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REVIEW

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Immunohistochemical evaluation of biomarkers with predictive role in acromegaly: a literature review

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Abstract

Acromegaly is a rare endocrine disorder, which despite the recent advances in diagnosis and management, remains a significant burden in terms of morbidity and mortality for patients because of the frequent aggressive evolution and lack of response to available first-line pharmacological therapy. A switch from the classical "trial and error" management to a personalized therapy approach has been proposed through early identification of biomarkers that could predict treatment response and biological behavior. Several such molecular markers have been extensively studied through immunohistochemistry (IHC), among them the somatostatin receptors type 2 (SSTR-2) and type 5 (SSTR-5), which are known to correlate with response to somatostatin analogues treatment, the SSTR-2 negative tumors usually being resistant to first-generation analogues, while SSTR-5 potentially being a predictive marker for the novel agent, Pasireotide. Based on cytokeratin (CK) immunostaining pattern, somatotropinomas have been classified into densely granulated adenomas (DGAs), which present a milder evolution and favorable outcomes after therapy, and sparsely granulated adenomas (SGAs), known to be more aggressive and frequently resistant to first-line treatment options. Other novel markers, such as the E-cadherin cell-adhesion protein, the aryl hydrocarbon receptor-interacting protein (AIP), the cytoskeleton molecule filamin A (FLNA) and the Ki-67 nuclear antigen have also been the highlight of IHC studies on growth hormone (GH)-producing tumors, with promising results regarding their predictive roles for the outcome of acromegalic patients. In this review, we aimed to summarize the current knowledge on the role of IHC for acromegaly, highlighting the most important biomarkers that could offer valuable information for predicting treatment response, biological behavior, and prognosis.

Keywords: acromegaly, pituitary, immunohistochemistry, biomarkers, treatment.

Introduction

Acromegaly is a rare endocrine disorder characterized by overproduction of growth hormone (GH), which leads to increased levels of insulin-like growth factor 1 (IGF-1) caused in most cases by a GH-secreting pituitary tumor, also called somatotropinoma, which is a benign neuroendocrine tumor in 99.9% of cases. The name "adenoma" was recently replaced with the term of pituitary neuroendocrine tumor (PitNET) because these types of tumors, unlike other typical "benign" neoplasms, can possess an aggressive local behavior and invade and destroy adjacent tissues [1–3]. While acromegaly has been traditionally known as a rare condition, with the latest data from a metanalysis reporting a prevalence of 2.8–13.7 cases per 100 000 inhabitants and a incidence of 0.2–1.1 cases per 100 000 inhabitants per year, it remains a heavy burden on both patients and the healthcare personnel involved in managing them due to the high morbidity and mortality caused by the multisystemic involvement of chronic IGF-1 excess and the frequent delay in diagnosis that can reach up to 6–10 years [4–6].

The diagnosis of acromegaly involves the use of serum IGF-1 measurement as a screening test when there is clinical suspicion, followed by confirmation through GH measurement after oral glucose load, with the latest recommended cut-off values for the nadir (lowest value) GH established at >0.4 ng/mL [7, 8]. Following biochemical diagnosis, a pituitary magnetic resonance imaging (MRI) will be recommended to detect the tumor and proceed with surgical treatment, usually done through a transsphenoidal approach. Primary surgical treatment remains the standard of care first-line therapy despite the recent advances done in pharmacological therapy [8, 9]. Despite this, the final rate of success of surgical treatment remains unsatisfactory and varies widely depending on the center's expertise, with remission rates of 30–70% being reported in various studies from different countries registries, usually lower in developed countries and for patients with less invasive tumors or microadenomas [10–13]. In patients with persistent active disease following surgery the therapeutic strategy involves the use of medical treatment, which includes the first-generation somatostatin receptor ligands (fg-SRLs)

Octreotide and Lanreotide, the newer SRL Pasireotide and the GH-blocking agent Pegvisomant. Dopamine agonists (DAs) may be beneficial and can be used for mild elevations of IGF-1 or added to the previously mentioned agents, but its effect is usually modest [8]. The most common practice is to start treatment "blindly" with fg-SRLs and switch to Pasireotide, GH-blockers or combination therapy in case of resistance to fg-SRL, which was reported to occur in between 20–70% of cases, with a mean of 45% [14, 15].

