scientific reports

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Immunohistochemical analysis of filamin a expression in acromegaly and its correlation with tumor characteristics and treatment response

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Acromegaly, caused by growth hormone-secreting pituitary tumors, often causes significant challenges in its management due to poor surgical outcomes and resistance to pharmacological treatment. The present study aims to explore the expression of Filamin A (FLNA), a cytoskeletal protein involved in somatostatin receptor signaling, and its clinical relevance in acromegaly. We conducted immunohistochemical (IHC) study on 34 GH-secreting pituitary tumors to evaluate FLNA expression intensity and its associations with somatostatin receptors (SSTR2, SSTR5), E-Cadherin, tumor characteristics obtained through imaging studies, and pharmacological treatment responses. Our findings revealed a 100% FLNA positivity rate, with moderate to strong FLNA expression correlating significantly with SSTR5 expression and the presence of suprasellar tumor extension, indicating a potential role in tumor invasiveness. Moreover, patients with macrodenomas presented significantly higher FLNA intensity compared to the ones with microadenomas. FLNA expression showed no significant association with SSTR2, E-Cadherin, surgical cure rate or first-generation somatostatin receptor ligand (fgSRL) responses. However, the series of patients treated with Pasireotide ($n = 4$) demonstrated a trend suggesting better biochemical control with higher FLNA expression. In conclusion, our results suggest that FLNA may be associated with tumor invasiveness in GH-secreting pituitary tumors. While data on Pasireotide-treated patients are exploratory, further studies are needed to assess FLNA's potential as a treatment response marker in acromegaly.

Keywords Pituitary, Acromegaly, Immunohistochemistry, Filamin A, Cytoskeleton

Acromegaly is a rare disorder caused in almost all cases by a pituitary neuroendocrine tumor (PitNET) that secretes growth-hormone (GH)[1]. While these tumors were classically considered histologically benign, recently a proposal suggested the nomenclature change from "adenoma" to "pituitary neuroendocrine tumor", considering that some of these tumors possess an aggressive local behavior and are resistant to multiple treatments[2]. Most patients with acromegaly are diagnosed with a pituitary GH-secreting macroadenoma, which often invades surrounding tissues, making complete surgical resection difficult. Transsphenoidal surgical resection is currently the mainstay treatment for most patients, but the outcomes vary, with success rates lower in patients with invasive tumors. Consequently, many patients eventually require pharmacological treatment, which typically involves the use of first-generation somatostatin receptor ligands (fgSRL) as the first-line therapy, followed by GH-blocker Pegvisomant or the newer somatostatin receptor ligand Pasireotide as second-line options in non-responsive patients[3,4].

Histological analysis of tumor tissue with the addition of immunohistochemistry (IHC) offers valuable insights for the classification of these tumors, based on hormone and transcription factors staining[2]. Furthermore, IHC

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evaluation for cytokeratin (CK) staining traditionally categorizes these tumors into densely (DG) and sparsely granulated (SG) types. The assessment of somatostatin receptors type 2 and 5 (SSTR2, SSTR5), which are the primary targets of the fgSRL and Pasireotide, provides additional valuable information for the prediction of treatment response[5,6]. Other IHC and histological markers, such as the Ki-67 index, p53, and the number of mitoses, are used to determine the aggressiveness and invasive potential of these tumors[7,8].

Somatostatin receptors belong to the G-protein coupled receptor family, with subtypes SSTR2 and SSTR5 being the main forms expressed in GH-secreting PitNETs. The binding of fgSRL – Octreotide and Lanreotide, or the newer generation SRL Pasireotide, to these receptors leads to the inhibition of cell-proliferation and hormone release[9–11]. While reduced SSTR expression is typically associated with poor response to SRL treatment, the molecular mechanisms responsible for pharmacologic resistance appear to be more complex. Some studies suggest that post-receptor alterations may play a significant role in treatment response, as evidenced by patients with high SSTR2 expression who still fail to respond to fgSRL. This observation supports the theory that other post-receptor molecules might be involved in the response to these agents[12–14].

Recently, increased focus was given to the role of cytoskeletal proteins involvement in the evolution, prognosis and treatment response of various types of tumors, including PitNETs. One of these proteins, Filamin A (FLNA), is a large, ubiquitously expressed cytoskeletal protein found in various tissues and cancers[12,15]. The role of FLNA in tumors has been controversial: some studies suggesting it promotes tumor growth and invasiveness, while others associate high FLNA expression with a favorable pharmacologic treatment response and less aggressive behavior[15–17]. FLNA binds to various proteins and is believed to play a significant role in the post-receptor signal transduction pathways.

In PitNETs, FLNA has been identified as an important functional protein, with roles in modulating the signaling of somatostatin receptor subtypes, SSTR2 and SSTR5, and dopamine receptor subtype 2 (DR2). By stabilizing these receptors to the cell membrane, FLNA enhances their ability to mediate the inhibitory effects of therapeutic agents, such as fgSRL and dopamine agonists, which are commonly used to treat acromegaly and other pituitary disorders[18–20].

