Original Paper

Silencing of HEPN1 is Responsible for the Aggressive Biological Behavior of Pituitary Somatotroph Adenomas

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Key Words

HEPN1 • Pituitary somatotroph adenoma • Invasion • Apoptosis • Proliferation

Abstract

Background/Aims: The pathogenic mechanisms underlying pituitary adenoma formation, progression, and invasion are poorly understood. To identify candidate tumor suppressor genes, we selected somatotroph adenomas as representative of pituitary adenomas. **Methods/Results:** We used genome-wide differential expression analysis in 15 invasive and 12 non-invasive somatotroph adenomas. HEPN1 reduction was more frequent in the invasive group, and this result was confirmed by qRT-PCR. To understand the function of HEPN1, the pituitary adenoma cell lines, GH3 and GT1.1, were stably transfected with short hairpin RNA (shRNA) targeting HEPN1 or ectogenic HEPN1 by lentivirus-mediated transfection. We found that HEPN1 overexpression in GH3 and GT1.1 cells inhibited cell proliferation, induced apoptosis, and attenuated invasive capacity, whereas HEPN1 silencing enhanced cell proliferation and invasion accompanied by decreased apoptosis. Western blot analysis revealed that HEPN1 overexpression decreased MMP-2, MMP-9, and Bcl-2 expression, but increased BAX, p53, and caspase-3 expression. In contrast, HEPN1 silencing increased MMP-2, MMP-9, and Bcl-2 expression, but decreased BAX, p53, and caspase-3 expression. **Conclusion:** Taken together, our results suggest that reduction of HEPN1 may play an important role in the progression of pituitary somatotroph adenomas. HEPN1 may thus be a candidate as a prognostic predictor or an anticancer therapeutic target for patients with somatotroph adenoma.

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Introduction

Pituitary adenomas, including somatotroph adenomas, constitute 10–15% of intracranial neoplasms [1]. Somatotroph adenomas are one of the most common types of pituitary adenomas, second only to prolactinomas [2]. With the exception of prolactinomas, surgical resection is the most effective treatment method for pituitary adenomas. Although pituitary adenomas are benign, some pituitary adenomas are considered to be aggressive or invasive, showing atypical behavior, such as invading adjacent tissues and proliferating rapidly [3]. Invasion of surrounding structures by pituitary adenomas increases the difficulty of complete resection and is an important reason for post-operative recurrence [4–6]. Although several biological markers for the aggressive manner of pituitary adenomas have been investigated, none of these factors are widely accepted as being responsible for invasiveness of pituitary adenomas [7, 8], and the pathogenic mechanisms underlying pituitary adenoma formation, progression, and invasion remain poorly understood. Mutations in classic oncogenes and tumor suppressor genes (TSGs), which might be prognostic factors or gene therapy targets, are rarely found in pituitary tumors [9–11], thus further investigation of new oncogenes and TSGs is needed.

To study the candidate oncogenes and TSGs involved in the invasion process of pituitary adenomas, we selected somatotroph adenomas as representative pituitary adenomas. Twenty-seven human somatotroph adenomas were selected, 15 of which were invasive adenomas and 12 were non-invasive, according to the modified Hardy classification [12]. We have used Affymetrix HG-U133 plus 2.0 arrays to identify genes with different transcription levels between invasive and non-invasive adenomas and normal pituitary tissues. The results of genome-wide differential expression analysis have not been previously reported. Aberrant reduction in transcription of hepatocellular carcinoma, down-regulated 1 (HEPN1), a novel candidate TSG, has been observed in invasive somatotroph adenomas. HEPN1 gene maps to the 3'-noncoding region of HEPACAM gene. While the expression of HEPACAM was downregulated or undetectable in many cancer cell lines, there is only one previous study on HEPN1 [13]. HEPN1 was first identified in human tumorous liver, and downregulated in human hepatocellular carcinoma [13]. When transfected into HepG2 cells, HEPN1 reduces cell viability and induces apoptosis [13]. However, silencing HEPN1 is associated with carcinogenesis of hepatocytes [13]; however, the mechanism underlying the anti-tumorigenesis effect of HEPN1 has not been determined.

