Epithelial–Mesenchymal Transition in the Resistance to Somatostatin Receptor Ligands in Acromegaly

Joan Gil1, Mireia Jordà1,†, Berta Soldevila2 and Manel Puig-Domingo1,2,3,†

1 Endocrine Tumours Lab, Program of Predictive and Personalized Medicine of Cancer (PMPPC), Germans Trias i Pujol Research Institute (IGTP), Badalona, Spain, 2 Department of Endocrinology and Nutrition, Germans Trias i Pujol University Hospital, Badalona, Spain, 3 Department of Medicine, Autonomous University of Barcelona, Bellaterra, Spain

Epithelial–mesenchymal transition (EMT) is a dynamic process by which epithelial cells lose their phenotype and acquire mesenchymal traits, including increased migratory and invasive capacities. EMT is involved in physiological processes, such as embryogenesis and wound healing, and in pathological processes such as cancer, playing a pivotal role in tumor progression and metastasis. Pituitary tumors, although typically benign, can be locally invasive. Different studies have shown the association of EMT with increased tumor size and invasion in pituitary tumors, and in particular with a poor response to Somatostatin Receptor Ligands (SRLs) treatment in GH-producing pituitary tumors, the main cause of acromegaly. This review will summarize the current knowledge regarding EMT and SRLs resistance in acromegaly and, based on this relation, will suggest new biomarkers and possible therapies to SRLs resistant tumors.

Keywords: epithelial-menchymal transition, somatostatin analogs, pituitary, E-cadherin, somatotroph adenoma, growth hormone, PitNETs, endocrine tumors

INTRODUCTION

Epithelial–mesenchymal transition (EMT) is a dynamic process that reorganizes the cell from an epithelial to a mesenchymal phenotype leading to functional changes in cell invasion and migration capacities (1). This process is triggered by microenvironment signals that cells receive which produce changes in gene expression and post-translational regulation mechanisms leading to the loss of epithelial characteristics (cell polarity, stable epithelial cell-cell junctions and interactions with extracellular matrix) and the acquisition of mesenchymal features (fibroblast-like morphology and increased migratory and invasive properties). Although it has been considered as a binary process for many years, EMT has been recently shown to occur through distinct transition cellular states that are driven by a network of transcription factors (EMT-TFs) (2, 3). SNAI1-2, TWIST, and ZEB protein families have been the most extensively studied EMT-TFs as they regulate the classical EMT focused on the repression of E-cadherin, the prototypic adhesion molecule; however, the list of EMT-TFs has largely grown in the last years (4).

EMT was first described in embryonic development as a process that enables the correct morphogenetic events during migration of epithelial cells from the original position to their ultimate destination. However, EMT also occurs in pathological situations such as cancer (2, 5).
During the progression of solid malignancies from benign tumors to locally invading tumors, and finally to metastatic neoplasms, EMT plays a key role. However, it seems that cancer-associated EMT is only activated partially and transiently, in contrast to developmental EMT (3). This attribute and the fact that EMT programs have been associated with other cellular programs such as cell survival, stemness and resistance to drugs (4), makes EMT difficult to study by only analyzing the expression of EMT-TF network.

Recent studies suggest the involvement of EMT in first generation Somatostatin Receptor Ligands (SRLs) resistance in GH-producing pituitary tumors (6–9). Here we review the role of EMT in pituitary adenomas and discuss the relationship between EMT and SRLs resistance in GH-producing pituitary tumors as well as offer new potential biomarkers and therapeutic options.

METHODS

We performed a systematic review with the aim of summarizing the current knowledge of EMT in GH-secreting adenomas with a special focus on SRLs resistance. We performed a search in MEDLINE database using its PubMed tool of the literature available until January 2021. We searched for the terms: “Epithelial-mesenchymal transition” OR “EMT” AND “Acromegaly” OR “Pituitary adenoma” OR “Pituitary tumor” AND/OR “Somatostatin” OR “Somatostatin receptor ligands” OR “Somatostatin analogs”.

EPITHELIAL–MESENCHYMAL TRANSITION IN PITUITARY TUMORS

It is well known the importance of the expression of determined transcription factors during pituitary organogenesis to give the final identity to every different hormone-secreting cell type (10), and EMT plays an important role in this embryogenic process (11). The PROP1 transcription factor, that is vital for the ontogenesis of somatotroph cells, was discovered to promote EMT during pituitary stem cell differentiation, making EMT an important step to obtain fully-functional differentiated pituitary cells (12, 13). These results have been validated by single-cell transcriptomic profiling of the different developmental lineages in human pituitary (14).

