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Mutation and genomic amplification of the PIK3CA proto-oncogene in pituitary adenomas

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Mutation and genomic amplification of the PIK3CA proto-oncogene in pituitary adenomas

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Abstract

The tumorigenesis of pituitary adenomas is poorly understood. Mutations of the PIK3CA proto-oncogene, which encodes the p110-α catalytic subunit of PI3K, have been reported in various types of human cancers regarding the role of the gene in cell proliferation and survival through activation of the PI3K/Akt signaling pathway. Only one Chinese study described somatic mutations and amplification of the PIK3CA gene in a large series of pituitary adenomas. The aim of the present study was to determine genetic alterations of PIK3CA in a second series that consisted of 33 pituitary adenomas of different subtypes diagnosed by immunohistochemistry: 6 adrenocorticotropic hormone-secreting microadenomas, 5 growth hormone-secreting macroadenomas, 7 prolactin-secreting macroadenomas, and 15 nonfunctioning macroadenomas. Direct sequencing of exons 9 and 20 assessed by qPCR was employed to investigate the presence of mutations and genomic amplification defined as a copy number ≥4. Previously identified PIK3CA mutations (exon 20) were detected in four cases (12.1%). Interestingly, the Chinese study reported mutations only in invasive tumors, while we found a PIK3CA mutation in one noninvasive corticotroph microadenoma. PIK3CA amplification was observed in 21.2% (7/33) of the cases. This study demonstrates the presence of somatic mutations and amplifications of the PIK3CA gene in a second series of pituitary adenomas, corroborating the previously described involvement of the PI3K/Akt signaling pathway in the tumorigenic process of this gland.

Key words: Pituitary adenomas; PIK3CA proto-oncogene; Genomic amplification; Somatic mutation

Introduction

The tumorigenesis of pituitary adenomas is poorly understood. The phosphatidylinositol 3-kinases (PI3Ks) are a group of heterodimeric lipid kinases composed of regulatory (85-kDa; p85) and catalytic (110-kDa; p110) subunits that play a key role in cell growth, proliferation, motility, and survival (1-3). The activation of PI3K results in phosphorylation of phosphatidylinositol-4,5-biphosphate (PIP₂) to phosphatidylinositol-3,4,5-triphosphate (PIP₃), which acts as an anchor for Akt serine/threonine kinase and 3-phosphoinositide-dependent protein kinase-1 (PDK1), and facilitates phosphorylation of Akt by PDK1, stimulating Akt activity and subsequent phosphorylation of several proteins involved in a variety of intracellular processes, including antiapoptosis (4,5). PIP₃ levels are tightly regulated by the action of phosphatases such as phosphatase and tensin homologue (PTEN) (6).

Mutations of the PIK3CA proto-oncogene, which encodes the p110-α catalytic subunit of PI3K, have been reported in various types of human cancers (7-10) and more than 80% of PIK3CA mutations are clustered in the helical domain encoded by exon 9 and in the kinase domain encoded by exon 20 (11), as shown with high prevalence in endometrioid, esophageal and pancreatic carcinomas (12-15).

A recent Chinese study (16) has described somatic mutations and amplifications of the PIK3CA gene in a large series of pituitary adenomas, implicating the PI3K/Akt signaling pathway in the tumorigenic process of this gland.
gland. Since a diversity of mutational spectra in different populations had been described for certain neoplasias (17, 18), in the present study we investigated genetic alterations in the PIK3CA proto-oncogene in a second series of pituitary adenomas.

Material and Methods

Tissue specimens and DNA isolation

Tissue collection was carried out in compliance with the Institutional Ethics Committee (CAPPesq) and in accordance to the Declaration of Helsinki, with informed and free written consent being required from each subject or subject’s guardian. From 1994-2009, tumor tissues were obtained from patients diagnosed with pituitary adenomas. During surgery, tumor fragments were collected into sterile containers and immediately frozen in liquid nitrogen. Thirty-three sporadic pituitary adenomas were obtained after surgery. The anatomo-pathological characteristics of these adenomas are shown in Table 1. Pituitary adenomas consisted of 6 adrenocorticotropic hormone (ACTH)-secreting microadenomas, 5 growth hormone (GH)-secreting macroadenomas, 7 prolactin (PRL)-secreting macroadenomas, and 15 nonfunctioning (NF) macroadenomas. As control, four normal pituitary tissues were obtained within 8 h post mortem from subjects without endocrine diseases. The tissue fragments were fragmented in a tissue pulverizer (Mikro-Dismembrator U, B. Braun Melsungen, Germany). Tumor DNA was extracted using the DNeasy kit (Qiagen, USA) according to manufacturer instructions.