We have studied the function of HEPN1 in pituitary adenoma cell lines (GH3 and GT1.1). We demonstrate that loss of HEPN1 promotes the proliferation and invasiveness of pituitary adenoma cells, inhibits cell apoptosis, thus underscoring the importance of HEPN1 in tumorigenesis and invasion and defining HEPN1 as a promising molecular target for gene therapy.

Materials and Methods

Ethics Statement

The use of Human tissue specimens was approved by the institutional research ethics committee of Second Military Medical University. All patients provided written informed consent, and samples were collected after surgical resection. All tissue samples included in this study were collected from tissues that remained after the completion of diagnosis and are otherwise discarded.

Human pituitary tissues and adenomas

Four normal human adenohypophyses were obtained at the time of autopsy from patients with no evidence of endocrinopathies. Histologic examinations were performed to exclude the possibility of incidental pathologies. Fifteen invasive somatotroph adenomas and 12 non-invasive adenomas were selected from our pituitary tumor tissue bank. All of the somatotroph adenoma specimens were obtained at the time of surgery at Changzheng Hospital (Shanghai, China). The patient sources did not receive seller

irradiation before tumor resection. All of the samples were frozen in liquid nitrogen and stored at -80 °C. The tumors were characterized based on the clinical, radiologic, histologic, and immunohistochemical features. Tumor size and invasiveness were defined on the basis of pre-operative radiologic studies and operative findings, and a modification of the Hardy classification [12], as follows: grade I tumors were microadenomas (<1 cm in diameter); and grade II tumors consisted of enclosed macroadenomas (>1 cm in diameter) with or without suprasellar extension. Both grade I and II tumors were defined as non-invasive. Grade III tumors exhibited local invasiveness with evidence of bony destruction and tumor within the sphenoid and/or cavernous sinus (CS). Grade IV tumors demonstrated CNS/extracranial spread with or without metastases. Grade III and IV tumors were considered to be invasive. Because there might be small defects in the wall of the CS, non-invasive pituitary adenomas are able to grow into the CS through defects and show an illusion of CS invasion [14]. Therefore, CS "invasion" on MRI was not considered a sufficient condition for invasion in the current study. Of the 27 tumors, 7 were grade I, 5 were grade II, 6 were grade III and 9 were grade IV.

Microarray preparation and data generation

Total RNA was isolated from 27 somatotroph adenomas and 4 normal pituitaries using TRIzol (Invitrogen), and then cleaned up with a QIAGEN RNeasy minikit according to the instructions of the manufacturer. RNA was quantified by spectrophotometry and RNA integrity was confirmed with the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). Microarray targets were prepared and labeled from 300 ng of total RNA using the MessageAmp premier RNA amplification kit (Applied Biosystems/Ambion, Austin, TX) following the manufacturer's instructions. Affymetrix HG-U133 Plus 2.0 arrays (Santa Clara, CA) were hybridized with 10 µg of cRNA and processed and scanned using standard Affymetrix protocols. RNA integrity was confirmed and cDNA was transcribed, labeled, and hybridized from individual samples on the Affymetrix Human Genome U133 Plus 2.0 arrays.

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

Total RNA, including the total RNA from normal pituitary tissues (BioChain Institute, Hayward, CA, USA) as a positive control, was reverse-transcribed using M-MLV transcriptase (Invitrogen, Carlsbad, CA, USA) with oligo(dT)12–18 primers. The reverse transcripts were used as templates for the analysis of gene expression using a thermal cycler (Takara Shuzo, Kyoto, Japan) and KOD-plus-polymerase (Toyobo, Osaka, Japan). The primers were as follows: 5'-ATT GCC CTC TCT CCT CAC ACA GTA G-3' (forward); and 5'-ACA ATC ACA CTA ATC GGC AGACGG-3' (reverse). The PCR conditions were as follows: 2 min at 94 °C, followed by 25–35 cycles of 94 °C for 30 s; 58–68 °C; and a final elongation step at 68 °C for 60 s. PCR products were separated on agarose gel and visualized by ethidium-bromide staining. The band intensity was quantified using a CS Analyzer 2.0 (ATTO, Tokyo, Japan).

HEPN1 transfection and silencing in GH3 and GT1.1 cells

Rat GH3 and mouse GT1.1 pituitary adenoma cells were obtained from the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in DMEM/F12 + 10% FBS supplemented with 100 U/ml of penicillin and 100 U/ml of streptomycin, and incubated at 37 °C and 5% CO₂.