Recent studies have highlighted FLNA's role in stabilizing and protecting somatostatin receptors from degradation. While the correlation between FLNA and SSTR2 expression has been inconclusive, with some evidence suggesting a link only in specific tumor subsets responsive to fgSRL, the association with SSTR5 was found more consistently, as recent studies have shown a stronger correlation between FLNA and SSTR5 expression in both somatotropinomas and corticotropinomas[21,22]. Additionally, research on corticotropinomas has indicated that FLNA might be essential for SSTR5 expression and Pasireotide-mediated signaling[20,21,23]. However, the precise role of FLNA in modulating treatment response and its potential in predicting tumor aggressiveness in GH-secreting PitNETs remains unclear and requires further investigation.

We aimed to conduct an IHC study to analyze the expression of FLNA in tumor samples from patients with acromegaly who underwent primary surgical treatment. Our primary objective was to examine the expression prevalence and intensity of FLNA in GH-secreting PitNETs, and the associations of FLNA with the expression of SSTR2, SSTR5, E-Cadherin, Ki-67 index and the CK granulation pattern. Additionally, we aimed to determine the clinical relevance of FLNA expression, by studying potential associations of FLNA with tumor characteristics such as diameter, invasiveness, post-surgical recurrence, and response to pharmacologic treatment.

Methods

Patients and tumors

We performed a retrospective observational study including consecutive patients with confirmed diagnosis of acromegaly who underwent transsphenoidal surgery in three tertiary centers from Romania between 2010 and 2023. The total sample size included in this study was 34 patients. Given the retrospective and observational design, no formal sample size calculation was performed. All consecutive eligible patients with available tumor tissue and clinical data were included. The diagnosis of acromegaly was based on elevated serum IGF-1 levels (adjusted for age and sex) and absence of adequate GH suppression during an oral glucose tolerance test (OGTT), in accordance with national clinical guidelines and international recommendations[24]. Inclusion criteria were: confirmed biochemical diagnosis of acromegaly and availability of sufficient quality tumor tissue for IHC analysis. Exclusion criteria were: insufficient or poor-quality tumor samples, missing essential clinical or paraclinical data, or prior treatment with somatostatin receptor ligands before surgery. Medical files and hospital discharge papers from the included patients were reviewed to collect clinical and paraclinical data from the patient's medical history. This included patient's age, gender at diagnosis, IGF-1 and GH nadir in the OGTT or GH mean over 24 h before surgery, and the imaging findings on the magnetic resonance imaging (MRI), reviewed by an expert radiologist: tumor maximum diameter, and the presence or absence of tumor invasions: cavernous sinus, optic chiasm compression. Furthermore, we assessed postsurgical cure by the IGF-1 and random GH levels measured 2–3 months after surgery. Patients were considered cured if the IGF-1 levels were in the reference range and the random GH level was less than 1 ng/ml. Patients with persistent acromegaly who were eventually treated pharmacologically with fgSRL were assessed for treatment response based on the IGF-1 levels after at least 3 months of treatment with maximum dose of Octreotide long acting release (LAR) or Lanreotide autogel. Some of the patients resistant to these agents were treated with Pasireotide LAR. Patients were categorized as responders: those who achieved IGF-1 levels below 1.3× the upper limit of the normal reference range (ULN), and non-responders, which included both patients with partial control (IGF-1 decrease > 50% compared to pre-treatment values, but remaining > 1.3×ULN), and patients considered biochemically uncontrolled (IGF-1 decrease < 50% from baseline values). Biochemical response to fgSRLs was thus defined as normalization of IGF-1 or a reduction to ≤ 1.3×ULN. This threshold reflects the classification criteria used in the clinical centers where patients were managed, according to the Romanian national treatment protocol for acromegaly, and is also consistent with the range of cut-offs reported in international literature, as highlighted

in the review by van Esdonk et al.[24,25]. MRI findings after surgery were assessed to determine the presence and maximum diameter of tumor rest.

Laboratory tests

Hormonal analysis was performed in specialized laboratory centers belonging to the units where the patients were under surveillance and treatment. The measurement was performed by the use of validated chemiluminescence immunoassays kits for both IGF-1 and GH. IGF-1 reference ranges were adjusted for age and gender, and results were provided as both absolute value and xULN.