While some of these medications share common mechanisms of action, there are also clear differences in how different classes of drugs act on inducing acromegaly disease control, from receptor-type activation to post-receptor signaling pathways and cytoskeleton involvement [16–18]. For patients with aggressive and invasive tumors resistant to first- and second-line therapies, sometimes the only option left is surgical reintervention or radiotherapy, both of which are frequently associated with poor prognosis, secondary side-effects, such as hypopituitarism, and high morbidity [7, 19]. A challenge for the modern management of acromegaly remains identifying patients responsive to specific types of treatment options early, to avoid the waste of time and costs associated with expensive and inefficient therapies which lead to impaired quality of life and worse prognosis for patients. To achieve this, a shift from the classical "trial and error" approach to a "precision medicine" has been proposed, which may be aided by the use various biomarkers that can stratify patients into different subgroups based on the prediction of biological behavior of the tumor and treatment response [20, 21].

Some of the biomarkers that have been extensively studied consist of demographic characteristics, pretreatment GH and IGF-1 levels, tumoral volume and cavernous sinus invasion, while other ones are mostly based on histological or molecular characteristics of the tumor, which could be studied by immunohistochemical or genetic techniques, such as quantitative polymerase chain reaction (qPCR) of tumoral samples [20, 22, 23]. While both immunohistochemistry (IHC) and qPCR techniques could offer highly specific details on the histopathological (HP) and molecular variations of acromegaly, these techniques are not yet validated, standardized and available on routine basis except for few reference centers, and are thus mainly applied for research purposes. IHC has the advantage of being a relatively low-cost morphological investigation through which the protein expression of certain biomarkers can be evaluated by using monoclonal or polyclonal antibody kits. Although being a semi-quantitative method, it can offer a valuable insight on the receptor profiling and on other membranous or intracellular proteins expression which may help predict the response to available treatment options in acromegalic patients [24–26]. A well-known example that has been the object of many IHC studies on acromegaly tumor samples is the evaluation of somatostatin receptors type 2 (SSTR-2) and type 5 (SSTR-5) expressions, both of which are known targets of SRLs [27]. While literature data remains heterogenous, most of the studies confirmed that a low SSTR-2 protein expression possesses a strong negative predictive value for treatment response to fg-SRLs. Despite this many acromegalic patients are still treated according to the current practice with fg-SRLs "blindly" and fail to respond, which could be prevented by the implementation of biomarkers-guided precision medicine [26, 28, 29].

Aim

The aim of the current paper was to review the available literature on immunohistochemical biomarkers that can predict disease behavior, treatment-response, and prognosis in acromegaly.

Materials and Methods

A literature search was conducted on *PubMed* using the terms "biomarkers, acromegaly, immunohistochemistry, predictors of treatment response, molecular markers" and extended by combination of the mentioned key terms to identify available published studies on this subject. The search was expanded for each identified biomarker with immunohistochemical evidence available through original research papers, reviews or metanalysis. Most of the articles included in this review are published in the 2010–2021 interval.

