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NOTCH RECEPTOR EXPRESSION PROFILE IN NORMAL AND PITUITARY ADENOMAS

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1 - Introduction

The pituitary gland regulates several tissue functions through the production and secretion of endocrine hormones by specialized cells. Pituitary adenomas are caused by dysregulation of cell proliferation and are classified according to the hormones they produce. These adenomas represent 10% of intracranial tumors, they cause morbidity and mortality due to critical localization, hormone secretion and mass effects like visual impairment, headache and compression of cranial nerves.

Evidence of deregulated signaling of Notch pathway has been reported in different types of cancer. However, expression, activation and function of the Notch system in normal and pituitary tumors require deeper studies.

2 - Objectives

The aim of the present study was to analyze the Notch receptor expression pattern in different pituitary tumor models to determine its role in the development and progression of these tumors in order to find future therapies.

3 - Methods

By qRT-PCR and Western Blot (WB) we analyzed the Notch receptor expression comparatively in somatolactotropicGH3 and prolactinomaMMQ rat cell lines, normal rat glands and xenografted tumors generated *in vivo* in nude mice by inoculation of GH3 cells.

We also generated rat prolactinomas *in vivo* by subcutaneous implantation of a 20 mg pellet of diethylstilbestrol (DES) in female 60-day-old Sprague-Dawley rats and analyzed the expression of Notch receptors by WB and Immunohistochemistry (IHC) comparatively with normal pituitary. We evaluated Notch receptor expression in the mouse corticotroph tumor cell line AtT20 and in adult mouse normal pituitary by qRT-PCR and WB. Additionally, surgically obtained pituitary adenomas and non-tumorous pituitaries were included in the study. Samples were preserved in RNAlater for subsequent measurement of mRNA or immediately fixed after surgery in formalin and embedded in paraffin for immunohistochemical analysis.

4 - Results

RAT MODELS

RT-qPCR

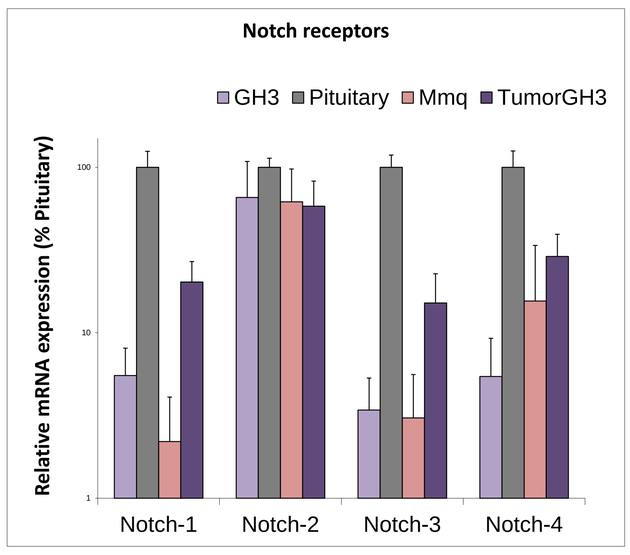

Figure 1. Relative mRNA expression of Notch receptors in rat tumor models. Notch mRNA levels were relativized to G3PDH. (N=5;6;5;4 in GH3, pituitary, MMQ and Tumor GH3, respectively)

WB

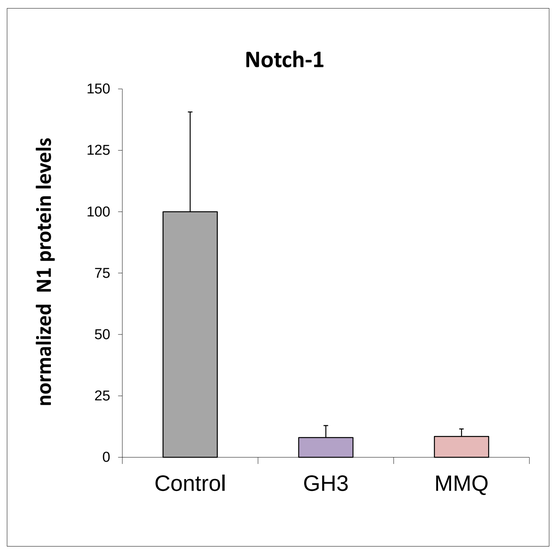

Figure 2. Notch 1 expression determined by Western Blot in rat prolactinomas cell lines. Notch1 levels were normalized to actina (N=3;2;2).