EMT is not only linked to pituitary through development, as E-cadherin has been related to hormone secretion in mature cells. E-cadherin reduces prolactin protein content through affecting trafficking of secretory granules (15). Furthermore, it has been also associated with follicle-stimulating hormone (FSH) content and subcellular localization in non-functioning pituitary tumors (16).

EMT also plays an important role in the aggressive biologic behavior of pituitary tumors. Pituitary tumors are the second most common primary brain tumors with invasive properties. The loss of E-cadherin, which is a key characteristic of EMT associated with poor prognosis and high grade tumors in almost all malignancies derived from epithelial cells, has also been reported in pituitary tumors. E-cadherin, a calcium-dependent cell to cell adhesion transmembrane protein, is part of a cell adhesion complex where it is associated with other proteins (α-, β-, γ- and p120-catenins) through an intracellular domain (17). Interestingly, E-cadherin can act as direct transcriptional regulator by nuclear translocation (18). In pituitary tumors, the loss of E-cadherin, specifically the loss of cytoplasmic E-cadherin, is frequently found concurrently with its detection in the nucleus (19). Importantly, nuclear staining E-cadherin is associated with tumor invasion, suggesting that cleavage of the extracellular domain of E-cadherin and nuclear translocation may participate in local invasion in pituitary tumors. Similarly, E-cadherin among other adhesion molecules was related to invasiveness and proliferative status of prolactinomas (20, 21). Other classical EMT markers, such as N-cadherin, SNAI1, SNAI2 and TWIST (21, 22) or β-catenin (23) have also been associated with a worse clinical course in pituitary tumors, especially indicating an invasive phenotype, although there is some controversy regarding this subject in acromegaly (21, 24).

Furthermore, different other non-classical molecules related to EMT have been characterized as part of the mechanisms allowing invasiveness in pituitary tumors, such as ADAM12 (a disintegrin and metalloprotease 12), which has been postulated as an EMT inducer in these tumors (25). ADAM12 overexpression is associated with pituitary tumor invasiveness, while its silencing prevents such biological behavior. Mechanistically, ADAM12 silencing impairs ectodomain shedding of epidermal growth factor receptor (EGFR) ligands and attenuated the EGFR/ERK signaling pathway. Inhibition of EGFR signaling resulted in EMT suppression similar to repression of ADAM12. Also, a recent study by Falch at al (26), in non-functioning gonadotroph tumors reported that those tumors harboring invasive and rapid growing characteristics showed overexpression of genes involved in EMT, in particular SPAG9, SKIL, MTDH, HOOK1, CNOT6L and PRKACB.

Surprisingly, pituitary tumor transforming gene 1 (PTTG1) has been related to EMT in non-functioning pituitary adenomas (27), just the opposite role of PTTG2 (28). Other authors suggested that the mechanism triggering the EMT in pituitary tumors is linked to the expression of S100A9, a member of the S100 family of EF-hand motif Ca2+-binding proteins, mediated by activation of AKT1 (29). In addition, it has been showed that the transcriptoma of USP8 wild-type corticotropinomas, characterized by increased invasiveness, was enriched in EMT signature (30).

In another study in GH-secreting adenomas, cyclin B1 (CCNB1) knock-down was found to decrease the mesenchymal marker N-cadherin and increase the epithelial markers E-cadherin and p120-catenin. Thus, inactivation of cyclin B1 results in a decreased proliferation and EMT, and an increased apoptosis (31). A similar approach was used in a different study where the expression of SMAD4 was found to regulate EMT in somatotropinomas. SMAD4 was associated with invasion, increased levels of vimentin and N-cadherin and, decreased E-cadherin (32).
Other genes have been related to the suppression of EMT and, therefore, invasion. It is the case of collagen type VI alpha 6 (COL6A6) that inhibits cell proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) through the binding of P4HA3 resulting in PI3K-Akt axis inhibition in pituitary adenomas (33).

