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Original contribution

Galectin-3 expression in pituitary adenomas as a marker of aggressive behavior☆

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Summary The purpose of this retrospective study was to investigate the role of galectin-3 (LGALS3) expression in predicting the recurrence and the progression potential of prolactin (PRL) and adrenocorticotropic hormone (ACTH)–producing pituitary adenomas and its correlation with the RUNX1 and RUNX2 transcription factors involved in the regulation mechanism of LGALS3 expression. Clinical, neuroradiologic, and follow-up data from 92 pituitary adenomas, including 59 PRL cell adenomas and 33 ACTH-functioning pituitary adenomas, were collected. The LGALS3 expression was analyzed by both immunohistochemistry and quantitative real time-polymerase chain reaction, whereas RUNX1 and RUNX2 were analyzed by quantitative real time-polymerase chain reaction only. The data obtained indicated that invasive growth with suprasellar extension, Ki-67 labeling index, and LGALS3 immunohistochemical and/or *LGALS3* messenger RNA levels are the most important histologic features for assessing a high risk of progression or recurrence of PRL- and ACTH-functioning pituitary adenomas. Multivariate Cox regression analysis assessed LGALS3 immunohistochemical positivity in at least 30% of neoplastic cells and/or *LGALS3* messenger RNA positivity ($P < .001$) as strong predictive factors of recurrence/tumor progression followed by a Ki-67 labeling index greater

Abbreviations: LI, labeling index; LGALS3, galectin-3; PRL, prolactin; ACTH, adrenocorticotropin; PA, pituitary adenoma; GH, growth hormone; TSH, thyrotropin; MRI, magnetic resonance imaging; FSH, follicle-stimulating hormone; LH, luteinizing hormone; IHC, immunnohistochemistry; ROC, receive operating characteristic; qRT-PCR, quantitative real time-polymerase chain reaction; Ct, cycle threshold; HPF, high-power field.

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than 3% (P = .019) in the 81 cases in which follow-up data were available. In addition, a significant correlation between LGALS3 and RUNX1 expression levels (P = .0435) was found. This retrospective immunohistochemical and molecular study demonstrated that LGALS3 expression appeared to be a predictive factor of the aggressive behavior of PRL- and ACTH-functioning pituitary adenomas, and its expression was correlated with RUNX1 expression levels.

1. Introduction

Galectin-3 (LGALS3) is a β-ganglioside binding lectin that is up-regulated during neoplastic progression and metastasis in several human malignancies such as in thyroid, colon, liver, and brain tumors [1]. Therefore, LGALS3 expression has been proposed as a potential diagnostic and/or prognostic marker in tumors located in different organs [1-4].

In the pituitary gland, a unique phenomenon is observed: LGALS3 is expressed in normal prolactin (PRL)- and adrenocorticotropin (ACTH)-producing cells as well as in folliculostellate cells. A different distribution of *LGALS3* gene products and LGALS3 protein in pituitary tumors (both adenomas and carcinomas) has been reported, with the highest level of LGALS3 expression in PRL and functioning ACTH pituitary adenomas (PAs) with respect to normal PRL- and ACTH-producing cells [5].

Previous studies on pituitary tumorigenesis have speculated that pituitary carcinoma develops from an adenoma through a stepwise series of genetic alterations [6] including overexpression of the *LGALS3* gene and the LGALS3 protein [5]. Furthermore, Jin et al [7] pointed out that, in ACTH-subtype adenomas, LGALS3 is a useful immunohistochemical marker for differentiating silent from functioning ACTH PAs.

Zhang et al [8] suggested that RUNX1 and RUNX2, RUNX family transcription factors that allow tumor proliferation and progression, are involved in the regulation mechanism of LGALS3 expression in both normal and neoplastic pituitary glands. Accordingly, RUNX1 and RUNX 2 up-regulate the *LGALS3* gene by direct binding to its promoter region, therefore partially contributing to pituitary tumor growth regulation.

Recently, Stilling et al [9], examining the expression of specific microRNAs in normal pituitary tissue and in ACTH tumors, found that microRNA-493, which binds the *LGALS3* and *RUNX2* genes, is up-regulated in ACTH carcinomas as compared with adenomas and normal pituitary tissue.

The purpose of this study was to investigate the role of LGALS3 overexpression in predicting the recurrence and the progression potential of PRL- and ACTH-functioning PAs using immunohistochemistry and quantitative real time-polymerase chain reaction (qRT-PCR) and its correlation with RUNX1 and RUNX2 transcription factors.

2. Material and methods

2.1. Cases

All PRL- and ACTH-functioning PAs present in the files of the Section of Anatomic Pathology of the Department of Biomedical and Neuromotor Sciences of the University of Bologna at Bellaria Hospital from January 1992 to December 2009 were retrieved. Moreover, 61 PAs of other main types (25 silent type I or II ACTH, 14 null cell, 6 gonadotropic cell, 11 growth hormone [GH; 6 densely and 5 sparsely granulated], 4 mammosomatotroph, and 1 thyrotropin [TSH]) were randomly selected as negative controls. To diagnose silent ACTH type I and silent ACTH type II, electron microscopy was used at the time of the original diagnosis. Thirteen of these 61 PAs (2 densely and 2 sparsely granulated GH, 3 silent ACTH, 3 null cell, 2 gonadotropic, 1 mammosomatotroph) were used as control cases of *LGALS3*, *RUNX1*, and *RUNX2* for quantitative RT-PCR analysis.

All patients with functioning ACTH PAs presented with Cushing disease and showed elevated serum cortisol levels, whereas those with silent PAs manifested clinical symptoms related to the tumor mass.

All patients of PRL cell tumors were treated with dopamine agonists in the preoperative period. Surgical resection became necessary either because of "resistance" to the drug or because of its adverse effects.

The same neurosurgeons (G.F., D.M.) operated on all the patients using the trans-sphenoidal approach.

The cases meeting the following criteria were selected: (1) the availability of enough material to allow morphologic and immunohistochemical characterization, (2) no radiation therapy before surgery, and (3) the availability of clinical information, including endocrinologic evaluation and neuroimaging data. This study was conducted according to the clinical standards of the 1975 and 1983 Declaration of Helsinki.

Tumor invasion was defined on the basis of 1 or more of the following parameters: preoperative imaging (magnetic resonance imaging [MRI] or computed tomography), intraoperative findings, and histology [10]. Dural invasion was not considered a feature of invasion because previous studies demonstrated that it is not related to the recurrence rate [10]. Suprasellar growth was considered an extension rather than an invasion. MRI findings were reviewed; PAs were classified by size into microadenoma (<10 mm) and macroadenomas (≥10 mm).