Full-length human HEPN1 complementary DNA (cDNA) and HEPN1 siRNA were subcloned into the pCMV-THM lentivirus vector (Invitrogen) to construct Lenti-HEPN1 and Lenti-HEPN1 shRNA. Conditioned medium containing lentiviruses was harvested 48 h after transfection of HEK293T cells. This medium was filtered and used to infect recipient cells in the presence of 10 µg/ml of polybrene.

Protein extraction and Western blotting

Proteins were extracted from subconfluent cultures and subjected to Western blot analysis. After blocking with 5% non-fat milk in phosphate-buffered saline with Tween (PBS-T) for 1 h at room temperature, the membranes (Protran; Schleicher & Schuell, Dassel, Germany) were blotted with primary antibody, followed by incubation with a peroxidase-conjugated secondary antibody, as described previously [15]. Bound antibodies were visualized using enhanced chemiluminescence (Bio-Rad, Richmond, CA, USA). The primary antibodies used were as follows: rabbit polyclonal antibody to HEPN1; mouse monoclonal antibody to p53; goat polyclonal antibody to BAX; goat polyclonal antibody to caspase-3; mouse monoclonal antibody to Bcl-2; goat polyclonal antibody to matrix metalloproteinase (MMP)-2; mouse monoclonal antibody to

MMP-9; and a rabbit polyclonal antibody to β -actin used as a gel loading control. These antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Cell proliferation assay

Cell growth was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Non-transfected GH3 and GT1.1 cells and stably transfected cells (Lenti-HEPN1, Lenti-shRNA, and Lenti-GFP) were replated onto 96-well plates at 4×10^3 cells/well and cultured overnight to allow for cell attachment. At daily intervals (24, 48, 72, 96, and 120 h), the number of viable cells was determined by MTT assay. Briefly, cells were incubated with 0.2 μ g/ml of MTT for 4 h in the dark at 37°C. After removal of the medium, the formazan crystals produced from MTT by live cells were dissolved in 150 μ l of dimethyl sulfoxide (DMSO), and the absorbance was measured at 570 nm with an Ultra multifunctional microplate reader (Tecan, Durham, NC, USA). Three independent experiments were performed in quadruplicate wells.

Apoptosis assay

Apoptosis was measured using an Annexin V/propidium iodide (PI) apoptosis detection kit (Bender MedSystem, Vienna, Austria). Briefly, cells cultured in 6-cm dishes were trypsinized, washed, stained with PI-conjugated anti-Annexin V antibody under darkness for 15 min at room temperature, then analyzed by flow cytometry (FACSCalibur; Becton-Dickinson, Mountain View, CA, USA).

Invasion assay

An equal number (1×10^5) of non-transfected cells as well as cells stably transfected with Lenti-shRNA, Lenti-HEPN1, or Lenti-GFP, were plated onto separate 24-well cell culture inserts coated with Matrigel with 8- μ m pores. Minimum essential medium (MEM) with 10% FBS was added to the lower chamber as a chemoattractant. After a 24-h incubation at 37°C under 5% CO₂ atmosphere, cells remaining adherent to the upper surface of the filter were removed using cotton swab. The cells on the lower surface of the membrane (the migrated cells) were fixed with 2.5% formaldehyde, stained with hematoxylin, and counted. The invasion rate was determined from three independent experiments.

Statistical Analyses

All data are expressed as the mean $\pm$ standard deviation (SD) of three independent experiments. Statistical analysis between two groups was performed using Student's t-test, and the comparison between three or more groups was performed using analysis of variance (ANOVA) analysis, followed by Dunnett's t-test. $P < 0.05$ was considered statistically significant.

Results

HEPN1 expression is significantly lower in invasive somatotroph adenomas

The average RPRM transcript intensity for normal pituitary was 72.5 compared with 12.3 in invasive somatotroph adenomas and 28.5 in noninvasive somatotroph adenomas (Fig. 1A). We next analyzed the expression of the HEPN1 gene by qRT-PCR in 27 human somatotroph adenomas and 4 normal pituitary tissues. The median level of HEPN1 mRNA expression (HEPN1/ β -actin) was 1.2 (range, 1.1–1.3) in normal pituitary tissues. We classified levels of expression of < one-half this value (HEPN1/ β -actin < 0.6) as significant reduction. Reduced expression of HEPN1 was detected more frequently in invasive adenomas than in non-invasive adenomas (Fig. 1B) and HEPN1 mRNA expression was significantly lower in invasive adenomas than in non-invasive adenomas (Fig. 1C).