IHC

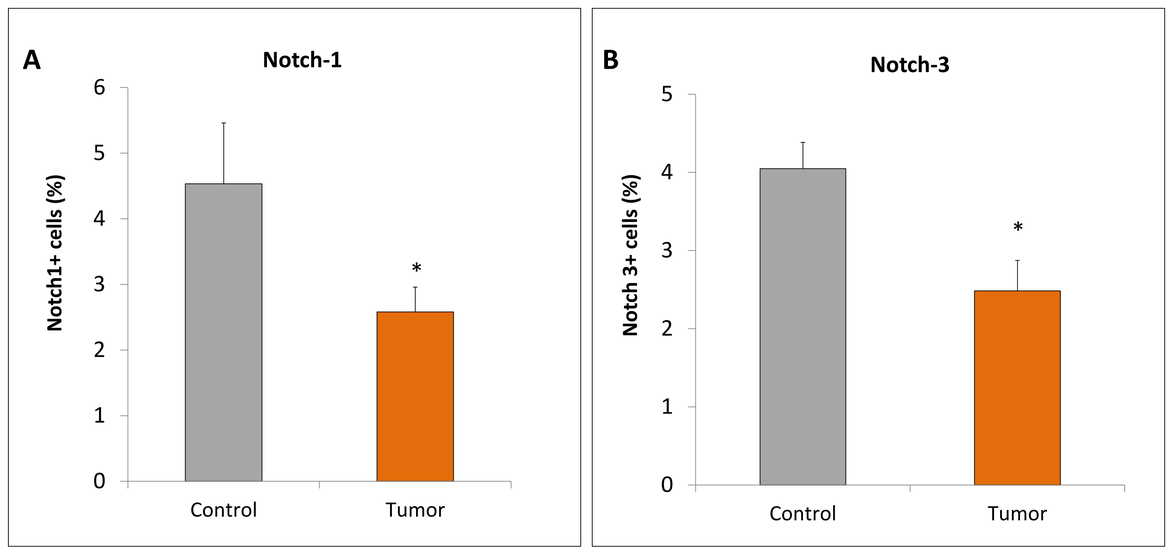

Figure 3. A. Notch1 positive cells expressed as percentage of total cells in control and DES rat pituitary (N=4;3) ($p<0,05$). **B.** Percentage of Notch3 positive cells in Control and DES rat pituitary (N= 2;3) ($p<0,05$).