Not only coding genes have been related to EMT in pituitary tumors, some microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have been shown to modulate EMT. MiR-149-5p and miR-99a-3p suppress the expression of EMT-related genes (34). miR-132, miR-15a and miR-16 also inhibit EMT in pituitary adenomas; in this case targeting SOX5 (35). Moreover, miR-424-3p inhibits EMT and invasion by targeting JAG1 (36). On the other hand, lncRNAs seem to be related to EMT enhancement rather than inhibition. For example, lncRNA SNHG6 induces EMT suppressing miR-944, which may inhibit RAB11A (37). Furthermore, lncRNA PVT1 enhances EMT and migration by activating Wnt/β-catenin (38). Finally, lncRNA SNHG1 promotes EMT and invasion by activation of TGFBR2/SMAD3 and RAB11A/Wnt/β-Catenin axis, and the inhibition of miRNAs such as miR-302/372/373/520 (39).

EMT is a dynamic process, not a binary process, with intermediary states (2). It is very unlikely that benign tumor cells undergo a complete mesenchymal transformation which is associated with metastatic tumors (5). Because of that, it is more likely that, as in the majority of neoplasms, pituitary tumors would exhibit partial EMT states (40). This would explain why in transcriptomic analysis some EMT markers are up-regulated while others do not, instead of showing a complete mesenchymal profile (9, 41).

It is noteworthy to highlight the importance of the tumor microenvironment in mediating EMT and, therefore, the aggressive behavior of pituitary adenomas. The alteration of the tumor microenvironment seems to be triggered by tumor chemokines that attract immune cells (42). Additionally, IL-6 and CCL2 produced by tumor associated fibroblasts have been associated with EMT-like morphological changes and aggressive behavior trough E-cadherin downregulation and ZEB1 upregulation in an in vitro study (43).

It is really important to confirm the link between EMT and AIP, since AIP-ZAC1 pathway is one of the main molecular mechanisms described for SRLs resistance (44). SRLs can activate AIP which inhibits adenylate cyclase, reducing cyclic AMP levels. On the other hand, AIP activates ZAC1. This molecule binds directly p53 and activates gene transcription; moreover, p53 arrests cell cycle, through p21 interaction, and increases apoptosis (45). Deeper explanation of the pathway could be found in some other reviews (45, 46).

E-CADHERIN LOSS IS AN OUTSTANDING BIOMARKER FOR SRL RESISTANCE IN ACROMEGALY

Somatostatin is secreted by the hypothalamus and inhibits hormone secretion and to a lesser extent pituitary cell growth by binding to different G protein-coupled receptors (SSTR1–5 [reviewed in Ben-Shlomo and Melmed (47)]. As remnant of its somatroph origin, somatotropinomas express somatostatin receptors, specially SSTR2 and SSTR5 (48). First-generation Somatostatin Receptor Ligands (SRLs), octreotide and lanreotide, so far the accepted first-line medical therapy in acromegaly despite that hormonal hypersecretion control of the disease is generally reported to be lower than 50% and both show a high affinity for SSTR2 receptor (49). Molecular characterization of the tumors has unveiled several explanations for such uneven response (50). However, many studies have proved the involvement of many other players and nowadays we do not know the whole picture of SRLs resistance in acromegaly.

EMT and epithelial plasticity have been associated with resistance to conventional, targeted and immune therapies in many cellular and preclinical models in different tumor contexts, although there is little evidence from clinical samples (51). In this line, different studies relate EMT and SRLs resistance in acromegaly. E-cadherin has been linked to SRLs response as an independent predictor by different studies and in different cohorts (8, 52, 53). In a fair comparison between several known biomarkers of SRLs response, E-cadherin showed the greatest performance in predicting postsurgical SRLs response, even greater than SSTR2 or Ki-67 (8). There is a general consensus that low levels of E-cadherin mRNA and protein indicate a poor responsive tumor to SRLs (8, 52, 53). Furthermore, E-cadherin loss seems to be related to the granulation pattern of the tumor, especially but not exclusively in GH-producing tumors (8, 53). It is worth saying that the histological granulation pattern of the tumor has been related to SRLs response for many years (54, 55). Interestingly, some studies have shown the association between E-cadherin downregulation and E-cadherin promoter hypermethylation in GH-secreting tumors, suggesting the involvement of epigenetic mechanisms (56–58). Another study pointed to the presence of progenitor mesenchymal cells derived from cancer stem cells as the cause of E-cadherin decrease and EMT induction (through TGFβRII increase) in somatotropinomas (59).

