; 2+, moderate; and 3+, strong staining.

Densitometric analysis of the Western blot film was done with a Bio-Rad Gel Doc. The protein bands were visualized with an anti-Gal-3 antibody, followed by an anti-rabbit secondary antibody. The relative densitometry units were calculated relative to β-actin for each lane.

Statistical analyses were done with the two-tailed t test and χ² test. P < 0.05 was considered significant.

RESULTS

Immunohistochemical and ISH Analysis. Analysis of Gal-3 immunoreactivity and mRNA expression in normal pituitary showed a positive reaction in some pituitary cells, including the FS cells (Fig. 1). Combined immunostaining and ISH revealed PRL and ACTH cells as well as FS cells positive for Gal-3 (Fig. 1B). ISH with a Gal-3 riboprobe showed that ~30–40% of pituitary cells were positive for Gal-3 (Fig. 1C), whereas ISH with the same probe was negative (Fig. 1D).

Immunohistochemical analysis of 148 pituitary adenomas and 14 pituitary carcinomas showed Gal-3 immunoreactivity restricted to PRL and ACTH benign and malignant tumors (Fig. 1, E–H; Table 1). All other tumor types were negative for Gal-3. The percentage of ACTH carcinomas positive for Gal-3 was significantly higher than the percentage of adenomas (Table 1). Only PRL and ACTH tumors expressed Gal-3 mRNA, as determined by ISH (data not shown).

Western blots for Gal-3 with a group of human pituitary tumors showed a strong band for PRL adenomas and for ACTH and PRL carcinomas (Fig. 2A), supporting the immunohistochemical findings.

p27-null Mice. p27-null mice develop hyperplasia and tumors of the ACTH-producing cells in the pituitary intermediate lobe. We examined the role of Gal-3 in pituitary hyperplasia in these null mice. Western blot analysis of p27-null mice and control littermates showed a 3–10-fold increase in Gal-3 levels in the neoplastic pituitaries (Fig. 2B). Immunostaining confirmed expression of Gal-3 in the pituitary, mainly in intermediate lobe tumors with focal staining in the anterior pituitary (data not shown). Sequential analysis of p21 and cyclin D1 also showed decreased expression of these cell cycle proteins compared with the control pituitaries (Fig. 2B).

TGFβ1 Treatment. Our previous studies had shown an important regulatory role of TGFβ1 on pituitary cell growth (14). Examination of the effects of TGFβ1 treatment (10⁻⁹ M) on Gal-3 expression by HP75 cells showed that TGFβ1 decreased Gal-3 and p27 protein expression, with a >75% decrease in Gal-3 in three separate experiments (Fig. 2C).

To determine whether the effects of TGFβ1 on Gal-3 was mediated
by p27, we dissociated pituitary cells from p27-null mice and treated them with TGFβ1 for 6 days. This produced a >3-fold decrease in Gal-3 protein expression level in two independent experiments (Fig. 2D).

**RNA Interference.** Treatment of HP75 cells with siRNA produced a 70% decrease in the expression of Gal-3 compared with the parental and control cells by Western blot analysis (Fig. 3A). Gal-3 siRNA treatment for 4 days produced a significant decrease in cell proliferation as measured by [3H]thymidine incorporation (Fig. 3B) and a significant increase in apoptosis (Fig. 3C) in four independent experiments.

Transfection of HP75 cells with pCNC10 Gal-3 antisense plasmid also produced an increase in apoptosis (1.76 ± 0.12%) compared with the vector (1.03 ± 0.01%) and parental (1.10 ± 0.06%) cells (P < 0.05) in three independent experiments.

**DISCUSSION**

We report here on the differential expression of Gal-3 in normal and neoplastic pituitary secretory cells. Gal-3 protein and mRNA were expressed in normal PRL and ACTH cells. Adenomas and carcinomas derived from PRL and ACTH cells also expressed Gal-3 protein, and there was a significant increase in the percentage of ACTH tumors expressing Gal-3 in pituitary carcinomas compared with adenomas. The finding of restricted Gal-3 expression by only PRL and ACTH cells may be significant because the majority of pituitary carcinomas, which are uncommon cancers, express mainly PRL or ACTH (19).