Effects of Lenti-HEPN1 or Lenti-shRNA on HEPN1 expression

After transfection, qRT-PCR analysis for HEPN1 expression demonstrated a 7.6-fold increase in HEPN1 transcripts in GH3 cells stably transfected with Lenti-HEPN1 and a 6.5-fold increase in stably transfected GT1.1 cells compared with non-transfected parental controls

Fig. 1. Level of expression of HEPN1 in somatotroph adenomas and normal pituitaries. (A) HEPN1 transcript expression levels assessed by using Affymetrix U133plus2.0 microarrays in 12 invasive somatotroph adenomas, 12 non-invasive adenomas and 4 normal pituitaries. The average HEPN1 transcript intensity in invasive adenomas or noninvasive adenomas was significantly lower than that in normal pituitaries (** $P < 0.01$). (B) Reduced expression of HEPN1 was detected less frequently in invasive adenomas than in non-invasive adenomas (80% vs. 33%, * $P < 0.05$). -, significant lower expression of HEPN1 mRNA; $\pm$, $0.6 \leq \text{HEPN1}/\beta\text{-actin} \leq 1.2$; and $+$, $\text{HEPN1}/\beta\text{-actin} > 1.2$. (C) HEPN1 mRNA expression in the invasion group was significantly lower than the non-invasion group (* $P < 0.05$).

Fig. 2. Effects of Lenti-HEPN1 and Lenti-shRNA transfection on HEPN1 expression. (A) Quantitative RT-PCR analysis demonstrates that HEPN1 mRNA was significantly overexpressed in pituitary adenoma cells stably transfected with Lenti-HEPN1 and suppressed in pituitary adenoma cells stably transfected with Lenti-shRNA compared with cells stably transfected with Lenti-GFP. (B) Expression of HEPN1 protein in GH3 and GT1.1 cells was examined by Western blotting. β -actin served as an endogenous control. ** $P < 0.01$ as compared with the control groups.

(Fig. 2A). Western blot analysis confirmed the increase in HEPN1 protein expression in GH3 Lenti-HEPN1 and GT1.1 Lenti-HEPN1 transfectants (Fig. 2B). In contrast, HEPN1 expression was reduced by 66.7% in GH3 Lenti-shRNA transfectants and 63% in GT1.1 Lenti-shRNA transfectants compared with HEPN1 expression in the control cell lines (Fig. 2A). HEPN1 protein was reduced by 65.7% in GH3 Lenti-shRNA transfectants and 62% in GT1.1 Lenti-shRNA transfectants compared with HEPN1 expression in the control cell lines (Fig. 2B).

Fig. 3. Effects of HEPN1 expression on pituitary adenoma cell proliferation and apoptosis. (A) Effect of HEPN1 overexpression and underexpression on GH3 cell proliferation was measured by MTT assay. Absorbance was read at 570 nm with an average of triplicate wells. (* $P < 0.05$ at 48, 72, 96, and 120 h for Lenti-HEPN1 versus Lenti-GFP or non-transfected GH3 cells; * $P < 0.05$ at 48 h, and ** $P < 0.01$ at 96 and 120 h for Lenti-shRNA versus Lenti-GFP or non-transfected parental GH3 cells). The results in GT1.1 cells were similar to GH3 cells. (B) Apoptosis in GH3 cells was measured by Annexin V/propidium iodide (PI) staining following Lenti-shRNA or Lenti-HEPN1 transfection. Early apoptotic cell populations were significantly increased (** $P < 0.01$) after Lenti-HEPN1 transfection and decreased (* $P < 0.05$) after Lenti-shRNA transfection. The results in GT1.1 cells were similar to GH3 cells. (C) Western blotting analysis for p53, Bax, Bcl-2, and caspase-3. Decreased expression of p53, Bax, and caspase-3 and increased expression of Bcl-2 were observed in GH3 and GT1.1 cells 72 h after Lenti-shRNA transfection as compared with the control groups (* $P < 0.05$). Increased p53, Bax, and caspase-3 and decreased Bcl-2 was observed in GH3 and GT1.1 cells stably overexpressing HEPN1 (* $P < 0.05$).