Somatostatin receptors type 2 (SSTR-2) and type 5 (SSTR-5)

Somatostatin is a hypothalamic produced polypeptide that under physiological conditions inhibits the secretion of GH from the anterior pituitary through binding on the G-protein coupled SSTRs that include five subtypes (SSTR1–5), each being encoded by different genes situated on different chromosomes [30]. The fg-SRLs under the long-acting formulas Octreotide long-acting release (LAR) and Lanreotide autogel are currently the mainstay of active acromegaly therapy. Fg-SRLs are known to have a higher affinity for SSTR-2 and less for SSTR-5, leading to biochemical disease control in approximately 40–45% of patients, while producing significant (>20%) tumor shrinkage effect in up to 66% of patients [31, 32]. Pasireotide, a newer, second-generation SRL, was shown to possess a higher affinity for SSTR-5 and is mainly used in patients resistant to fg-SRL, which frequently reaches biochemical control after the therapeutical switch to Pasireotide [33, 34].

Many studies have demonstrated that higher SSTR-2 protein expressions on GH-producing tumors correlate both *in vivo* and *in vitro* with the efficacy of SRLs treatment [24, 28, 29, 35]. In 2013, Gatto *et al.* demonstrated on 25 somatotropinoma tissue probes from acromegalic patients with persistent disease after surgery that a high immuno-reactive score (IRS), which takes in account both the percentage of positive cells as well as the intensity of staining, can predict biochemical control defined as IGF-1 normalization for 86% of patients after six months Octreotide treatment [24]. Wildemberg *et al.* found that SSTR-2 was expressed on 100% of the tumors in a large immunohistochemical study done on 88 tissue probes from confirmed acromegaly patients. Possibly, the most important finding in their study was that SSTR-2 protein expression presented a 100% negative predictive value and 60% positive predictive value for the biochemical response to fg-SRLs [29]. This finding was also confirmed by Plockinger *et al.* who found that all SSTR-2 negative patients in their study presented resistance to fg-SRLs treatment [35]. Fg-SRLs pre-treatment for patients waiting

for surgery is a strategy commonly practiced in some centers, which could influence the intensity of SSTR membranous expression through a down-regulation mechanism. While pre-treated patients usually have a lower SSTR expression, Venegas-Moreno *et al.* found in their study that patients with low SSTR-2 protein expression responded worse in terms of IGF-1 decrease after six months of fg-SRLs therapy than patients with higher expressions, but no differences in this regard were observed between pre-surgery treated patients and treatment-naïve ones, in concordance with other studies [28, 29, 35]. While there is clear evidence that SSTR-2 is a reliable predictor of response to fg-SRLs, data regarding the role of SSTR-5 is less established. Considering that Pasireotide has a higher affinity for this type of receptor than fg-SRLs, it was postulated that patients with a low SSTR-2/SSTR-5 ratio who usually fail to respond to fg-SRLs could benefit from Pasireotide treatment [34, 36]. Despite this, results from an *in vitro* study done on many primary cultures of GH-secreting tumors have found that both Octreotide and Pasireotide GH-lowering effects inversely correlated with SSTR-5 expression on IHC protein level, but not on gene levels, which was surprising because Pasireotide is a known SSTR-5 preferential ligand [37]. Nonetheless, in an *in vivo* study done on 11 Pasireotide-treated acromegaly patients who initially failed to respond to fg-SRLs, Iacovazzo *et al.* interestingly found that SSTR-5 negative patients remained unresponsive, while higher SSTR-5 IHC scores correlated with a better response in terms of IGF-1 decrease after Pasireotide [38]. The same authors confirmed their previous findings in a more recent study, where they found that none of the patients with absent SSTR-5 expression responded to Pasireotide [39]. Despite all these findings that highlight the importance of SSTR-5 as a predictor of Pasireotide responsiveness, there are other authors that suggested that Pasireotide efficacy may be driven by SSTR-2 rather than SSTR-5. In such a study done by Muhammad *et al.*, it was confirmed that IGF-1 reduction after Pasireotide treatment was positively associated with SSTR-2 rather than SSTR-5 protein expression and with the response to fg-SRLs [40]. The main difference between the patients group included in this study and the other ones mentioned earlier was that most included patients were partially responsive to fg-SRLs, whereas in Iacovazzo *et al.* study only fg-SRLs resistant patients were evaluated for Pasireotide response [38, 40]. All things considered, it can be suggested that routine evaluation for IHC expression of SSTRs may be beneficial to facilitate the switch to "targeted therapy", by subcategorizing patients into fg-SRLs responsive and resistant, for which Pasireotide could be a reasonable first-line therapy choice [41]. While the evidence for the predictive role of SSTR-2 in SRLs response appears to be clear, results on SSTR-5 are still contradicting, probably also due to the limited evidence and heterogenous patients' populations used in different studies [20]. Another limitation for implementing a reliable routine IHC evaluation for SSTRs on GH-producing tumors remains the lack of an agreement on a standardized IHC scoring system and technique, the heterogeneity of SSTR expression in different tumor samples and many multiple confounding factors that could influence SSTR expression, such as SRLs pretreatment [20, 31, 42, 43]. Finally, it is reasonable to affirm that absent or low SSTR-2 expression strongly predicts treatment resistance to fg-SRLs and such patients could benefit from early treatment with second-line options, such as Pegvisomant or Pasireotide, while a higher SSTR-5 expression might favor response to the latter. Despite this, many patients remain treatment resistant despite expressing high SSTRs, suggesting there could be other molecular factors involved in modulating treatment response, and the simplified idiom of "more receptor equals better response" doesn't fully apply to acromegaly pharmacological therapy [20, 24, 38, 43, 44].