Immunohistochemistry

The IHC staining of the formalin-fixed paraffin tissue samples included were performed in a laboratory of the Advanced Medical and Pharmaceutical Research Center of the George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, using the BOND-MAX Fully Automated IHC and ISH Staining System. After the sectioning of blocks into cores and tissue preparation for antibody penetration, including paraffin removal and dehydration, the sections were subjected to IHC analysis using the following antibodies: Recombinant Anti-Filamin A antibody, clone EP2405Y (Abcam Cat# ab76289, RRID: AB_1523618), Cytokeratin (CK) 8/18, Clone 5D3 (Leica Biosystems Cat# NCL-5D3-BIOTIN, RRID: AB_876934), used to distinguish between DG and SG tumors; E-Cadherin, Clone 36B5 (Leica Biosystems Cat# NCL-E-Cad, RRID: AB_442084), Recombinant Anti-Somatostatin Receptor 2 Antibody, Clone UMB1 - BSA (Abcam Cat# ab134152, RRID: AB_2737601), Recombinant Anti-Somatostatin Receptor 5 Antibody, Clone UMB4 (Abcam Cat# ab109495, RRID: AB_10859946) Ki67, clone- MM (Leica Biosystems Cat# PA0118, RRID: AB_10555423).

The IHC slides were independently evaluated by a pituitary-specialized senior pathologist and a junior researcher who had undergone prior dedicated training in IHC scoring. Discrepant scores were reviewed jointly, and the final consensus results were validated by the senior pathologist. For FLNA, an IHC scoring system was applied, previously used in a study by Coehlo et al.[21]. Only the intensity of the cytoplasmatic staining was evaluated: 3- strong, 2- moderate, 1- mild, 0- negative. Based on the CK pattern, tumors were classified as DG, SG, or CK negative. The Ki-67 index was assessed based on the percentage of positive stained nuclei in several fields with at least 1000 cells. SSTR2, SSTR5 and E-Cadherin were assessed by using the immunoreactivity score (IRS), a scoring system which is commonly applied for various IHC markers, which was detailed in a previous study by our team. The IRS considers both the percentage of positive cells, and the staining intensity, and the final calculated IRS can range from 0–12[26].

Ethics

We obtained the approval from the ethics committees of all hospitals from where we obtained the tumor samples. The Scientific-Research Ethics Committee of George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mures approved this study, with the decision number 2401, from 22.06.2023. Participating patients provided informed consent prior to the inclusion in our study. All procedures and experimental protocols were conducted in compliance with the regulations of our institutions, and adhering to the principles outlined in the Declaration of Helsinki.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism version 8.4.3 software. Results for categorical variables are presented as absolute numbers and percentages. For continuous variables with a normal distribution, we reported mean values with standard deviations (± SD). For data with a non-normal distribution, we reported medians with ranges (minimum–maximum values). The Kolmogorov-Smirnov test was used to assess data distribution. To compare groups, we used the Kruskall-Wallis or Mann-Whitney test for non-parametric data and Student's t-test for parametric data. For binary and categorical data, we applied the Chi-square test or Fisher's exact test, depending on distribution. A significance level of $p < 0.05$ was considered statistically significant.

Results

Patients and tumors characteristics

We included 34 patients with biochemically confirmed acromegaly, each with sufficient quality of the tumor tissue stored as paraffin blocks for IHC analysis. The general characteristics of the sample are presented in Table 1. The mean age at the time of surgery was 46.81 years, with the majority of patients being of female gender (61.8%). Due to incomplete or missing imaging or follow-up data, presurgical tumor size and biochemical outcomes were available for 30 out of the 34 patients. Among these, 26 were diagnosed with macroadenomas and 4 with microadenomas, with a mean maximum tumor diameter of 23.44 mm prior to surgery. None of the patients in our cohort received somatostatin analogues as primary therapy prior to surgery.

Postsurgical biochemical outcomes indicated that 83.33% ($n = 25$) of patients had persistent active acromegaly requiring pharmacological treatment, while only 16.66% ($n = 5$) were surgically cured, as indicated by normalized IGF-1 a few months after the surgery. Among those requiring medical treatment, 23 patients were started on fgSRL: Octreotide LAR or Lanreotide autogel, and 2 were treated with dopamine agonists.

Data about the treatment response to fgSRL after at least six months of therapy at the maximum dose was available for 21 patients. Of these, 57.1% were considered resistant to fgSRL treatment based on their biochemical response, and 4 patients were eventually treated with Pasireotide LAR.

Immunohistochemistry findings

The percentage of tumors that stained positive for each studied marker is summarized in Table 2. All tumors were immunohistochemically positive for FLNA. As the membranous staining was inconsistent and negative, hence

Parameter	Value
Total number of patients	34
Patients with complete presurgical MRI data and postsurgical biochemical outcomes available	30
Age at surgery (years)	46.81 ± 12.06
Gender	
Female	21 (61.8%)
Male	13 (38.2%)
Size of the tumor	
Macroadenoma	26 (86.66%)
Microadenoma	4 (13.33%)
Tumor maximum diameter before surgery (mm)	23.44 ± 11.44
Extension and Invasion	
Suprasellar extension	21 (70%)
Cavernous sinus invasion	15 (50%)
Sphenoidal sinus invasion	4 (13.33%)
Optic chiasm compression	8 (26%)
IGF-1 levels	
Before surgery (ng/ml)	828.1 ± 336.3
After surgery (ng/ml)	520.77 ± 359.16
IGF-1 levels (×ULN)	
Before surgery (ng/ml)	3.43 ± 1.44
After surgery (ng/ml)	2.13 ± 1.53
Treatment	
Surgically cured patients	5 (16.66%)
Patients with persistent biochemical disease postsurgery	25 (83.33%)
Patients controlled on fgSRL treatment	12 (57.1%)
Patients resistant to fgSRL treatment	9 (42.9%)
Patients uncontrolled on fgSRL treated with Pasireotide	4 (19.04%)
Tumor rest	
On postsurgical MRI	20 (66.6%)
Maximum diameter postsurgery (mm)	19.70 ± 13.67

Table 1. General characteristics of the sample.