Mutation analysis

Exons 9 and 20 of PIK3CA were analyzed by direct sequencing on an ABI 3130X Genetic Analyzer (Applied Biosystems, USA) after PCR amplification of genomic DNA. PCR was carried out in 50 µL containing 100 ng genomic DNA, 0.2 mM of each primer, 200 µM deoxynucleotides, 1X buffer and 1 U DNA Taq polymerase (GE Healthcare, USA). The nucleotide sequences for the primers were designed using the Primer3 software: exon 9 forward 5’-CAAAGCAATTTCACACAGAGATCC-3’; exon 9 reverse 5’- GTAAAAACATGCTGAGATCAGCCACAT-3’; exon 20 forward 5’- TGGAATGCCAGTACCGCATCATTG-3’ and exon 20 reverse 5’- GGTCTTTGGCTGTGCTGAGAAGTATG-3’. Fluorescence changes were monitored after each cycle. Melting curve analysis was performed at the end of each reaction to confirm PCR product’s identity (72°C ramping to 99°C at 0.2°C/s with continuous fluorescence readings). Standard curves were constructed to assess the amplification efficiency of PIK3CA and COL7A1 using duplicate serial dilutions with seven different DNA concentrations (400 to 6.25 ng) from a reference sample of DNA obtained from normal pituitary tissue. Since equal amplification efficiencies of target and reference genes were attained (E >0.9), the mathematical model 2-ΔΔCt was used to evaluate the relative genomic amplification of PIK3CA. All samples were run in duplicate. DNA obtained from a pool of four normal pituitary tissues was included in the assay as a calibrator sample and DNA extracted from the MCF-7 cell line was used as a positive control for PIK3CA amplification. DNA amplification was defined as values ≥4 (16).

Results

PIK3CA mutations

Previously reported PIK3CA mutations were detected in four cases (12%): 3 of 15 (20%) NF pituitary adenomas and 1 of 6 (16.6%) ACTH-secreting tumors, both in exon 20 (Table 2). No mutations were found in exon 9.

Table 1. Demographic data and anatomo-pathological findings of 33 patients with pituitary adenomas who participated in the present study.

| Pituitary adenomas | N  | Gender (male/female) | Age (years) | Tumor size (micro/macroadenoma) |
|--------------------|----|----------------------|-------------|--------------------------------|
| ACTH-secreting     | 6  | 4/2                  | 32.3 (13-61)| 6/0                            |
| GH-secreting       | 5  | 2/3                  | 30.2 (23-55)| 0/5                            |
| PRL-secreting      | 7  | 1/6                  | 31 (20-64)  | 0/7                            |
| Nonfunctioning     | 15 | 6/9                  | 44 (21-70)  | 0/15                           |

ACTH = adrenocorticotropic hormone; GH = growth hormone; PRL = prolactin; microadenoma = ≤10 mm; macroadenoma = ≥10 mm.
Amplification of *PIK3CA*

The number of copies of the *PIK3CA* gene in each tumor sample is shown in Figure 1. Genomic amplification was observed in 21.2% (7/33) of the cases; in 4 of 5 (80%) GH-secreting adenomas, 1 of 6 (16.6%) ACTH-secreting adenomas and in 2 of 15 (13.3%) NF pitu-

Table 2. *PIK3CA* mutations found in four pituitary adenomas.

| Exon | Functional domain | *PIK3CA* DNA mutation | Amino acid | Subtype of tumor |
|------|------------------|-----------------------|------------|-----------------|
| 20   | Kinase domain    | GCC > GAC, C2994A     | Ala$^{2995}$Asp, A > D | ACTH           |
| 20   | Kinase domain    | GCC > GGT, C3031T     | Gly$^{1007}$Gly, G > G | NF             |
| 20   | Kinase domain    | TCT > CCT, T3053C     | Ser$^{1015}$Pro, S > P | NF             |
| 20   | Kinase domain    | CAA > CGA, A3108G     | Gln$^{1033}$Arg, Q > R | NF             |

ACTH = adrenocorticotropic hormone-secreting adenoma; NF = nonfunctioning pituitary adenoma.

**Figure 1.** *PIK3CA* gene copy number detected by qPCR in different subtypes of pituitary adenomas. Positive amplification is defined as ≥4 copies. ACTH = adrenocorticotropic hormone-secreting adenoma; GH = growth hormone-secreting macroadenoma; PRL = prolactin-secreting macroadenoma; NF = nonfunctioning pituitary adenoma.
itary adenomas. No genomic amplification was detected in PRL-secreting adenomas.

Discussion

Several studies have implicated PIK3CA somatic mutations and genomic amplification in the tumorigenic process of different human cancers (19-26). In the present study, we investigated mutations and genomic amplification of the PIK3CA proto-oncogene in 33 sporadic pituitary adenomas. During our experiments, another group of investigators evaluated the association of these genetic alterations in pituitary tumors (16). Given the diversity of mutational spectra in different populations for certain neoplasias (17,18), we decided to continue to examine the incidence of these mutations and amplifications in pituitary tumors of Brazilian patients.

The overall frequency of PIK3CA gene mutations and amplification in the Chinese study of 353 pituitary tumors was 2.3% (8.8% of the invasive tumors and 0% of the non-invasive ones) and 28% (26.3% of the noninvasive tumors and 32.9% of the invasive ones), respectively (16), while in the current series, PIK3CA mutations and amplification were found in 12.1 and 21.2% of the cases, respectively.

Although it is difficult to compare studies that differ so much in the size of the evaluated population, the present study corroborates the previous report (16) that genetic alterations in the PIK3CA gene are found in pituitary tumors. We only detected mutations in one ACTH-secreting and three NF pituitary adenomas, while Lin et al. (16) also found mutations in two PRL-secreting tumors. Mutations in GH-secreting tumors were not found in any of the two series. Interestingly, the Chinese study reported mutations only in invasive tumors, while we found a PIK3CA mutation in one noninvasive corticotroph microadenoma. Recent reports have shown that PIK3CA knockdown reduces proliferation and invasiveness in different cell lines (27-29).

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