Granulation pattern and cytokeratin

GH-secreting PitNETs are classically divided histologically into sparsely granulated adenoma (SGA) and densely granulated adenoma (DGA) based on the granulation staining pattern of GH-containing secretory granules and the cytokeratin (CK) filaments distribution. While initially this categorization was done by using electron microscopy, it was eventually proven that immunohisto-chemical evaluation for CK by using the CAM 5.2 keratin staining can be used successfully to distinguish between the two histological subtypes [45]. The two histological subtypes are classically known to be associated with different clinical phenotypes and disease evolution [46]. First of all, the surgical cure rate was shown to be lower in SGA compared to DGA in various studies, recent data from two large studies reporting a 14–42% success rate for SGA, while for DGA the cure rate was between 60–65% [47, 48]. Another difference observed between the two subtypes was that SGA type of tumors occur more frequently in younger patients, are of larger volume at diagnosis and present with higher invasiveness rates compared to the DGA [46, 48]. As expected, patients with DGA also present a better response rate to medical treatment with fg-SRLs, as shown in multiple studies [47–49]. While the success rates of second-line therapies for the two subtypes are less established, there is some evidence that patients with SGA who failed to respond to fg-SRLs managed to achieve biochemical control with Pegvisomant therapy, but data comparing the response rate to Pegvisomant between the two subtypes is still lacking [48]. Interestingly, results from a study by Iacovazzo *et al.* concluded that patients with SGA responded better to Pasireotide treatment than the ones with DGA (80% *vs* 16.7%). These results were observed in another study by Lasolle *et al.*, where SGA patients seemed to be better responders to Pasireotide irrespective of SSTR-5 expression [50]. Although the number of evaluated patients was too low and lacks statistical significance, these preliminary findings might pave the path to a transition to personalized therapy in acromegaly, as SGA patients could potentially be successfully treated as first-line choices with Pasireotide or Pegvisomant [38]. The underlying mechanisms behind this different behavior of the two histological entities are still being debated. Some authors have suggested that cell-adhesion molecules, such as the E-cadherin might be involved, as there is some evidence that found that SGA presented with reduced E-cadherin expressions, which could contribute to a more aggressive tumoral phenotype [48, 51, 52]. Moreover, patients with SGA have been found

to possess lower SSTR-2 IHC expression rates compared to DGAs, which might explain the frequent resistance to fg-SRLs therapy in the SGA group [27, 39]. Interestingly, a study by Mayr *et al.* observed that SGAs express SSTR-5 stronger than DGAs [53], while other studies failed to confirm this finding [54], yet the exact mechanisms of how the granulation pattern subtype influences the receptor profile of GH-producing tumors remains to be elucidated.