IHC marker	% positivity
FLNA	100%
SSTR2	94.11%
SSTR5	97.05%
E-Cadherin	68.96%
CK- DG tumors	41.17%
CK- SG tumors	50%
CK- negative	8.82%

Table 2. FLNA, SSTR2, SSTR5, E-Cadherin- positivity prevalence, granulation pattern- distribution. IHC- immunohistochemistry, SSTR- somatostatin receptor, CK- cytokeratin, DG- densely granulated, SG- sparsely granulated, FLNA- Filamin A.

difficult to interpret, we only considered the cytoplasmatic staining of FLNA for the final results. As mentioned, no tumor from our lot had negative FLNA staining (score 0). Mild FLNA staining (score 1) was observed in 11 patients (32.35%), while 15 patients (44.12%) presented moderate staining (score 2), and 8 patients (23.53%) had strong staining (score 3). Examples of the FLNA staining intensity scores (1, 2, and 3) from selected patients in our study are shown in Fig. 1, which contains IHC images illustrating these scores, alongside with a negative control image. Positive staining for SSTR2 was observed in 94.11% of tumors, and SSTR5 positivity was higher at 97.05%. E-Cadherin was detected in 68.96% of the tumors. Based on their granulation patterns, half of the tumors (50%) were SG and 41.17% exhibited a DG pattern. Notably, three tumors (8.82%) were CK-negative. Only two patients had a Ki-67 index above 3, both presenting with large, invasive tumors.

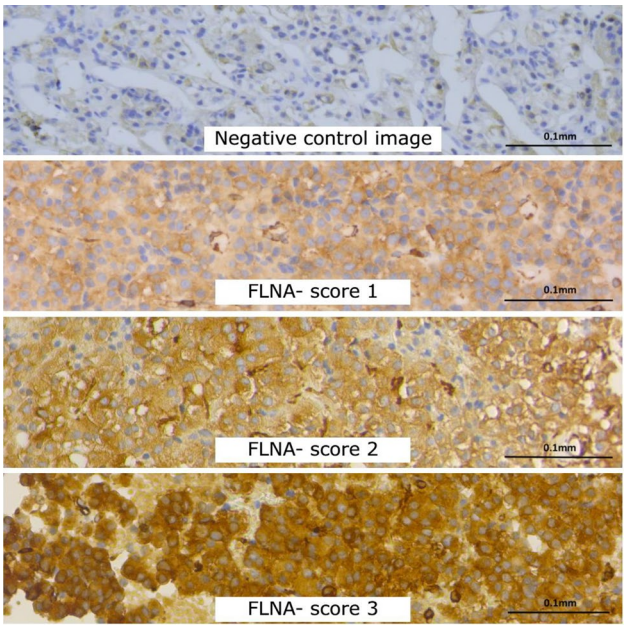

Fig. 1. Representative IHC images of FLNA with intensity scores (1–3) and negative control, magnification x20. Scale bar = 0.1 mm (applies to all panels). FLNA- Filamin A.

Parameter	FLNA- weak (1)	FLNA- moderate (2)	FLNA – strong (3)	*p* value
Ki-67 index median (min-max)	0.5 (0–1.25)	0.5 (0–2)	0.5 (0–1.5)	0.930
SSTR2 IRS- median (min-max)	9 (2–12)	9 (4–12)	12 (4.5–12)	0.640
SSTR5 IRS- median (min-max)	**8 (2–8)**	**12 (8–12)**	**12 (4–12)**	**0.031***
E-Cadherin IRS- median (min-max)	3 (0.75–8)	1 (0–6)	4 (0–12)	0.241
CK- DG- number (%)	5 (35.7%)	5 (35.7%)	4 (28.6%)	0.439
CK- SG- number (%)	6 (35.3%)	9 (52.9%)	2 (11.8%)	

Table 3. Associations between FLNA staining intensity and SSTR2, SSTR5, E-Cadherin IRS, Ki-67 index, granulation pattern subtype. *- Kruskall-Wallis test was applied, $p < 0.05$. SSTR- somatostatin receptor, CK- cytokeratin, DG- densely granulated, SG- sparsely granulated, FLNA- Filamin A.

