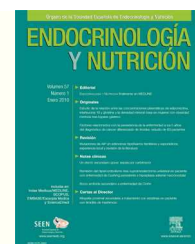


# ENDOCRINOLOGÍA Y NUTRICIÓN

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## EDITORIAL

### Current role and future perspective of molecular studies in pituitary tumors

### Papel actual y perspectiva futura de los estudios moleculares en los tumores hipofisarios

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The molecular study of pituitary tumors has contributed important knowledge regarding not only their oncogenic mechanisms, but also by identifying markers potentially associated with their biological and clinical behavior. Some of these markers could turn out to be crucial in making difficult therapeutic decisions, such as the indication of radiation therapy to prevent tumor growth and the selection of a particular molecular-targeted therapy like somatostatin analogs (SSA). The subcellular and molecular analysis of pituitary adenomas is not an easy task. Besides the evident inherent technological complexities, the available biological material is frequently scarce, since many of these tumors, rather than being excised, are aspirated by the neurosurgeon. Immunohistochemical (IHC) analysis of these lesions using specific antibodies allows both, their classification according to the peptide hormones they synthesize and the establishment of the cell lineage they stem from.<sup>1</sup> For instance, a negative Pit-1 immunostain confirms that the lesion in question is indeed a gonadotrophinoma or a null cell adenoma.<sup>1</sup> IHC to determine the Ki-67 index also enables us to estimate the proliferative potential of a particular adenoma. Dot-pattern cytokeratin immunostaining can also be related to adenoma aggressiveness and to the finding of sparse granularity on electron microscopy.<sup>1</sup> Even though IHC

for pituitary hormones and to a lesser extent, for Ki-67 and cytokeratin have become routine in the evaluation of these tumors, the identification of molecular alterations, be it by real-time polymerase-chain reaction (PCR) or by direct sequencing, remains in the domain of specialized research laboratories.

Pituitary tumors result from a complex interaction of molecular events that includes activating mutations of oncogenes, inactivating mutations of tumor suppressor genes (TSG), the action of regulatory hormones and the relative loss of the physiological feedback inhibition of hormonal synthesis and cellular proliferation.<sup>2,3</sup> Although most pituitary tumors are sporadic, it is in the few tumors that occur either on a hereditary basis or in the context of well-established genetic syndromes, where a more precise knowledge of the mechanisms that lead to neoplastic transformation has been generated.<sup>4</sup> In the case of type 1 Multiple Endocrine Neoplasia (MEN1) the responsible oncogenic mechanism involves inactivating mutations of the TSG called Menin, mapped to the chromosomal region 11q13.<sup>2–4</sup> Menin is a nuclear protein that interacts with Jun D and suppresses transactivation.<sup>2–4</sup> Pituitary adenomas in the context of MEN 1 tend to occur at a younger age and to be more aggressive; yet, no MEN1 mutations have been found in the more frequent sporadic tumors.<sup>2–4</sup> An analogous situation is found in pituitary adenomas occurring as part of the Carney Complex (CC), a condition in which the molecular abnormality consists of inactivating mutations of the 1- $\alpha$  regulatory subunit of

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protein kinase A (PKAR1A).<sup>2-4</sup> The evaluation of patients with hereditary pituitary adenomas not fulfilling the criteria for MEN1 or CC, enabled the identification of a loss of heterozygosity (LOH), precisely close to the 11q13, that suggested the nearby presence of a locus coding for yet a different TSG.<sup>2,3</sup> The use of linkage disequilibrium analysis led to the identification of the gene that codes for the Aryl Hydrocarbon Receptor Interacting Protein (AIP).<sup>5</sup> AIP modulates the subcellular localization of this receptor and interacts with other proteins such as the Heat Shock Protein 90 (HSP90), and attenuates phosphodiesterase activity, thereby regulating cAMP-mediated intracellular signaling.<sup>5</sup> The prevalence of germ line AIP mutations in patients with Familial Pituitary Adenoma Syndrome (FIPA) could be as high as 20%.<sup>6,7</sup> Phenotypically, affected individuals tend to be younger and to have larger and more aggressive tumors than their sporadic counterparts.<sup>6,7</sup> Despite the very low prevalence of AIP mutations in sporadic pituitary adenomas, they do occur in a non-negligible proportion of patients who are diagnosed before age 30.<sup>8,9</sup>