Recent evidence suggests that molecular mechanisms involved in the pathogenesis of human pituitary tumors are heterogeneous and may be related to tumorigenesis in specific cell types (9, 11). Although alterations in a few genes, such as pituitary transforming gene (PTTG), are associated with most subtypes of pituitary tumors (20), mutations in the α subunit of Gs transmembrane signaling protein is mostly present in a subset of GH tumors (9, 11). Nonfunctional (FSH, LH, and null cells) tumors are associated with molecular alterations in p16 (hypermethylation), but these alterations are not found in GH tumors (11). Alterations in p27 expression are seen primarily in ACTH-producing cells in human and rodent pituitaries (14, 16). These recent observations suggest another level of complexity in tumorigenesis within specific organs, i.e., cell-type-specific molecular changes, and would be consistent with our observations that Gal-3 expression is largely restricted to the two most common cell types associated with human pituitary carcinomas (PRL and ACTH cells).

Our studies also provide direct evidence for a role of Gal-3 in pituitary cell proliferation and in apoptosis; Gal-3 siRNA reduced Gal-3 expression associated with inhibition of cell proliferation and increased apoptosis in the cultured HP75 cells. Transfection of pituitary HP75 cells with pCNC10 Gal-3 antisense also increased apoptosis.

Gal-3 expression was markedly increased in p27-null mice, which develop pituitary hyperplasia and tumors of intermediate lobe ACTH cells, suggesting a direct role of Gal-3 in pituitary tumorigenesis. The increased expression of Gal-3 in human pituitary carcinomas, which are defined by the presence of metastatic disease in the brain or sites outside of the brain, supports a role of Gal-3 in pituitary tumor progression. Similar findings in breast carcinoma cell lines and other tumors (6–8) support a broad role of Gal-3 in tumor progression. A recent study using the BT549 cell line showed that Gal-3 phosphorylation was necessary for some of its activities, including inhibition of apoptosis (6).

The experiments with TGFβ1 treatment indicate a critical role of this cytokine in regulating pituitary cell proliferation, which is mediated in part by Gal-3 protein in pituitary cells. The marked proliferative activity leading to the development of pituitary hyperplasia and tumors in p27-null mice may be linked to the markedly enhanced Gal-3 levels as well as lower p21 and cyclin D1 protein levels in the pituitary tumors. The in vitro analysis of cultured pituitary cells showed a direct inhibitory effect of TGFβ1 on Gal-3 expression in the p27-null mice, suggesting that the inhibitory effects of this cytokine on Gal-3 do not require p27.

The HP75 cells were derived from a nonfunctional pituitary adenoma that produced FSH and LH (15). Recent studies have also detected pro-opiomelanocortin mRNA and the transcription factor Neuro D1, which is important for ACTH cell development, in these cells, the detection of Gal-3 expression would therefore be consistent with ACTH production, as in the primary pituitary tumors in this study.

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1 R. Y. Osamura, personal communication.
In summary, there is a differential pattern of Gal-3 protein and mRNA expression in pituitary tumors with PRL, ACTH, and FS cells as well as PRL and ACTH tumors expressing this lectin. A higher percentage of ACTH carcinomas compared with adenomas expressed Gal-3 protein, whereas the levels of Gal-3 protein were markedly increased in pituitary tumors from p27-null mice. RNA interference experiments showed that Gal-3 has a direct effect on cell growth and apoptosis in cultured pituitary cells. These results indicate that Gal-3 has an important role in pituitary cell proliferation and may serve as a possible therapeutic target to prevent pituitary tumor progression and carcinoma development because of the poor prognosis associated with pituitary carcinomas (19).

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REFERENCES


2255

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