Associations between FLNA, paraclinical and IHC markers

The results regarding the associations of FLNA expression with the other IHC markers are summarized in Table 3. We found a statistically significant association between FLNA and SSTR5 expression, with tumors having moderate and strong FLNA staining exhibiting higher SSTR5 IRS scores ($p = 0.0321$). No significant associations were found between FLNA intensity and SSTR2, E-Cadherin, Ki-67, or granulation patterns. Although SSTR2 expressions tended to be higher in patients with moderate or strong FLNA staining, the differences were not statistically significant.

No significant associations were observed between FLNA expression and gender or age at the time of surgery. As shown in Fig. 2, Patients with FLNA intensity scores of 2 and 3 had slightly larger tumors compared to those with score 1, although the difference did not reach statistical significance ($p = 0.153$). Similarly, presurgical IGF-1 levels appeared to be higher in patients with more intense FLNA staining, as illustrated in Fig. 3, but this did not reach statistical significance.

Table 4 summarizes the main associations between FLNA scores and various tumor characteristics, surgical outcomes, and response to fgSRL. All patients with microadenomas ($n = 4$) presented weak FLNA staining, while macroadenomas generally had higher FLNA scores, this difference being statistically significant. A significant association was also found between FLNA intensity and suprasellar extension, with higher FLNA scores more frequently observed in tumors with this type of extension. Although no significant associations were identified between FLNA expression and invasion of the cavernous sinus, sphenoid sinus, or optic chiasm compression, it was observed that all patients with sphenoidal sinus invasion ($n = 4$) were in the moderate FLNA intensity group. Also, the association between FLNA and sphenoidal sinus invasion was close to statistical significance ($p = 0.0715$). No significant associations were observed between FLNA expression and surgical success, although it is noteworthy that all five patients who achieved surgical cure exhibited low to moderate FLNA staining. We didn't find significant associations between FLNA expression and the biochemical response to fgSRL treatment.

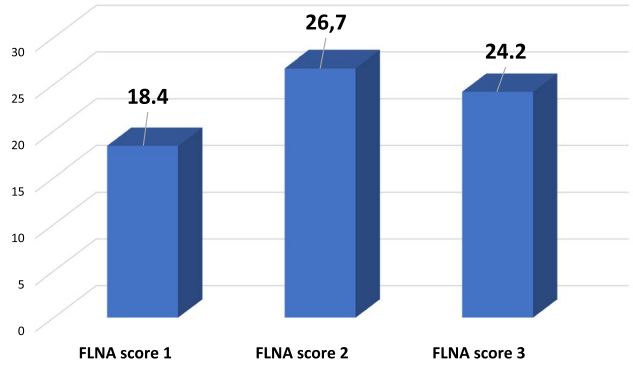

Fig. 2. Max tumor diameter (mm) before surgery and FLNA scores. $p = 0.153$ (Kruskall-Wallis test was applied), FLNA- Filamin A.

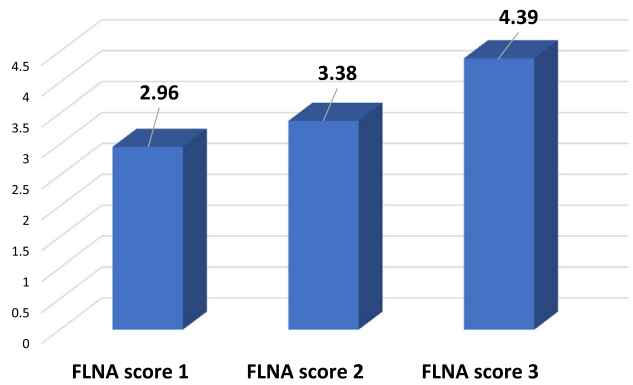

Fig. 3. IGF-1 levels (xULN) before surgery and FLNA scores. $p = 0.472$ (Kruskall-Wallis test was applied), FLNA- Filamin A.

Patients treated with Pasireotide

Four patients in our sample received Pasireotide LAR treatment for at least six months making it possible to evaluate their biochemical response to this agent. All four patients were female, and their ages, tumor sizes at surgery, and IHC characteristics are detailed in Table 5. Notably, the two patients who achieved optimal biochemical control under Pasireotide had moderate and intense FLNA staining and the highest SSTR5 expression score (IRS = 12), with both tumors displaying a SG pattern. In contrast, the two patients who were resistant to Pasireotide presented weak FLNA staining (score 1), with one having an SSTR5 IRS of 8 and the other staning negative for SSTR5.

Discussions

In this study, we investigated the protein expression of FLNA through IHC in GH-secreting PitNETs and its association with somatostatin receptor subtypes, granulation pattern, E-Cadherin and the Ki-67 index. Furthermore, we also investigated the link between FLNA and tumor characteristics (tumor size and invasiveness,) and with the response to pharmacological treatment with first- and second-generation somatostatin receptor ligands.