Taking into account that E-cadherin is routinely assessed in pathology departments as diagnostic tool for other cancer types (60), it would be easy to implement it as biomarker of response to SRLs to better define acromegaly treatment (61).

BEYOND E-CADHERIN LOSS: INVOLVEMENT OF OTHER EPITHELIAL–MESENCHYMAL TRANSITION MOLECULES IN SRL RESISTANCE IN ACROMEGALY

Since E-cadherin loss is a marker of advanced EMT, some authors have further investigated this phenomena in GH-producing tumors. Lekva and colleagues analyzed the transcriptome of tumors with very high and very low levels of E-cadherin and identified several EMT-related genes. Interestingly, in vitro, the expression of these genes were not
regulated by E-cadherin but by Epithelial Splicing Regulatory Protein1 (ESRP1) (6). ESRP1 has been characterized as an important contributor to EMT by mediating alternative splicing in EMT affecting the maintenance of epithelial features (62). It is important to mention that several studies have proved the relation of splicing and SRLs resistance in acromegaly (7, 63–65). ESRP1, thus, may be a master regulator of the EMT, SRLs response and other pathological processes in acromegaly (7).

Lekva and colleagues also investigated genes that were differentially expressed upon treatment with SRLs in different EMT contexts. They found that RAR Related Orphan Receptor C (RORC) was overexpressed in phenotypically epithelial tumors but not in mesenchymal ones (9). Moreover, RORC expression was associated with SRLs response, a result that has been confirmed by finding that RORC is a biomarker of SRLs response improvement after surgical debulking (66).

On the other hand, patients harboring AIP-mutated somatotropinomas tend to be diagnosed at a younger age with larger, more aggressive, and SRLs resistance tumors (44). Some studies have shown that AIP is an important mediator of SRLs response (45), and AIP expression has been found to be a potent SRLs response predictor (44). In this context, it was interesting to prove that the transcriptome of ten somatotropinomas and five normal pituitaries revealed EMT as one of the most significantly altered pathways in AIP-mutated tumors. Furthermore, the cell-conditioned media of AIP-knockdown cells increases migration of macrophages (41), reinforcing the role of tumor microenvironment in inducing EMT and a more aggressive phenotype.

### CYTOSKELETON, EPITHELIAL–MESENCHYMAL TRANSITION, AND SRL RESISTANCE IN ACROMEGALY

One of the main characteristics of EMT is the reorganization of cell polarity through changes in the cytoskeleton, which is composed of the actin cytoskeleton, the microtubule network and the intermediate filaments that provide structural design and mechanical strength. The cytoskeleton is known to play an important role in EMT during cancer progression (67). Concretely, reelin proteins perform their function through filament A (FLNA) to regulate the actin cytoskeleton reorganization. ReelinA promotes the conversion of FLNA from an actin branching protein into an F-actin bundler, and ReelinB combined with FLNA organize a unique perinuclear actin network at the apical surface during the EMT (68, 69). Interestingly, in SNAI1-induced EMT, it has been proved that the changes in nuclear morphology and in the cytoskeleton structure correlate with decreased expression of FLNA (70).

FLNA plays an important role in GH-producing tumors since it has been related to pituitary tumors migration and invasion (71) and, more importantly, also to SRLs resistance (72). Additionally, it has been proved that FLNA mediates octreotide-induced SSTR2 trafficking through endosomal proteins in acromegaly. Moreover, FLNA influences the number of available SSTR2 at the surface of the cell (73).

For more detailed explanation of the cytoskeleton involvement in SRLs resistance, we recommend the review by Peverelli et al. published in 2015 in this same journal (74).

### EPITHELIAL–MESENCHYMAL TRANSITION-RELATED THERAPIES

The involvement of EMT in acromegaly pathogenesis and SRLs resistance offers new therapeutic approaches that should be explored. As an example, CCNB1 overexpression in acromegaly can be targeted with resveratrol, inhibiting CCNB1 and reverting its effects on invasion (31). Interestingly, Pasireotide, a second generation SRL, has been associated with a reduction of EMT-associated chemokines in tumor associated fibroblasts, suggesting an anti-tumor effect targeting the microenvironment (43). In contrast, other first generation SRLs do not appear to affect EMT (59).