E-cadherin

Among the molecular markers that have been recently proposed to be involved in the prediction of treatment response, tumor aggressiveness and invasiveness in somatotropinomas is the cell adhesion protein E-cadherin. This marker has been studied in various types of tumors, where a loss of E-cadherin function is associated with an aggressive biological behavior and enhanced metastatic ability of the tumor [55, 56]. The role of E-cadherin in pituitary tumors has been highlighted in several recent studies, and it appears that the loss of its membranous expression might be linked to a more aggressive tumoral behaviors, increased invasiveness, and a poor response to SRLs treatment [51, 52, 55]. Multiple studies have found that SGA presents significantly lower expressions of E-cadherin than the DGA. As expected, low or absent E-cadherin expressions were strongly linked to fg-SRLs treatment resistance, but while some authors consider E-cadherin as an independent predictor of treatment response [51], others suggest it's more likely a surrogate marker of SGA subtype, as the two histological features were proven to be strongly linked [52]. In a recent immunohistochemical study by Venegas-Moreno *et al.*, it was observed that loss of E-cadherin expression at the membrane level was associated with poor response to fg-SRLs in terms of IGF-1 decrease after 3- and 6-month treatment. While most tumors with low or absent E-cadherin failed to respond to fg-SRLs, around half of those with high expression were also resistant, which led to the conclusion that E-cadherin function is not sufficient to modulate the response to treatment, and other molecular factors might be involved in treatment-resistant tumors [51]. Another interesting discovery about the role of E-cadherin in the modulation of treatment response to SRLs found in this study is that tumors with low E-cadherin expressions presented higher SSTR-5 IHC scores [51]. This finding could be attributed to the histological subtype differences, as SGAs were found to carry higher SSTR-5 expressions than DGA in some studies [53]. Considering all these discoveries, it might be reasonable to assume that patients with tumors expressing low E-cadherin might be better candidates for Pasireotide or Pegvisomant treatment than fg-SRLs, but evidence from literature about a possible relationship between second-line acromegaly drug responsiveness and E-cadherin expression is missing.

Ki-67 proliferation index

Ki-67 is a nuclear antigen expressed in all active phases of the cell cycle and a well-known marker of tumor aggressiveness, proliferation, and invasiveness. Several studies confirmed the involvement of the Ki-67 index in pituitary tumors, revealing that higher Ki-67 expressions are associated with increased risk of recurrence and tumor aggressiveness, which leads often to the necessity of multiple therapeutic interventions and a poor prognosis for the patient [57, 58]. Recent findings from the literature also highlighted the role of the Ki-67 in predicting treatment response in GH-secreting tumors. The first study to evaluate this aspect by Fusco *et al.* in 2008 found that acromegalic patients with high Ki-67 index were less likely to respond to Octreotide treatment [58]. Later, a study by Kasuki *et al.* found that Ki-67 is a strong negative predictor of SRLs treatment response in a lot of acromegaly patients. The novel finding in this study was that Ki-67 was proven to be an independent predictor for acromegaly SRLs treatment response, as there were no significant associations between Ki-67 and SSTR-2 expressions [59]. Interestingly, it was found that patients with a SGAs presented higher Ki-67 expressions than the ones with DGAs, suggesting that the mechanisms behind the increased aggressiveness and poor treatment response in these patients might involve molecular markers from the post-receptor signaling pathway, which work independently and by different mechanisms than the classical receptor-protein markers, such as the SSTRs [58, 59]. These findings were confirmed in a larger study by Puig-Domingo *et al.*, where Ki-67 expression negatively correlated with fg-SRLs response in a group of 100 acromegalic patients [25]. Another interesting finding that might change the perspective on the way how fg-SRLs influence disease control was found in a study by Selek *et al.*, where authors compared the Ki-67 index of double-operated acromegalic patients between the first and second intervention and found that SRLs treatment appear to significantly decrease the Ki-67 index in a manner independent of tumor features, SRLs dosage and treatment duration. These findings could indicate a beneficial role of SRLs in decreasing the proliferation rate of tumoral cells even in patients with high Ki-67 index, even though these patients could initially be considered poor responders in terms of biochemical control [60]. The Ki-67 index was also found to be associated with tumor invasiveness and volume in a more recent immunohistochemical study on 31 acromegalic patients [61], while in another study by Alimohamadi *et al.*, Ki-67 index correlated only with the radiological evidence of invasiveness of the tumor and but not with the volume [62]. Although controversies remain, and large multicenter studies with statistical power on this subject are still lacking, the integration of routine immunohistochemical evaluation of the Ki-67 proliferation index for all patients with somatotropinomas is recommended by most authors, as it could provide valuable information for the post-surgical prognosis and recurrence risk of patients [57, 63]. Moreover, the Ki-67 index could also help in guiding the choice of medical treatment for acromegalic patients, as it was proven to be a strong independent predictor for fg-SRLs treatment resistance. There is some scarce evidence that suggested high Ki-67 expression might be associated with Pegvisomant resistance, but the number of patients evaluated was too low (n=6) [39]. Pasireotide on the other hand seems to be a viable choice for patients with the SGA subtype which usually possess higher Ki-67 index [25, 58, 59]. Despite this, further studies are needed to clarify the role of the newer second-line treatment options like Pasireotide and GH-blockers as