FLNA is a large cytoskeletal protein that has an important structural role in maintaining cell structure by linking actin filaments, thus maintaining the mechanical stability and flexibility of cells. Recent studies found that FLNA also has important functional roles through its involvement in various cellular processes, such as cell signaling, migration, and adhesion. It was found to act as a scaffold for various transmembrane proteins, including G-protein-coupled receptors (GPCRs), a family of receptors that includes somatostatin and dopamine receptors[15,22,27]. The role of FLNA in tumor behaviour remains controversial, as some studies found high expressions of this protein in highly aggressive cancers of the prostate, or in metastatic melanomas, while in other studies a low FLNA expression was noticed in different types of malignancies, such as gastric cancer[17,28,29]. These contradicting findings have made the hypothesis that FLNA may play a dual role in tumors depending on its subcellular localization, in the cytoplasm or nucleus. Specifically, higher cytoplasmic FLNA expression is thought to be associated with increased tumor growth and invasiveness, while nuclear FLNA expression might have a tumor suppressing role.

Our study focused exclusively on the cytoplasmic expression of FLNA, given its predominant presence in this cellular compartment at the review of the IHC slides. FLNA was positive in all 34 tumors included, with varying

Tumor type			
	Macroadenoma	Microadenoma	
FLNA score 1	7 (53.8%)	4 (46.2%)	***p* = 0.0186***
FLNA score 2	14 (100%)	0	
FLNA score 3	5 (100%)	0	
Suprasellar extension			
	present	absent	
FLNA score 1	4 (36.4%)	7 (63.6%)	***p* = 0.0078***
FLNA score 2	12 (85.7%)	2 (14.3%)	
FLNA score 3	5 (100%)	0 (0%)	
Cavernous sinus invasion			
	present	absent	
FLNA score 1	4 (36.4%)	7 (63.6%)	$p = 0.5210$
FLNA score 2	8 (57.1%)	6 (42.9%)	
FLNA score 3	3 (60%)	2 (40%)	
Sphenoidal sinus invasion			
	present	absent	
FLNA score 1	0	11 (100%)	$p = 0.0715$
FLNA score 2	4 (28.6%)	10 (71.4%)	
FLNA score 3	0	5 (100%)	
Optic chiasm compression			
	present	absent	
FLNA score 1	3 (27.3%)	8 (72.7%)	$p = 0.7214$
FLNA score 2	3 (21.4%)	11 (78.6%)	
FLNA score 3	2 (40%)	3 (60%)	
fgSRL response			
	responders	non-responders	
FLNA score 1	5 (71.4%)	2 (28.6%)	$p = 0.5199$
FLNA score 2	5 (45.5%)	6 (54.5%)	
FLNA score 3	2 (66.7%)	1 (33.3%)	
Surgically cured			
	yes	no	
FLNA score 1	2 (18.2%)	9 (81.8%)	$p = 0.5361$
FLNA score 2	3 (21.4%)	11 (78.6%)	
FLNA score 3	0 (0%)	5 (100%)	

Table 4. Associations between FLNA and tumor characteristics and treatment outcomes. *- Chi-square test was applied, $p < 0.05$, FLNA- Filamin A.

Patient /gender	Age at surgery (years)	Tumor size presurgery (mm)	Response to Pasireotide	FLNA score	SSTR2 IRS	SSTR5 IRS	CK granulation	Ki-67 (%)
1/f	36	26	controlled	2	4	12	Sparsely	2
2/f	52	28	controlled	3	2	12	Sparsely	1.5
3/f	54	24	uncontrolled	1	2	8	Sparsely	2
4/f	44	45	uncontrolled	1	0	0	Densely	0

Table 5. Series of patients treated with Pasireotide. SSTR- somatostatin receptor, CK- cytokeratin, FLNA- Filamin A.

degrees of staining intensity, similar to the results found in the study by Coelho et al.,where 89% of tumors were found positive for FLNA across a similar range of intensities[21]. Nevertheless, considering that no cases were fully negative for FLNA (score 0), and that tumors with score 1 showed minimal cytoplasmic staining, it is possible that FLNA negative cases (score 0) and weak cases (score 1) may represent a biologically similar category. The high prevalence of FLNA positivity in PitNETs was further confirmed by a recent study by Toledo et al., which reported increased FLNA expression in tumoral tissues compared to normal pituitary tissue[30].

In our study cohort, we observed a predominance of female gender (61.8%), with the average age at diagnosis falling in the fifth decade, consistent with epidemiological data on acromegaly[31]. A high prevalence of macroadenomas at diagnosis (86.66%) was observed, which was expected given the significant diagnostic delays

commonly associated with acromegaly[32]. Notably, the surgical cure rate was remarkably low, with only 16.66% (5 patients) achieving remission post-surgery, while the majority continued to present with active disease requiring pharmacological treatment. The low surgical remission rate in our cohort likely reflects a combination of selection bias and the high prevalence of late-diagnosed, invasive tumors. Furthermore, the surgical cure rates observed are consistent with other studies from our country, where surgical remission rates were also low (14.8–26.9%), underscoring similar challenges in real-life clinical practice of patients with acromegaly[33,34], though generally, reported surgical cure rates from registries in Western European countries are significantly higher, ranging from 40% to 60%[3,35,36]. Resistance to fgSRL is a frequent issue in clinical practice, which led to a recent interest in identifying biomarkers that could predict patient response[13,37]. In our study, 57.1% of patients responded to fgSRL therapy, while the remaining 42.9% (9 patients) were resistant and subsequently received second-line treatments. Among the fgSRL non-responders, four patients eventually underwent Pasireotide treatment and their outcomes and correlations with IHC markers were analyzed separately in this study.

