

Role of ACTH receptor in adrenocortical tumor formation

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Abstract

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Adrenocorticotrophin (ACTH) is the major regulatory hormone of steroid synthesis and secretion by adrenocortical cells. The actions of ACTH are mediated by its specific membrane receptor (ACTH-R). The human ACTH-R gene was recently cloned, allowing systematic determination of its sequence, expression and function in adrenal tumorigenesis. The presence of oncogenic mutations of the ACTH-R gene in adrenocortical tumors has been reported. Direct sequencing of the entire coding region of the ACTH-R gene of sporadic adrenocortical adenomas and carcinomas did not reveal constitutive activating mutations, indicating that this mechanism is not frequent in human adrenocortical tumorigenesis. Recent studies demonstrated allelic loss of the ACTH-R gene in a subset of sporadic adrenocortical tumors using a *PstI* polymorphism located in the promoter region of the ACTH-R gene. Loss of heterozygosity of the ACTH-R was analyzed in 20 informative patients with a variety of benign and malignant adrenocortical tumors. Three of them showed loss of heterozygosity of the ACTH-R gene. In addition, Northern blot experiments demonstrated reduced expression of ACTH-R mRNA in these three tumors with loss of heterozygosity, suggesting the functional significance of this finding at the transcriptional level. Deletion of the ACTH-R gene seems to be involved in a subset of human adrenocortical tumors, contributing to cellular dedifferentiation.

Key words

- Adrenocorticotrophin receptor
- Adrenocortical tumors
- Tumorigenesis

Introduction

Pituitary adrenocorticotrophin (ACTH) is the major regulator of adrenocortical zona fasciculata and reticularis function and is a significant growth factor for normal adrenocortical cells. The actions of ACTH are mediated by its specific membrane receptor (ACTH-R) (1). The ACTH-R belongs to a subfamily of the G protein-coupled receptor superfamily, the melanocortin receptor family, which consists of the ACTH receptor (or MCR2), MSH-R (or MCR1) and three other

receptors (MCR-3 to 5) (1,2).

Human ACTH-R is a 297-amino acid protein with seven transmembrane domains (1). The melanocortin receptors are the smallest G protein-coupled receptors because they have a short NH₂-terminal extracellular domain, short COOH-terminal intracellular domain, unusually short fourth and fifth transmembrane-spanning domains, and a small hydrophobic second extracellular loop (2). In addition, the melanocortin receptors lack several amino acid residues present in most G protein-coupled receptors.

ACTH-R couples to heterotrimeric guanine nucleotide-binding protein that activates adenylyl cyclase (1,2). The human ACTH-R gene was recently cloned and was found to be an intronless gene mapped on chromosome 18p11.2 (1,3). Inactivating mutations in the ACTH-R gene were identified in several families with hereditary isolated glucocorticoid deficiency, a rare autosomal recessive disorder characterized by early onset of primary adrenocortical insufficiency, usually without mineralocorticoid deficiency (3).

Most of the adrenocortical neoplasms were shown to be monoclonal in composition, suggesting that they may arise by transformation of adrenocortical cells due to somatic mutations (4,5). The pathogenesis of these tumors is still not understood. So far, abnormalities of the p53 tumor suppressor gene, guanine nucleotide-binding proteins (Gs and Gi proteins) and overexpression of insulin-like growth factor-II were identified in up to 30% of adrenocortical tumors and thus might be implicated in their pathogenesis (4,6).

No evidence for oncogenic mutations in the ACTH-R

Naturally occurring activating mutations have been reported in G protein-coupled receptors, such as that of TSH receptors in thyroid tumors, suggesting that these receptors can be mitogenic and play a role as oncogenes. When such receptors are constitutively activated by mutations, they transform cells in an agonist-independent fashion. To examine whether the ACTH-R gene could be an oncogene in human adrenocortical tumors, we analyzed the presence of activating point mutations of the ACTH-R gene in a subset of adrenocortical tumors (7). Twenty-five patients with adrenocortical tumors (17 adenomas and 8 carcinomas) and two human adrenocortical cell lines (SW13 and NCI-H-295) were studied. No constitutive activating point mutation or even

silent polymorphism in the entire coding region of the ACTH-R were identified in any of the tumors or cancer cell lines studied. Similar results were later reported by Light et al. (8) in 13 adrenocortical tumors and 3 nodular hyperplasias. These findings indicate that activating mutations of the ACTH-R gene do not represent a frequent mechanism of human adrenocortical tumorigenesis.