treatment options for patients with high Ki-67 positivity rates.

Aryl hydrocarbon receptor-interacting protein (AIP)

Aryl hydrocarbon receptor-interacting protein (*AIP*) gene mutations have been extensively studied and identified in patients with a predisposition for familial and sporadic pituitary PitNETs [64]. Besides this well-known role of *AIP* gene mutations in hereditary somatotropinomas, it was discovered that AIP protein expression evaluated by IHC is indicative of increased invasiveness and might be associated with fg-SRLs resistance, which is unrelated to *AIP* mutations [12, 65, 66]. Some authors have suggested that AIP expression might be an independent predictor of fg-SRLs resistance, although AIP deficient tumors also appear to have lower SSTR-2 expressions [65]. These findings were confirmed in another study that also evaluated the response to Pasireotide for tumors resistant to fg-SRLs, but no differences were found between tumors with strong AIP expressions compared to the AIP deficient ones [38]. Another interesting discovery about the link between AIP and SSTR activation was found by Chahal *et al.*, in a study that compared Lanreotide pretreated acromegalic patients with a matched control group and found that tumors from patients who were treated expressed AIP significantly stronger on IHC compared to the control group, which might suggest that fg-SRLs effects could also be modulated by AIP involvement [67]. Limitations for using AIP protein expression on a routine basis to predict the biological behavior of acromegaly remain mainly due to the absence of a reproductible and standardized IHC technique, while further research into the role of AIP in modulating medical therapy response and tumor characteristics is needed for integrating this biomarker in the molecular diagnosis of somatotropinomas in the future [12, 52].