FLNA has been proposed as a key factor in SSTR2-mediated signaling, influencing important processes such as cell cycle arrest and apoptosis in somatotroph tumor cells, which underpin the antiproliferative and pro-apoptotic effects of fgSRLs[16,22]. However, our study did not reveal any significant associations between FLNA IHC expression and the biochemical response to fgSRL treatment. This contrasts with some previous studies, which have reported a functional interaction between FLNA and SSTR2 in PitNETs[21,22,38]. Several factors may account for this discrepancy. One hypothesis is that protein modifications, such as phosphorylation, known to influence FLNA's interaction with SSTR2, may affect its function in ways that are undetectable through conventional IHC. Since IHC does not differentiate between phosphorylated and non-phosphorylated forms, the total protein expression level may not fully reflect FLNA's functional status. Moreover, current evidence regarding FLNA expression in somatotropinomas, particularly from IHC-based studies, is limited and heterogeneous, highlighting the need for larger, well-characterized cohorts. Interestingly, in the study by Coelho et al., similar to ours, there were no significant associations between FLNA expression and SSTR2, except for the subset of patients who were responsive to fgSRL treatment and had not been pretreated with fgSRL[21]. Although in our study SSTR2 expression tended to be higher in patients with moderate to strong FLNA staining, these differences did not reach statistical significance. Additionally, while in a recent research by our group, we identified E-Cadherin as the strongest predictor of fgSRL response[26], the current study did not find significant associations between FLNA expression and E-Cadherin, granulation pattern, or Ki-67 index. A notable result from our study is the statistically significant association between FLNA and SSTR5 expression, reinforcing the findings of Coelho et al., who observed this link at both protein and mRNA levels[21]. These findings suggest that FLNA may play an important role in stabilizing or enhancing the function of SSTR5 in somatotropinomas.

The multireceptor ligand Pasireotide is a newer somatostatin receptor ligand used to inhibit hormonal secretion and cell proliferation in both ACTH- and GH-secreting PitNETs. This agent displays a markedly higher affinity for SSTR5 compared to fgSRLs such as octreotide, which may explain its differential efficacy in certain patients[39,40]. While solid evidence for biomarkers predicting response to this drug in acromegaly is limited, there is some evidence suggesting that higher SSTR5 expression and the presence of SG tumors might be associated with a favourable response[41–43]. The correlation FLNA expression and SSTR5 has also been observed in studies on corticotropinomas. Additionally, Treppiedi et al. demonstrated that FLNA is crucial for Pasireotide's ability to inhibit ACTH secretion, a key factor in treating Cushing's disease. In their study, silencing FLNA in cells rendered Pasireotide ineffective in reducing ACTH secretion, cell viability, and inducing apoptosis. These findings suggest that FLNA could be a significant biomarker for predicting treatment response to Pasireotide in ACTH secreting PitNETs[23]. While our study included only a small series of four patients treated with Pasireotide for a sufficient duration to assess biochemical response, we observed that those who responded to the treatment exhibited high to moderate FLNA staining, while the non-responders showed low FLNA expression. These observations are preliminary and based on a very small sample; they may suggest a possible association between FLNA expression and Pasireotide responsiveness in somatotropinomas, but further studies with larger cohorts are required to validate this potential link.The exact molecular mechanism through which FLNA is involved in SSTR5-mediated signaling remains to be established in future research.

We examined the relationship between FLNA staining and tumor invasiveness by assessing the presence of various types of invasions and the tumor maximum diameter before surgery. Tumors with moderate to strong FLNA staining (scores 2 and 3) showed larger mean diameters (27.7 mm and 24.2 mm) compared to those with weak staining (18.4 mm), although this difference did not reach statistical significance and should be interpreted with caution. Furthermore, there was a significant difference in FLNA expression between micro- and macroadenomas, with microadenomas having lower FLNA scores. While this finding is novel, it's relevance might be limited due to the small number of patients with microadenomas in our sample, and the lack of a clear association with tumor diameter. A noteworthy finding in our study was the association between higher FLNA expression and suprasellar tumor extension. Although suprasellar expansion is not a definitive marker of invasiveness and may reflect tumor volume or sellar anatomy, its exclusive presence in tumors with moderate to strong FLNA staining suggests a possible biological link that warrants further investigation. This observation contrasts with the study by Coelho et al., who found no link between FLNA protein or mRNA expression and tumor invasiveness[21]. Conversely, Toledo et al. reported that increased FLNA expression in PitNETs was linked to a more migratory and invasive cellular phenotype. In their rat model, FLNA expression levels increased during the later stages of pituitary hyperplasia/adenoma development. Furthermore, their study on human PitNETs found that a cleaved form of FLNA was associated with more aggressive tumors, further indicating a potential role for FLNA in promoting invasiveness[30]. In the study by Sickler et al. on corticotropinomas that examined the link between FLNA and tumor invasiveness, a significant association between FLNA expression and sphenoidal sinus invasion was found[20]. Although our study identified a significant association primarily with suprasellar extension, it is noteworthy that none of the patients with weak FLNA expression (score 1) in our cohort had