RT-qPCR

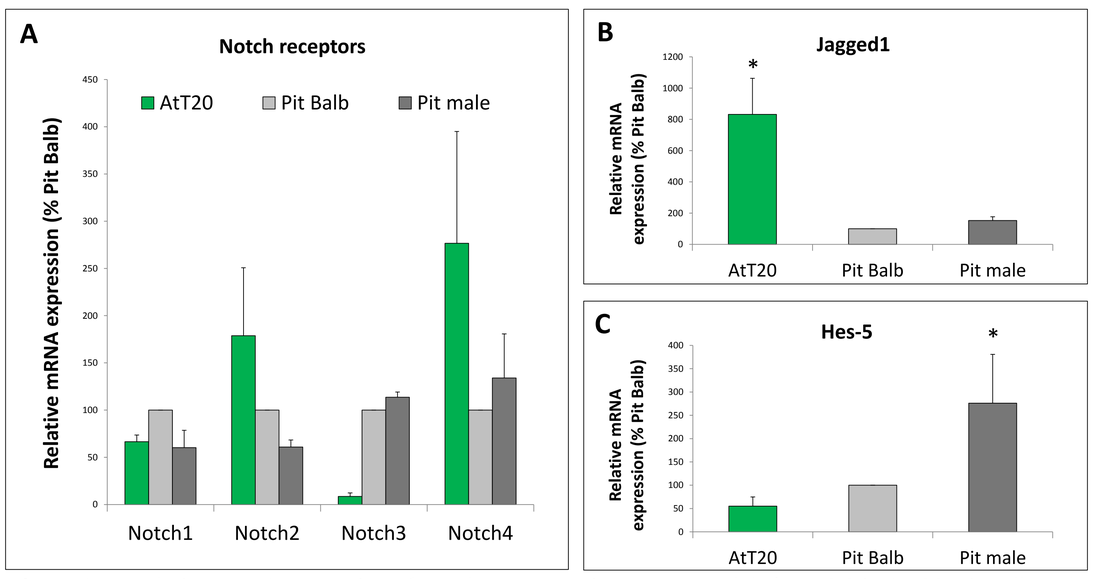

Figure 4. A. Relative mRNA expression of Notch receptors in mouse models (N=7;3;3 in AtT20, Pit Balb and Pit male, respectively). **B.** Relative mRNA expression of Jagged1 in mouse models (N=7;3;2 in AtT20, Pit Balb and Pit male, respectively) ($p<0,05$). **C.** Relative mRNA expression of Hes-5 in mouse models (N=7;3;2 in AtT20, Pit Balb and Pit male, respectively) ($p<0,05$). mRNA levels were relativized to G3PDH.

WB

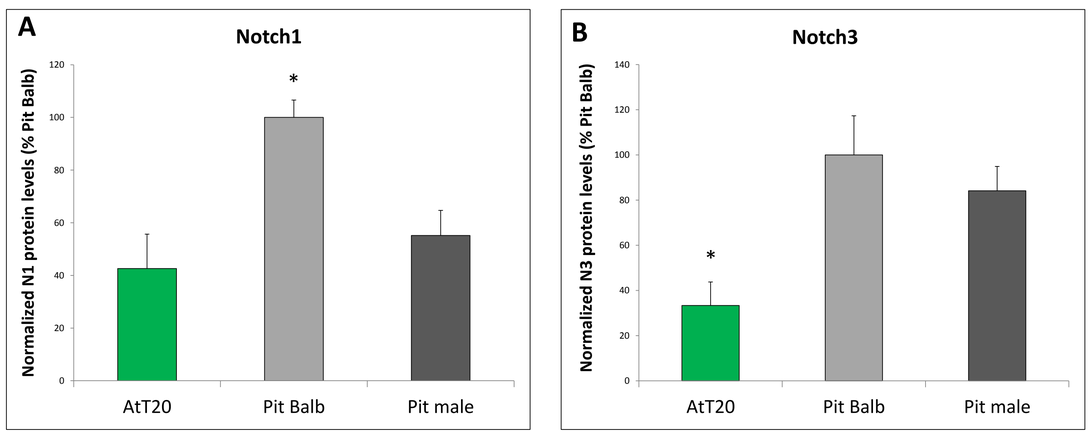

Figure 5. A. Notch1 receptor expression determined by Western Blot in mouse models (N=3) ($p<0,05$ for Pit Balb vs AtT20 and Pit male). **B.** Notch3 receptor expression determined by Western Blot in mouse models (N=3) ($p<0,05$ for AtT20 vs Pit Balb). Notch levels were normalized to actin levels and expressed as percentage of Pit Balb.