Cytoskeleton: filamin A, beta-arrestins

Research about the involvement of cytoskeleton molecules in the pharmacological treatment resistance of pituitary tumors has been of increasing interest recently. Several defects in the post-receptor signaling pathways that might be involved in generating resistance to SRLs in acromegaly patients have been proposed, with the focus shifted on actin-binding proteins, such as the filamin family, which consists of three homologous subtypes: filamin A, B and C. Filamin A (FLNA) is an ubiquitously expressed cytoskeleton protein in the human body, and among its multiple functions, it was found *in vitro* that FLNA is involved in the signal transduction and stabilization of several receptors expressed frequently in pituitary tumors, such as SSTR-2, SSTR-5 and the dopamine receptors D2 (DRD2) [68–70]. Early studies have shown that FLNA is necessary to maintain proper DRD2 function in lactotroph PitNETs, and DA-resistant tumors were more likely to express low FLNA [71]. Similarly, it was shown that FLNA is involved in the expression and signaling of SSTRs in somatotropinomas [72], but while some *in vivo* studies failed to find a correlation between FLNA expression at protein levels and SSTR-2, in a recent study by Coelho *et al.* a positive correlation was observed between FLNA expression and SSTR-2 in acromegalic patients who weren't pre-treated before surgery and achieved optimal biochemical response with fg-SRLs [69]. In the same study, another important novel discovery was that FLNA positively correlated with SSTR-5 expression regardless of pharmacological responsiveness or pre-treatment history, which might be a potentially clinically relevant finding considering the SSTR-5 was recently highlighted as a potential marker of response to the second-generation SRL, Pasireotide [69]. No associations have yet been discovered between FLNA and clinical characteristics or tumor invasiveness. Further studies are required to understand the complex role of this novel marker in modulating the response to available treatment options and possibly to discover molecular targets for new pharmacological treatment options in acromegaly.

Beta-arrestins are multifunctional proteins that bind to several intracellular molecules and have been discovered to play a role in the internalization of various G-protein coupled receptors, among them SSTRs as well. It has been postulated that high beta-arrestin activity might be involved in pharmacological treatment resistance through desensitization of the SSTR-2 receptors [73, 74]. In a study by Gatto *et al.*, it was discovered that low beta-arrestin levels strongly correlated with a favorable treatment response to fg-SRLs in a group of 32 acromegalic patients [75], while a later study by Coelho *et al.* failed to confirm these findings [74]. It is noteworthy that all these preliminary studies only used mRNA expression through RT-PCR for the evaluation of beta-arrestin, and it remains to be seen if IHC exploration of these proteins can be successfully used and plays any predictive role in GH-secreting PitNETs [74, 75].

Dopamine receptors

Dopamine receptors (DRs) are another type of G-protein coupled receptors widely expressed in pituitary tumors. Among the family of DRs, the subtype DR-2 has been most extensively studied as it was found to be the primary target of DAs, the main pharmacological treatment in prolactinomas. While the role of DR-2 has received more attention in studies involving lactotroph tumors or non-secreting pituitary tumors, it was found that this type of receptor is expressed in most GH-producing tumors as well [28, 29, 76]. While the expression of DR-2 is considered to be associated with DA response in lactotroph tumors, its role in predicting pharmacological treatment response in acromegaly to SRLs or DA is less clear. In a recent study by Soukup *et al.*, it was found that low DR-2 expression is associated with increased invasiveness in a group of acromegalic patients [52]. However, no associations were found in this study or others between DRD2 expression and fg-SRLs treatment response or SSTRs expression. Further studies are needed to clarify the involvement and usefulness of DR-2 in modulating the response to available pharmacological treatment for acromegaly [28, 29, 52].

GH–prolactin cosecretion

Immunohistochemical staining for other pituitary hormones is a useful practice for the evaluation of acromegaly patients as it can identify tumors with hormonal cosecretion, the most frequent one being GH–prolactin cosecretion. While the exact relationship between GH–prolactin cosecretion

and other clinical and HP characteristics is not clear, several studies found that these types of tumors present a better response to DA in terms of IGF-1 decrease, thus prolactin staining could potentially have a role in choosing the optimal medical treatment for patients with active acromegaly [77, 78] (Figure 1; Table 1).