Several EMT regulating TFs (SNAI1, SNAI2, TWIST…) can induce a therapy-resistant intrinsic mechanism (overexpression of drug efflux pumps) as well as an extrinsic one (gaining resistance to apoptosis inducing agents). This explains why EMT is often related to drug resistance in tumors (2). However, EMT features are emerging as novel therapeutic targets in cases of resistance to current therapies (75). Some of the drugs proposed to inhibit EMT in clinical phases are well-known for endocrinologists such as metformin (76). Others have been proposed to be useful in acromegaly to target GDNF-RET/PIT1/p14ARF/p53 pathway, like Sorafenib (77).

EMT offers target opportunities in different levels: inhibiting stimuli from the tumor microenvironment, inhibiting extracellular mediators and their corresponding receptors, inhibiting or activating intracellular signaling pathways, and inhibiting transcription factors that indirectly induce EMT (78). On this last regard, the usage of an inhibitor of STAT3 could very much benefit acromegaly therapy since it would act reverting EMT process (79–81) and directly inhibiting GH hypersecretion (82). More than a dozen of different therapies targeting EMT are being tested in clinical trials, however the vast majority are used in combination with regular chemotherapy since it is expected to recover sensitivity of more conventional drugs upon EMT inhibition (78). In acromegaly, the main concern rather than the proliferation and formation of metastasis is the normalization of hormone levels; this is the reason why rather than the expected antiproliferative effects of these drugs, we would expect a resensitization to SRLs. However, nowadays it is unknown if this effect would be achieved and which molecules should be targeted.

### DISCUSSION

It is very likely that EMT plays an important role in acromegaly pathogenesis, but also in the modulation of pharmacologic response, thus inducing SRLs resistance in particular. However, most of this relationship is unknown since the molecular pathways relating EMT and SRLs signaling are not really understood and sufficiently explored. We are only beginning to
unveil this relationship and we have, by now, been able to find some of the key molecules, but the whole picture remains elusive. To add a little more complexity to the acromegaly and EMT relationship, it is worth to mention that one of the surprising effects of GH is the induction of EMT (83), closing the circle around EMT and acromegaly. The induction of EMT by GH seems to be mediated by the tumor microenvironment involving not only tumor cells but multiple non-tumoral cell types (84, 85). Importantly, the study of EMT has provided some interesting biomarkers to predict SRLs response in acromegaly, for example, E-cadherin and RORC. Furthermore, as we could only glimpse for now, EMT in acromegaly is involved in a lot of processes like stemness, apoptosis, secretory vesicles trafficking, cytoskeleton organization, invasion capacities and aberrant splicing. All of them are, to some extent, individually related to SRLs resistance and that makes very difficult to delimitate the action of EMT. It forces to contemplate EMT as a dynamic process with deep connections with a multitude of different cellular programs. Moreover, the presence of intermediate EMT states in tumors, which generates tumor heterogeneity, is probably key in the contribution of EMT to treatment resistance.

Further studies of the EMT process would not only provide in-deep knowledge about the dedifferentiation of GH-secreting tumors and the SRLs desensitization, but will certainly offer alternative treatments to the SRLs. Several EMT inhibitors are currently been tested in clinical trials for other malignancies. The plastic and dynamic nature of EMT increases the difficulty in determining the appropriate therapeutic and diagnostic windows. However, targeting EMT blockade as an adjuvant therapy could potentially increase the effectiveness of the GH-secreting tumors to SRLs.

In conclusion, EMT is a process that plays an important role in the heterogeneity of pituitary adenomas and is associated with a more aggressive phenotype. Furthermore, it has been linked to SRLs response in somatotropinomas. Thus, EMT-related therapies may be taken into consideration in the treatment of acromegaly, especially in SRLs non-responder patients. This could be an opportunity to find new therapies for pituitary adenomas; however, the increasing therapeutic options for acromegaly may overwhelm clinicians making more difficult the choice of the best molecule(s) to target. In this regard, some authors have developed a universal and quantitative EMT scoring based on transcriptomic data that allows the prediction of response to different pharmacological treatments (86). We think that this type of tools should be the basis of the future medicine in acromegaly; the “trial-and-error” approach to decide the appropriate drug would no longer be an option (87–90).