Fig. 4. HEPN1 expression regulates cell invasive capacity in the Matrigel model. (A) Matrigel assays showing the effect of Lenti-shRNA and Lenti-HEPN1 on the invasive potential of GH3 and GT1.1 cells. The bar graph indicates the mean number of invaded cells at 24 h after top-side cell seeding. The results demonstrated that Lenti-HEPN1 suppressed invasion of GH3 and GT1.1 cells, while Lenti-shRNA potentiated invasion of GH3 and GT1.1 cells compared with control groups (** $P < 0.01$). (B) Western blot of MMP-2 and -9 expression. Decreased MMP-2 and -9 expression was observed in Lenti-HEPN1-transfected GH3 and GT1.1 cells, whereas Lenti-shRNA transfected cells exhibited increased MMP-2 and -9 protein expression 48 h after transfection. (* $P < 0.05$ and ** $P < 0.01$).

HEPN1 inhibits proliferation and induces apoptosis in GH3 and GT1.1 cells via improvement in p53 expression

The proliferation of GH3 and GT1.1 cells was examined by MTT assay 2-5 days after transfection with lentivirus containing Lenti-shRNA, Lenti-HEPN1, Lenti-GFP, and non-transfected cells. Lenti-shRNA transfection promoted GH3 and GT1.1 cell growth. In contrast, Lenti-HEPN1 transfection suppressed GH3 and GT1.1 cell growth (Fig. 3A).

Also, flow cytometry (Annexin V-FITC-PI) was used to study the effect of Lenti-HEPN1 transfection on apoptosis. Lenti-shRNA transfection inhibited GH3 and GT1.1 cell apoptosis, while Lenti-HEPN1 transfection promoted GH3 and GT1.1 cell apoptosis (Fig. 3B).

Because GH3 and GT1.1 cell growth was inhibited and apoptosis was induced by HEPN1, we further studied the HEPN1 effect on the level of p53, BAX, BCL-2, and caspase-3, which are related to apoptosis in GH3 and GT1.1 cell lines. After a 72-h transfection, a decrease in BAX, p53, and caspase-3 and an increase in BCL-2 were observed in Lenti-shRNA transfected GH3 and GT1.1 cells compared with the control groups. A decrease in BAX, p53, and caspase-3 and an increase in BCL-2 were observed in Lenti-HEPN1 transfected GH3 and GT1.1 cells (Fig. 3C).

Reduction of HEPN1 promotes cell invasion might via an increase in MMP-2 and -9 expression

To investigate the effect of HEPN1 inhibition or overexpression on pituitary adenoma cell invasion, Transwell migration was performed. Exogenous expression of HEPN1 reduced Transwell migration compared with the other transfected groups (Fig. 4A). The processes of cell migration and infiltration that characterizes metastasis require several extracellular matrix (ECM) matrix metalloproteinases (MMPs), especially MMP-2 and -9. In pituitary adenomas, thus we examined the expression of MMP-2 and -9. As shown in Fig. 4B, GH3 and GT1.1 cells transfected with Lenti-HEPN1 demonstrated significantly increased MMP-2 and -9 protein expression, while Lenti-shRNA transfectants exhibited decreased expression. Thus, reduction of HEPN1 might increase MMP-2 and -9 expression, corresponding with the improved invasive capacity in the Matrigel invasion model.

Discussion

The mechanisms responsible for progression and invasion of pituitary adenomas are poorly understood. Mutations in classic oncogenes and TSGs are rarely found in pituitary tumors [9-11], so identification of candidate oncogenes and TSGs is an important method to understand pituitary tumorigenesis and invasion.