sphenoidal sinus invasion. Moreover, all four cases that did present with sphenoidal sinus invasion were found with moderate FLNA staining (score 2). All these findings combined with the ones from our study highlight the potential role of this protein as a prognostic biomarker for invasiveness and risk or recurrence in PitNETs, while the exact role of FLNA in specific subtypes of PitNETs warrants further investigation.

The main limitation of our study is the small number of patients included, which underscores the need for further research on larger cohorts of somatotropinomas to confirm our findings. This limited sample size resulted from strict inclusion criteria, as only patients with both high-quality tumor tissue and complete long-term clinical data were eligible. Furthermore, subgroup analyses (such as surgical remission or microadenoma comparisons) should be interpreted with caution due to the small number of cases in certain categories.

Additionally, our evaluation of FLNA was limited to IHC and focused solely on cytoplasmic protein expression through staining intensity, without assessing nuclear FLNA, mRNA expression, or post-translational modifications. recent findings suggest that post-translational modifications, such as phosphorylation, may critically affect FLNA's functional role in receptor signaling. For instance, Peverelli et al. showed that cAMP/PKA-induced phosphorylation of FLNA inhibits SSTR2 signal transduction in GH-secreting pituitary tumor cells, potentially contributing to resistance mechanisms beyond receptor density or localization[38]. Another important limitation is the absence of the Knosp-Steiner classification for tumor invasiveness, due to the retrospective nature of our study and the variability in MRI reports, which lacked sufficient detail to calculate the score consistently for all patients. Despite these limitations, we believe our study brings exploratory value regarding FLNA expression in somatotropinomas, especially through its inclusion of a series of Pasireotide-treated patients. Although based on a very small subgroup, this is, to the best of our knowledge, the first report to assess FLNA expression in acromegaly patients undergoing Pasireotide therapy. While no conclusions can be drawn, these preliminary observations may serve as a useful starting point for future research in this underexplored area. Future studies with larger patient cohorts treated with Pasireotide would be valuable in confirming the relevance of FLNA as a biomarker for treatment response. Furthermore, to better understand the relationship between FLNA and tumor invasiveness in pituitary tumors, future studies, preferably prospective in nature, would be useful to explore the causal relationship and the molecular mechanisms through which this protein may contribute to increased aggressiveness.

In conclusion, our study demonstrated that FLNA is widely expressed at the cytoplasmatic level in GH-secreting PitNETs and is correlated with SSTR5 expression, suggesting a role in stabilizing this receptor subtype. While no significant associations were found with SSTR2, E-Cadherin, Ki-67, or the response to fgSRL, our findings suggest that FLNA expression may be associated with certain tumor behavioural features in somatotropinomas. Preliminary observations in a small subgroup of Pasireotide-treated patients suggest a potential link between FLNA levels and treatment response, but further research is required before proposing FLNA as a predictive biomarker in this context. One of our main findings was the significant link between higher FLNA expression and suprasellar tumor extension, suggesting a possible association between FLNA expression and tumor expansion patterns. Future studies are required to further explore the role of this cytoskeletal protein in driving tumor behaviour and treatment responsiveness in patients with PitNETs and explore the usefulness of assessing it as predictive biomarker, or the development of novel targeted therapies that specifically modulate its function, with the ultimate goal of achieving a personalized treatment approach for patients with acromegaly or other types of PitNETs.

Data availability

The data that support the findings of this study are not publicly available due to patient confidentiality but are available from the corresponding author on reasonable request.

Received: 18 February 2025; Accepted: 13 October 2025
Published online: 19 November 2025

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Author contributions

All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were conducted by all authors. Dr. Gliga Maximilian Cosma, Dr. Chinezu Laura. The initial draft of the manuscript was written by Dr. Gliga Maximilian Cosma, with all authors providing critical revisions and feedback on previous versions. Prof. Dr. Pascanu Maria Ionela and Dr. Chinezu Laura supervised the study and contributed to the final manuscript review. All authors have read and approved the final version of the manuscript.

Funding
This study received no external funding.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
This study was approved by the The Scientific-Research Ethics Committee of George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mures. Informed consent was obtained from all participating subjects.

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