**AUTHOR CONTRIBUTIONS**

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

**FUNDING**

Work in our laboratory is supported by the Instituto de salud Carlos III (PM 15/00027) and Novartis through the REMAH (Registro Español Molecular de Adenomas Hipofisarios) consortium to the SEEN (Sociedad Española de Endocrinología y Nutrición).

**REFERENCES**

1. Yang J, Antin P, Berx G, Blanpain C, Brabletz T, Bronner M, et al. Guidelines and definitions for research on epithelial–mesenchymal transition. *Nat Rev Mol Cell Biol* (2020) 6. doi: 10.1038/s41580-020-0237-9
2. Nieto MA, Huang R-Y-J, Jackson RA, Thiery JP. EMT: 2016. *Necel Cell Biol* 2016:16621–45. doi: 10.1016/j.cell.2016.06.028
3. Pastushenko I, Blanpain C. EMT Transition States during Tumor Progression and Metastasis. *Trends Cell Biol* (2019) 29:212–26. doi: 10.1016/j.tcb.2018.12.001
4. Stemmpler MP, Eccles RL, Brabletz S, Brabletz T. Non-redundant functions of EMT transcription factors. *Nat Cell Biol* (2019) 21:102–12. doi: 10.1038/s41556-018-0196-y
5. Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. *Nat Rev Cancer* (2018) 18:128–34. doi: 10.1038/nrc.2017.118
6. Lekva T, Berg JP, Fougner SL, Olstad OK, Ueland T, Bollerslev J. Gene expression profiling identifies ESRP1 as a potential regulator of epithelial mesenchymal transition in somatotroph adenomas from a large cohort of patients with acromegaly. *J Clin Endocrinol Metab* (2012) 97:E1506–14. doi: 10.1210/jc.2012-1760
7. Lekva T, Berg JP, Lyle R, Heck A, Ringstad G, Olstad OK, et al. Epithelial Splicing Regulator Protein 1 and Alternative Splicing in Somatotroph Adenomas. *Endocrinology* (2013) 154:3331–43. doi: 10.1210/en.2013-1051
8. Puig-Domingo M, Gil J, Sampedro Nuñez M, Jordà M, Webb SM, Serra G, et al. Molecular profiling for acromegaly treatment: a validation study. *Endocr Relat Cancer* (2020) 27. doi: 10.1530/ERC-18-0565
9. Lekva T, Berg JP, Heck A, Lyngvi Fougner S, Olstad OK, Ringstad G, et al. Attenuated RORC expression in the presence of EMT progression in somatotroph adenomas following treatment with somatostatin analogs is associated with poor clinical recovery. *Plos One* (2013) 8:e66927. doi: 10.1371/journal.pone.0066927
10. de Moraes DC, Vaisman M, Conceição FL, Ortiga-Carvalho TM. Pituitary development: a complex, temporal regulated process dependent on specific transcriptional factors. *J Endocrinol* (2012) 215:239–45. doi: 10.1530/JEO-12-0229
11. Dubois PM, ElAmraoui A. Embryology of the pituitary gland. *Trends Endocrinol Metab* (1995) 6:1–7. doi: 10.1016/1043-2760(94)00090-Q
12. Pérez Millán ML, Brinkmeier ML, Mortensen AH, Camper SA. PROP1 triggers epithelial-mesenchymal transition-like process in pituitary stem cells. *Elife* (2016) 5. doi: 10.7554/eLife.14470
13. Cheung LYM, Davis SW, Brinkmeier ML, Camper SA, Pérez-Millán ML. Regulation of pituitary stem cells by epithelial to mesenchymal transition events and signaling pathways. *Mol Cell Endocrinol* (2017) 445:14–26. doi: 10.1016/j.mce.2016.09.016
14. Zhang S, Cui Y, Ma X, Yong J, Yan L, Yang M, et al. Single-cell transcriptions identifies divergent developmental lineage trajectories during human pituitary development. *Nat Commun* (2020) 11:5275. doi: 10.1038/s41467-020-19012-4
15. Kusumoto K, Kikuchi M, Fujisawa K, Horiguchi K, Kouki T, Kawanishi K, et al. Effect of E-cadherin Expression on Hormone Production in Rat Anterior Pituitary Lactotrophs In Vitro. *Acta Histochem Cytchem* (2010) 43:83–8. doi: 10.1267/ahc.10001
16. Kolnes AJ, Øystese KAB, Olarescu NC, Ringstad G, Berg-Johnsen J, Casar-Borota O, et al. FSH Levels Are Related to E-cadherin Expression and Subcellular Location in Nonfunctioning Pituitary Tumors. *J Clin Endocrinol Metab* (2020) 105:2587–94. doi: 10.1210/clm/dgaz281
17. Møge RM, Ishiyama N. Integration of Cadherin Adhesion and Cytoskeleton at Adherens Junctions. *Cold Spring Harb Perspect Biol* (2017) 9. doi: 10.1101/ cshperspect.a027338