Among the examples of oncogene activation, the GSP- $\alpha$  (GNAS) mutations, which result in constitutive activation of the GHRH receptor, can explain both the neoplastic transformation and the hormonal hypersecretion in a variable proportion of patients with sporadic acromegaly<sup>3</sup>; up to 40% of Caucasians harbor such molecular abnormalities,<sup>10</sup> whereas the prevalence is 7% in Oriental<sup>11</sup> and 18% in Mestizo<sup>12</sup> patients with the disease. GNAS mutations are also the molecular basis for the hormonal hypersecretion occurring in the context of the McCune-Albright syndrome.<sup>3</sup> Interestingly, somatotrophinomas positive for GSP- $\alpha$  mutations are usually densely granulated on electron microscopy, and tend to be less invasive and more responsive to treatment with SSA.<sup>10,12</sup> Pituitary Transforming Gene (PTTG1) codes for a securin that regulates the separation of sister chromatids during mitosis. PTTG1 modulates p53 participation in DNA repair, and when over-expressed can lead to aneuploidy and deregulation of the cell cycle.<sup>13</sup> PTTG1 mRNA has been found increased in both functioning and non-functioning pituitary adenomas.<sup>13</sup>

In this issue of *Endocrinología y Nutrición* Sánchez-Tejada and colleagues from the University of Alicante General Hospital, report an interesting study where they evaluated the mRNA expression of PTTG1 and the IGF-1 receptor (IGF-1R) by real time RT/PCR in 47 pituitary adenomas, seeking correlations with both the Ki-67 labeling index and variables such as tumor volume and extension, as well as re-growth during a mean follow up of 46 months.<sup>14</sup> The sample included 27 gonadotrophinomas, one null cell adenoma, 10 somatotrophinomas, 4 corticotrophinomas, 3 prolactinomas and one thyrotrophinoma<sup>14</sup>; it is important to highlight the heterogeneous nature of the studied adenomas, since we know that oncogenic mechanisms do vary among the different tumor types. Their main finding was a trend toward a higher IGF-1R mRNA expression among the non-functioning adenomas, which appeared to be associated, upon multivariate analysis, with tumor recurrence during follow up.<sup>14</sup> The IGF-1R is a tyrosine kinase that signals through the MAPK and Akt/PI3K pathways, both of which are involved in cell cycle regulation.<sup>15</sup> IGF-1R expression in pituitary adenomas had been previously evaluated, both at the mRNA and protein levels.<sup>16,17</sup> According to these earlier studies, GH-secreting

adenomas express less IGF-1R, when compared to non-tumoral pituitary tissue, while expression is increased in nonfunctioning tumors.<sup>16,17</sup> PTTG1 mRNA expression has been consistently reported in all types of pituitary adenomas perhaps with the exception of ACTH-secreting tumors.<sup>13</sup> Thus, although the results of our colleagues from Alicante are concordant with previously reported data in regards to IGF-1R expression, their finding of a low PTTG1 mRNA expression in almost all the tumors they studied is indeed surprising. Possible explanations for this discrepancy include technical issues inherent to their PCR methods, but my main criticism is related to the lack of data in normal pituitary.

It is crucial to remember that the expression of a particular mRNA does not necessarily imply that the corresponding protein is also appropriately expressed and processed. We recently reported the results of a large high-throughput IHC study in a tissue microarray of over 70 nonfunctioning pituitary adenomas where we found significant PTTG1 nuclear immunoreactivity in virtually all of them, which nevertheless did not correlate with any clinical or imaging feature of tumor aggressiveness.<sup>18</sup> In contrast to Sánchez-Tejada's results,<sup>14</sup> in this study the Ki-67 index was significantly associated with both, tumor size and the risk of tumor recurrence.<sup>18</sup>

It would be very helpful to have molecular studies that could give us a hint as to which particular pituitary adenoma will behave aggressively, so we could initiate therapeutic or even preventive measures as early as possible. Studies such as the one appearing in this issue of *Endocrinología y Nutrición* contribute important, albeit somewhat contradictory information regarding the biological nature of pituitary adenomas. Although the routine clinical application of molecular studies in this field is still not feasible, it is clear to me that it will eventually be; when that time comes, it will undoubtedly help us to deliver a more comprehensive care to our patients.

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