Expression of ACTH-R mRNA in adrenocortical tumors

The ACTH-R is mainly expressed in the adrenal cortex but has been identified in extra-adrenal tissues, including human skin and mouse adipose tissue (9,10). The ACTH stimulates the expression of its own receptor in the adrenocortical carcinoma cell line in a time- and dose-dependent manner (9). Recently, Reincke et al. (10) examined the expression of ACTH-R and P450_{scc} (side chain cleavage) in a variety of adrenocortical tumors by Northern blot and reverse transcriptase PCR. Forty-two patients, including 22 patients with adenomas, 8 with normal adrenals, 6 with diffuse adrenocortical hyperplasias and 6 with carcinomas, and 2 carcinoma cell lines were studied. It was demonstrated that plasma ACTH is not the major factor influencing ACTH-R mRNA expression in neoplastic adrenal tissues (10). Mean ACTH-R mRNA expression showed significant differences among adenomas, hyperplasias and carcinomas. Compared to normal adrenal tissue, ACTH-R mRNA expression was low in non-functional adenomas and carcinomas, intermediate in adrenocortical hyperplasias and cortisol-producing adenomas and high in aldosteronomas (10). In adenomas, ACTH-R mRNA expression correlated closely with the expression of P450_{scc} mRNA, suggesting regulation by similar factors (10). However, carcinomas and cancer cell lines did not show a positive correlation between these two parameters. This finding may be explained as a result of

tumor dedifferentiation associated with the malignant phenotype of these neoplasms.

These findings suggest that most adrenocortical tumor tissues express ACTH-R mRNA and that in benign adrenocortical tumors there is a close positive correlation between ACTH-R and P450scc mRNA which is missing in adrenocortical carcinomas.

Loss of heterozygosity of the ACTH-R gene in adrenocortical tumors

The lack of activating point mutations of the ACTH-R gene and *in vitro* findings showing that ACTH had a low potential for stimulating cell proliferation and tumorigenesis, have raised the possibility that mutational loss of the ACTH-R by deletion could result in loss of differentiation and clonal expansion of malignant cell clones.

Using a *PstI* polymorphism in the promoter region of the ACTH-R gene, Reincke et al. (11) analyzed loss of heterozygosity of the ACTH-R gene in sporadic benign and malignant adrenocortical tumors. The rate of heterozygosity for this polymorphism in 99 unrelated Caucasian individuals was 53.5%. Forty-one patients with adrenal diseases were studied and 20 of them (16 adenomas and 4 carcinomas) were heterozygous or informative for the *PstI* polymorphism of the ACTH-R gene. Three of these 20 tumors (1 oncocytic non-functional adenoma and 2 carcinomas) showed loss of heterozygosity for *PstI* polymorphism (11). Analysis of the flanking region of the ACTH-R using the polymorphic microsatellite markers (D18S37 and D18S40)

showed that this deletion was confined to the ACTH-R gene locus. Both patients with adrenocortical carcinomas and loss of heterozygosity had advanced tumor stages and showed a more rapid course than patients with carcinomas and no loss of heterozygosity. In addition, these tumors with loss of heterozygosity showed greatly reduced ACTH-R mRNA levels in Northern blot experiments, suggesting functional significance of this finding at the transcriptional level.

The pathogenesis of adrenocortical tumors is not well understood. However, the molecular mechanisms that lead to adrenocortical tumor formation are being increasingly studied. Elucidating the mechanism of pathogenesis might provide new prognostic factors and eventually new approaches to therapy. In general, cancer is believed to result from a series of genetic alteration that ultimately disrupt the complex mechanisms controlling growth. One abnormal feature associated with adrenocortical tumors is the autonomous or ACTH-independent capacity of steroid synthesis and secretion. Indeed, steroid production is excessive even when there is no circulating ACTH. The potential abnormalities of the ACTH-R were investigated and recent studies have indicated that oncogenic mutations of the ACTH-R gene do not play a role in adrenal tumor formation. Conversely, deletions of the ACTH-R gene are present in a subset of adrenocortical tumors, suggesting that the ACTH-R gene can be involved in adrenocortical tumorigenesis, contributing to cellular dedifferentiation and malignant phenotype.

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