Based on our results of gene-wide differential expression analysis, which have not been reported before, HEPN1, a novel candidate TSG, exhibited an aberrant reduction in transcription in invasive somatotroph adenomas tissues. Before our study, others have reported microarray analyses used in somatotroph tumors. In the study of Evans et al. [16], there is no details about HEPN1. However, HEPN1 is a gene that identified in 2003 [13], so it is possible that Evans' complementary DNA (cDNA) microarray analysis may not include HEPN1. In the study of Morris et al. [17], they used Affymetrix GeneChip HG-U133A in a total of five human pituitary adenomas of each of the main subtypes (somatotroph adenoma, prolactinoma, corticotropinoma, and non-functioning pituitary adenoma) and 5 normal human pituitary autopsy specimens. The cyclin-dependent kinase inhibitor 2C, or p16, was down-expressed in the somatotroph adenoma, prolactinoma and non-functioning pituitary adenoma arrays. However, in the realtime quantitative PCR (RQ-PCR) analysis, and p16 was not significantly under-expressed in corticotropinomas. HEPN1 was not included in the 92 genes under-expressed ≥ 2.0 -fold. This difference between the study of Morris et al. and ours may attribute to the difference of tumor samples or experimental error of microarray. The microarray results were further confirmed by q-RT-PCR, suggesting that HEPN1 reduction may have significant value in somatotroph adenoma tumorigenesis and invasion. Another possibility is that the down-regulation of HEPN1 in somatotroph adenomas is a consequence of HEPN1 being poorly expressed in GH-secreting cells generally, as the normal pituitary contains a mixed population of cells. Immunohistochemistry studies may exclude this possibility, but there is no commercial product of HEPN1 antibody used for immunohistochemistry at present. There are no previous reports on the effect of HEPN1 in pituitary adenomas. To understand the function of HEPN1 in pituitary somatotroph adenomas, we overexpressed HEPN1 in murine pituitary adenoma cell lines (GH3 and GT1.1). When transfected into pituitary adenoma cells, HEPN1 reduced cell viability,

induced apoptosis accompanied by enhanced expression of BAX, p53, and apoptotic effector caspase-3, and inhibited expression of anti-apoptotic Bcl-2. Conversely, HEPN1 silencing by Lenti-shRNA transfection inhibited cell apoptosis, promoted cell growth accompanied by inhibited expression of apoptotic factors Bax, p53, and caspase-3, and enhanced expression of anti-apoptotic factor Bcl-2 [18]. It is widely accepted that Bax [18], p53 [19], caspase-3 [20] and Bcl-2 [18] play an important role in regulating apoptosis. These results suggest that loss of HEPN1 may contribute to somatotroph adenoma growth by apoptosis inhibition.

Furthermore, in GH3 and GT1.1 cells, HEPN1 inhibited cell invasion with MMP-2 and -9 downregulation. In contrast, HEPN1 silencing by Lenti-shRNA transfection promoted cell invasion with increased expression of MMP-2 and -9. Remodeling of the ECM is considered an important step in pituitary tumor invasion and angiogenesis [21, 22]. MMPs can degrade and reorganise the ECM. In addition, the capsule of pituitary tumors is enclosed by dura, while the capsule is mainly composed of type IV collagen. MMP-2 and -9 which are both type IV collagenases capable of promoting tumor invasion by breaking down the basement membrane and dura [23]. Gong et al. [7] showed that MMP-9 expression can be used to distinguish invasive from non-invasive pituitary tumors, and reflect the extent of invasiveness in pituitary tumors according to tumor subtype, size, and extension. Liu et al. [24] reported that MMP-2 is associated with aggressiveness and invasion in pituitary tumors, but not tumor size or secretory function. In the present study, we demonstrated that silencing of HEPN1 leads to enhanced invasive capacity in GH3 and GT1.1 cells, possibly through increased expression of MMP-2 and -9.

Collectively, these findings demonstrate that HEPN1 performs multiple functions as a TSG through suppression of proliferation and invasion, and induction to apoptosis. In conclusion, silencing of HEPN1 may contribute to the progress and invasion of human pituitary somatotroph adenomas. In pituitary adenoma cell lines, HEPN1 silencing promotes proliferation, inhibits apoptosis by decreasing p53, BAX, and caspase-3 expression, and promotes invasiveness by increasing the expression of MMP-2 and -9. Our study indicates that HEPN1 might be a potential prognostic predictor or gene therapy target for patients with invasive somatotroph adenomas; however, further investigations are required to study the details of the effect of HEPN1 on pituitary adenoma progression.

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