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Research paper

Expression pattern of the Hedgehog signaling pathway in pituitary adenomas

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HIGHLIGHTS

- Hedgehog signaling pathway is downregulated in non-functioning pituitary adenomas.
- Somatotropinomas display an overexpression of Hedgehog related genes.
- Expression of Notch 3 and Jagged-1 is down-regulated in somatotropinomas.

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ABSTRACT

Several studies have demonstrated the role of Wnt and Notch signaling in the pathogenesis of pituitary adenomas, but data are scarce regarding the role of Hedgehog signaling. In this study we investigated the differential expression of gene targets of the Hedgehog signaling pathway.

Formalin-fixed, paraffin-embedded specimens from adult patients who underwent transphenoidal resection and normal human pituitary tissues that were obtained from autopsies were used. Clinical information and data from pre-operative MRI scan (extracellular tumor extension, tumor size, displacement of the optic chiasm) were retrieved from the Hospital's database. We used a customized RT^2 Profiler PCR Array, to investigate the expression of genes related to Notch and Hedgehog signaling pathways (PTCH1, PTCH2, GLI1, GLI3, NOTCH3, JAG1, HES1, and HIP).

A total of 52 pituitary adenomas (32 non-functioning adenomas, 15 somatotropinomas and 5 prolactinomas) were used in the final analysis. In non-functioning pituitary adenomas there was a significant decrease (approximately 75%) in expression of all Hedgehog related genes that were tested, while Notch3 and Jagged-1 expression was found significantly increased, compared with normal pituitary tissue controls. In contrast, somatotropinomas demonstrated a significant increase in expression of all Hedgehog related genes and a decrease in the expression of Notch3 and Jagged-1.

There was no significant difference in the expression of Hedgehog and Notch related genes between prolactinomas and healthy pituitary tissues.

Hedgehog signalling appears to be activated in somatotropinomas but not in non-functioning pituitary adenomas in contrast to the expression pattern of Notch signalling pathway.

1. Introduction

Pituitary adenomas account for 10–16% of intracranial tumors [8]. They cause significant morbidity by compression of regional structures and inappropriate secretion of pituitary hormones [2,10]. Most pituitary tumors are sporadic while some have been described as part of genetic syndromes such as multiple endocrine neoplasia type 1, McCune-Albright syndrome, or Carney complex

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[21]. Clonal analysis has shown that almost all sporadic tumors are monoclonal in origin from a genetically mutated single cell [1,11], but the underlying pathogenetic cause remains currently unknown.

During pituitary development, components of highly evolutionary conserved intracelluar signaling pathways Notch, Hedgehog and Wnt are expressed in the invaginating Rathke pouch [18,19,23,26,27]. In the adult pituitary, dysregulation of Notch and Wnt/beta-catenin signalings has been implicated in the pathogenesis of pituitary adenomas [9,14,16]. In particular, expression of the Notch 3 receptor and the Jagged 1 transcription factor of the Notch signaling [28] were found significantly elevated in non-functioning pituitary adenomas compared with normal pituitary tissue [14]. Gene microarrays and proteomic analyses have also demonstrated that Notch 3 gene and protein expression are increased in human clinically non-functioning pituitary adenomas [6,17] but not in GH- and PRL-secreting adenomas.

Hedgehog (Hh) family of signaling proteins in mammals include Sonic Hh (Shh), Indian Hh (Ihh) and Desert Hh (Dhh) that act through the trans-membrane receptors Patched 1 (Ptc1) 1 and Patched 2 (Ptc2). Upon activation of the pathway intracellular signal is transmitted through the transcription factors of Gli family zinc fingers (Gli1, Gli2, Gli3). Target genes include several components of the pathway itself including Gli 1, which is considered the earliest response of Hh activation, Gli3 which is regulated by a negative manner [15], Ptch1 and Hedgehog interacting protein (HIP). HIP is a transmembrane glycoprotein that functions as a putative antagonist of the pathway binding to Hh proteins with similar affinity to Ptch1 and sequester them in a negative feedback loop [3,5]. In primary cell cultures derived from pituitary adenomas exogenous Shh increases secretion of GH, PRL and ACTH from, somatotropinomas, lactotropinomas and corticotropinomas respectively [25], and exerts antiproliferative effects. In addition Ptch1 and Ptch2 receptor proteins are expressed at variable levels in pituitary adenomas [25] but data are scarce regarding other components of the intracellular signaling.

