Retinoid X Receptors (RXRs) mRNA Expression in Human Pituitary Adenomas

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Abstract
Retinoid-X receptors (RXRs) are transcriptional factors that belong to the steroid/thyroid hormone receptor (TR) superfamily. It has been demonstrated that those nuclear receptors act as ligand-activated transcription factors in pituitary cells. To determine whether RXRs play roles in the cell differentiation of pituitary adenomas, we have investigated the expression of RXRγ mRNA in various types of pituitary adenomas using in situ reverse transcriptase-polymerase chain reaction (RT-PCR). The synergistic function on promoters of specific hormones between these nuclear receptors and pituitary specific transcription factor, Pit-1, has been noticed in in vitro experiments. The colocalization between RXRγ mRNA and Pit-1 protein was examined by combined in situ RT-PCR and immunohistochemistry. RXRγ mRNA was detected in normal pituitary gland as well as all five growth hormone- (GH)-secreting adenomas and five thyroid stimulating hormone (TSH) secreting adenomas, two of four prolactin- (PRL) secreting adenomas, one of two adrenocorticotropin- (ACTH) secreting adenomas, one of four nonfunctioning adenomas. By in situ hybridization and in situ RT-PCR followed by immunohistochemistry, the colocalization of Pit-1 mRNA with RXRγ as well as RXRγ mRNA with Pit-1 was observed in adenoma cells of GH-secreting adenomas and TSH-secreting adenomas. We suggest that RXRγ may play a role in cell differentiation and hormonal transcription synergistically with Pit-1 in normal and neoplastic human pituitaries.

Key Words: Retinoid; nuclear receptor; pituitary neoplasm; mRNA; in situ RT-PCR.

Introduction
Retinoids are known to affect cell growth and differentiation by binding to nuclear receptors that function as ligand-inducible transcriptional enhancer factors. Retinoid-X receptors (RXRs) are transcription factors that belong to the steroid/thyroid hormone receptor superfamily [1-3], and 9-cis retinoic acid has been identified as their ligand [2,4,5]. RXRs act as homodimers [6] or as heterodimers with thyroxine receptors (TRs), retinoic acid receptors (RARs), vitamin D receptors [7-10] and other receptors, and mediate gene transcription. For the rat growth hormone gene, endogenous RXRs can function as transcriptional factor that regulate the transcription of growth hormone (GH) [11,12]. The signaling crosstalk between RXRs and other receptors, including TRs, RARs, vitamin D receptors, and estrogen receptor, has been reported [2,4,5,8]. The three known isoforms of the RXRs are RXRα, RXRβ, and RXRγ. Each has a distinct pattern of expression and chromosomal location of their encoding genes. RXRα and RXRβ messenger ribonucleic acids (mRNAs) are widely expressed in...
various tissues [2,3]. In contrast, the immunohistochemical expression of RXRγ mRNA is restricted to tissues, such as skeletal muscle, heart, brain, and fetal pituitary [8,12]. We previously produced isoform-specific polyclonal anti-RXR antibodies, and reported the expression of RXR proteins in rat pituitaries [13] and human pituitary adenomas [14]. Interestingly, in rat pituitary gland, RXRγ protein expression was observed predominantly in thyrotropes [13], but in human pituitary, RXRγ protein expression was observed both somatotrophs and thyrotropes [14].

Pituitary specific transcription factor Pit-1 binds to and transactivates the pituitary GH, prolactin (PRL), and thyroid-stimulating hormone (TSH) genes [15,16]. Analysis of the proximal Pit-1 gene promoter indicates that the presence of autoregulatory sites of its own expression [17] and also contains cAMP-responsive elements (CREs) that mediate the transcriptional actions of cAMP [17a]. Specific DNA-dependent interactions have been observed between Pit-1 and nuclear receptors, such as thyroid hormone receptor [18], retinoic acid receptor [19], and estrogen receptor [20] to promote GH and PRL transcription. It may be speculated that these nuclear receptors act synergistically with Pit-1 in cell differentiation and cell proliferation in pituitary cells.

In situ hybridization is a useful technique in demonstrating gene expression in individual cells. However, it has limitations in detecting small amounts of mRNAs. Recently, combined in situ hybridization and reverse transcriptase polymerase chain reaction (in situ RT-PCR) methods were applied to endocrine cells. In this study, we aimed at evaluating whether RXRγ and Pit-1 synergistically associated with functional differentiation of human pituitary adenomas by combined immunohistochemistry, in situ hybridization, and in situ RT-PCR.

**Subjects and Methods**

Five nontumorous human pituitaries were obtained from autopsy cases within 6 h postmortem from patients without endocrinological abnormalities and studied. Sixty human pituitary adenomas (male 28, female 32, aged from 21 to 79 yr) were obtained during transsphenoidal surgery. Sixteen of these patients had GH-secreting adenomas and symptoms of acromegaly, 14 patients had PRL-secreting adenomas with serum PRL levels ranging from 120 to 2430 ng/mL, 6 patients had TSH-secreting adenomas and hyperthyroidism, 5 patients had adrenocorticotropic- (ACTH) secreting adenomas with typical Cushing's syndrome, and 19 patients had nonfunctioning adenomas, which clinically presented no evidence of anterior pituitary hormone excess, and did not show high serum concentration any of anterior pituitary hormones, except mild hyperprolactinemia (<100 μg/L). Eleven patients with nonfunctioning adenomas had visual disturbance, three patients had headache, and four patients were incidentally shown to have pituitary tumors by computerized imaging.

The tissues were routinely fixed in 10% formalin for 8–24 h and embedded in paraffin. Serial sections were prepared for hematoxylin and eosin staining, avidin–biotin complex (ABC) peroxidase method, or the indirect immunoperoxidase method. Polyclonal antibodies for RXRγ were raised against synthetic peptides RXRγ [2,5]. The characterization of the RXRγ antibody was confirmed by Western blotting and immunoprecipitation as described previously [13]. Immunohistochemical preabsorption tests were performed to confine the specificity of the immunohistochemical reaction by preincubating the diluted antibodies with the corresponding antigens overnight at 4°C prior to the immunohistochemical procedure [14].