HUMAN PITUITARY TUMORS

IHC

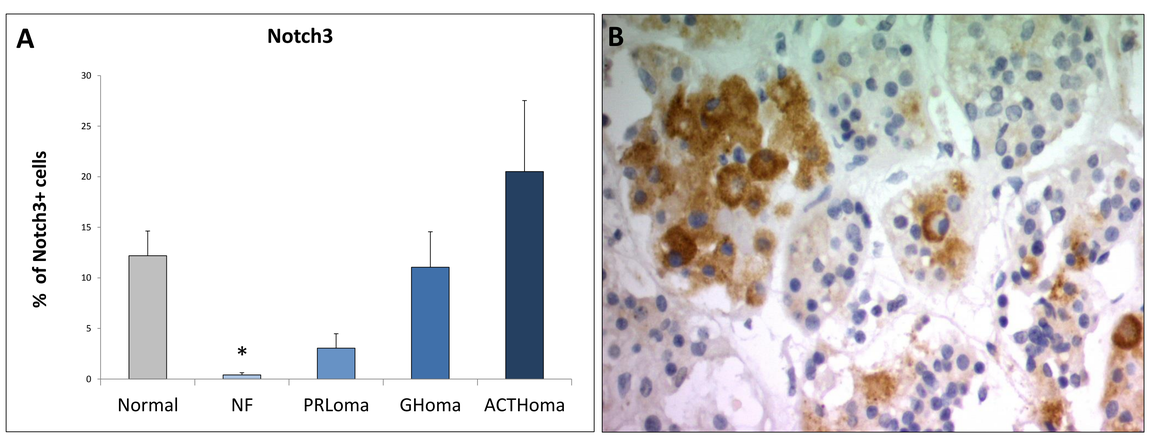

Figure 6. A. Notch3 positive cells expressed as percentage of total cells, in different types of human pituitary tumors. (N=3;2;2;4;3 in Normal, Nonfunctioning (NF), Prolactinoma (PRLoma), Somatotrophinoma (Ghoma) and Corticotrophinoma (ACTHoma), respectively) ($p<0,05$ for NF vs Normal and ACTHoma). **B**. Representative staining of Notch3 in histological section of corticotroph tumor.

RT-qPCR

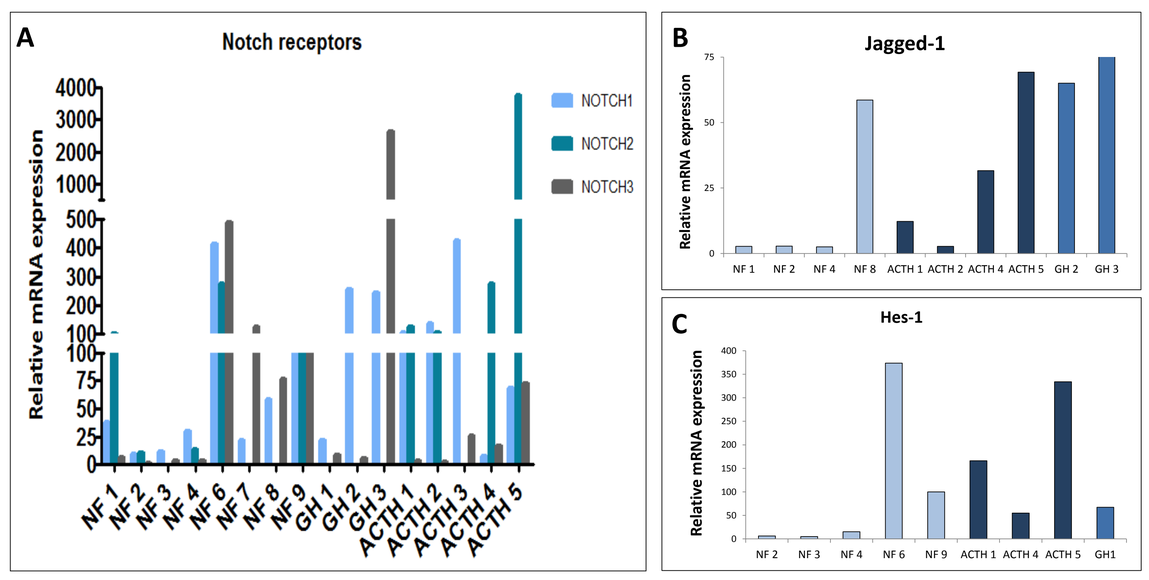

Figure 7. A. Relative mRNA expression of Notch receptors in pituitary tumors. **B.** Relative mRNA expression of Jagged1 in pituitary tumors. **C.** Relative mRNA expression of Hes-1 in pituitary tumors. mRNA levels were relativized to G3PDH.

5 - Discussion

The expression profile of Notch receptors resulted different in tumor models in relation to normal glands. In rat pituitary tumors models, the mRNA levels of all Notch receptors were lower than in the normal pituitary; these results were confirmed by WB for Notch1 and for Notch1 and Notch3, by IHC (DES model). In accordance with rat models, mouse cell line AtT20 showed lower expression of Notch1 and Notch3 in comparison with normal gland. Otherwise, the high expression of Jagged1 in AtT20 suggests the activation of the pathway.

In human pituitary adenomas, the mRNA expression pattern of Notch pathway components was different among the different adenoma types. There was a trend of higher Notch-3 protein level in ACTH-secreting adenomas relative to other pituitary tumors. Interestingly, Notch-3 was detected in normal pituitary as well.

6 - Conclusions

Our results show that at the pituitary Notch system participates both in pathological and physiological states. Our work contributes to the understanding of Notch signaling in pituitary adenoma development expanding the potential therapeutic approaches of these adenomas.