In this study we sought to investigate the expression of transmembrane, cytoplasmic and nuclear components of the Hedgehog signaling pathway in pituitary adenomas, in order to shed more light in the potential implication of this pathway in the pathogenesis of the disease. In addition we also searched for the expression of certain components of the Notch signaling that have been implicated in pituitary adenomas (Notch 3 and Jagged 1) in an effort to characterize a potential interaction between the two pathways in functioning and non-functioning pituitary adenomas.

2. Material and methods

2.1. Patients

This study was approved by the Scientific Review Board of AHEPA University Hospital (protocol number 51741).

We studied the medical records of patients who were diagnosed with pituitary adenomas and underwent transphenoidal resection at the Neurosurgery Department of AHEPA University hospital from 2009 to November 2014. Diagnosis was determined on the basis of clinical, biochemical, and radiologic findings before surgery, as well as morphologic and immunohistochemical data during and after surgery. The following characteristics were recorded for each patient: year of diagnosis, age at diagnosis, gender, pre-operative clinical manifestations, tumor size, optic chiasm displacement and extrasellar extention based on pre-operatve MRI scans, where available.

2.2. Human tissues

All formalin-fixed, paraffin-embedded (FFPE) specimens available from adult patients (age > 20 years at the time of the surgery) with confirmed pituitary adenoma by histopathological examination that were identified from the medical records in the time-period we searched, were retrieved from the pathology database of AHEPA University hospital. All pathology slides were reviewed by two pathologists (C.P, G.K.) to confirm the final diagnosis.

Normal human pituitary tissues were obtained from autopsies performed postmortem in healthy subjects from the Forensic & Toxicology Department of Aristotle University of Thessaloniki. The integrity of proteins up to 12 h postmortem was examined by performing immunohistochemistry for all anterior pituitary hormones.

2.3. Extraction

Once the archived pathology samples were identified and diagnosis confirmed, the workflow included (a) specimen processing, (b) total RNA extraction, (c) cDNA synthesis, (d) Real-time PCR arrays and (e) statistical analysis.

(a) Manual micro-dissection was performed from each FFPE block to enrich for epithelial lesional tissue before total RNA extraction. One hematoxylin and eosin (H&E) slide and up to 5 unstained slides (10 microns thick) were generated from each FFPE block. After an overnight deparaffinization, the tissue area of interest was scraped off the slide into an eppendorf tube to facilitate total RNA extraction.
(b) Total RNA from the target tissue area was extracted using the commercial RNeasy FFPE kit (Qiagen GmbH, Hilden, Germany), according to the manufacturers' instructions.
(c) cDNA synthesis was conducted by using the commercial RT^2 First Strand Kit (Qiagen), according to the manufacturers' instructions. Samples were kept at -80 °C for the subsequent Real-time PCR analysis.
(d) Real-time PCR analysis was conducted by using customized RT^2 Profiler PCR Arrays (Qiagen GmbH, Hilden, Germany), according to the instructions of the manufacturer. SYBR Green was used as fluorescence pigment. Lyophilized primer pairs specific for one of the 9 genes (PTCH1, PTCH2, GLI1, GLI3, NOTCH3, JAG1, HES1, HIP, GADPH), were present in the 8 wells of each of the 9 columns of the 96 well plates. GADPH was used as a reference gene. The 3 remaining columns of the 96 well plate was used for the quality control of the reverse transcription and polymerase chain reaction efficiency, of the Real-time PCR (positive control) and of the genomic DNA contamination. All runs were performed in duplicate. Only samples with good amplification signal and melt curves were included in the statistical analysis.
(e) Statistical analysis

Data were analyzed by specific software supported by SaBiosciences (RT^2 profiler PCR array data analysis version 3.5, GeneGlobe Data Analysis). The real-time PCR modules transform threshold cycle (C_T) values to calculated results for genes.

All analyses were based on fold-change ($2^{-\Delta\Delta Ct}$) defined as the normalized gene expression ($2^{-\Delta Ct}$) in the test sample (pituitary adenomas) divided the normalized gene expression ($2^{-\Delta Ct}$) in the control sample (normal pituitaries). Fold-change values less than one was indicative of down-regulation of the gene expression, and fold-change values greater than one was indicative of an up-regulation. Values greater than 2 or less than 0.5 were considered by the software statistical significant (p values < 0.05). The p values were calculated based on a Student's t-test of the replicate ($2^{-\Delta Ct}$) values for each gene in the control group (normal pituitary tissue) and pituitary adenomas. Spearman's rank correlation was perfomed to assess associations between mRNA expression of the tested genes in pituitary adenomas and tumor size, radiologic parameters (extra-sellar extention or optic chiasm displacement)