Table 1 – ***Immunohistochemical biomarkers in acromegaly and correlation to treatment response and tumor invasiveness***

Marker	Tumor invasiveness	Fg-SRLs response	Pasireotide response	Pegvisomant response	Dopamine agonists
SSTR-2	0	++	+	0	0
SSTR-5	0	0	++	0	0
DR-2	0	0	0	0	+
SGA	++	-	+	+	0
DGA	--	+	-	0	0
Ki-67	++	--	+	-	0
E-cadherin	--	++	-	0	0
AIP	-	+	0	0	0
FLNA	0	+	+	0	+

++: Strong evidence of positive correlation; +: Limited or indirect evidence of positive correlation; --: Strong evidence of negative correlation; -: Limited or indirect evidence of negative correlation; 0: No evidence or inconclusive data; AIP: Aryl hydrocarbon receptor-interacting protein; DGA: Densely granulated adenoma; DR-2: Dopamine receptor type 2; Fg-SRLs: First-generation somatostatin receptor ligands; FLNA: Filamin A; SGA: Sparsely granulated adenoma; SSTR: Somatostatin receptor.

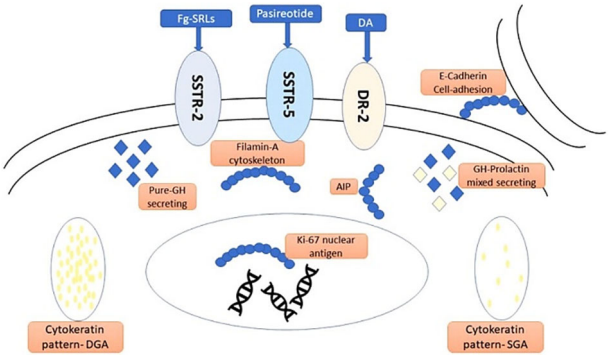

Figure 1 – ***Simplified scheme of membranous receptors, intracytoplasmic and nuclear proteins that can be evaluated through IHC in GH-producing PitNETs: potential biomarkers for treatment response, disease behavior and prognosis. DA: Dopamine agonist; DGA: Densely granulated adenoma; DR-2: Dopamine receptor type 2; Fg-SRLs: First-generation somatostatin receptor ligands; GH: Growth hormone; IHC: Immunohistochemistry; PitNETs: Pituitary neuroendocrine tumors; SGA: Sparsely granulated adenoma; SSTR: Somatostatin receptor.***

Conclusions

Immunohistochemical evaluation of somatotroph PitNETs has an undisputable role in assisting the optimal choice of management tailored to the patient's tumor HP characteristics. Considering that the biological behavior and the response to treatment remains highly variable among acromegalic patients, identifying certain biomarkers through IHC may aid in categorizing patients into different prognostic and treatment-response groups with the final aim of reducing the morbidity and mortality of these patients by efficient and early personalized treatment.

SSTRs remain among the most powerful markers known so far in predicting acromegaly treatment response to fg-SRLs, a high SSTR-2 expression usually predicting fg-SRLs success while tumors with low SSTR-2 and high SSTR-5 are more likely to respond favorably to Pasireotide. The granulation pattern categorization into DGA and SGA, and the Ki-67 index are other useful histological markers for predicting tumor aggressiveness and invasiveness, as SGAs with high Ki-67 index are more likely to be resistant to fg-SRLs. High expression of the E-cadherin adhesion molecule is usually correlated with a good response to fg-SRLs and seems to be associated with the DGA histological subtype, while absent or low E-cadherin is a predictor of fg-SRLs resistance but could be related to favorable Pasireotide therapy as early evidence found an association with high SSTR-5 expression. The novel cytoskeleton marker FLNA appears to be involved in the post-receptor signaling pathway of somatostatin and DRs, and a novel association with SSTRs was found, yet the exact role of this marker's IHC expression in predicting treatment response and clinical behavior of somatotropinomas remains to be elucidated in future studies.

While all these IHC biomarkers have been intensively studied recently, and further novel ones are likely to be discovered in the near future, the challenge remains to establish a reliable and reproductible methodology for the IHC technique which can be eventually integrated into routine clinical practice.

Conflict of interests

The authors have no conflict of interests to disclose.

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