18. Du W, Liu X, Fan G, Zhao X, Sun Y, Wang T, et al. From cell membrane to the nucleus: an emerging role of E-cadherin in gene transcriptional regulation. *J Cell Mol Med* (2014) 18:1712–9. doi: 10.1111/jcmm.12340

19. Elston MS, Gill AJ, Conaglen JV, Clarkson A, Cook RJ, Little NS, et al. Nuclear accumulation of e-cadherin correlates with loss of cytoplasmic membrane staining and invasion in pituitary adenomas. *J Clin Endocrinol Metab* (2009) 94:1436–42. doi: 10.1210/jc.2008-2075

20. Qian ZR, Li CC, Yamasaki H, Mizusawa N, Yoshimoto K, Yamada S, et al. Tumor microenvironment defines the invasive phenotype of AIP-mutation-positive tumors. *Oncogene* (2019) 38:5381–95. doi: 10.1038/s41388-019-0779-9

21. Grigore A, Jolly M, Jia D, Farach-Carson M, Levine H, et al. Tumor budding; The name is EMT. Partial EMT. *J Clin Med* (2016) 5:51. doi: 10.3390/jcm5050051

22. Barry S, Carlsten E, Marques P, Stiles CE, Gadeleta E, Berney DM, et al. Tumor microenvironment defines the invasive phenotype of AIP-mutation-positive tumors. *Endocr Relat Cancer* (2017) 29:141–54. doi: 10.1002/erc.2380

23. Venegas-Moreno E, Luque RM, Neto LV, Machado D, Naiki M, et al. Quantitative analysis of somatostatin receptor subtypes (1–5)–gene expression levels in somatotropinomas and correlation to in vivo hormonal and tumor volume responses to treatment with octreotide LAR. *J Endocrinol* (2019) 235:R101–7. doi: 10.1530/JEO-19-0791-0

24. Ben-Shlomo A, Melmed S. Pituitary somatostatin receptor signaling. *Trends Endocrinol Metab* (2010) 21:123–33. doi: 10.1016/j.tem.2009.12.003

25. Taboada GF, Luque RM, Neto LV, Machado D, Naiki M, et al. Quantitative analysis of somatostatin receptor subtypes (1–5)–gene expression levels in somatotropinomas and correlation to in vivo hormonal and tumor volume responses to treatment with octreotide LAR. *J Endocrinol* (2019) 235:R101–7. doi: 10.1530/JEO-19-0791-0

26. Santamaria PG, Moreno-Bueno G, Cano A. Contribution of Epithelial Plasticity to Therapy Resistance. *J Clin Med* (2019) 8:676. doi: 10.3390/ jcm8050676

27. Fougner SL, Lekva T, Borota OC, Hald JK, Bolverslev J, Berg JP. The expression of E-cadherin in somatotroph pituitary adenomas is related to tumor size, invasiveness, and somatostatin analog response. *J Clin Endocrinol Metab* (2010) 95:2334–42. doi: 10.1210/jc.2010-0002

28. Fougner SL, Casar-Borota O, Heck A, Berg JP, Bolverslev J. Adenoma granulation pattern correlates with clinical variables and effect of somatostatin analogue treatment in a large series of patients with acromegaly. *Clin Endocrinol (Oxf)* (2012) 76:96–102. doi: 10.1111/j.1365-2265.2011.04163.x

29. Kiseljak-Vassiliades K, Carlson NE, Borges MT, Kleinschmidt-DeMasters BK, Lilliehi KO, Kerr JM, et al. Growth hormone tumor histological subtypes predict response to surgical and medical therapy. *Endocrine* (2015) 49:231–41. doi: 10.1007/s12020-014-0383-y

30. Qian ZR, Sano T, Yoshimoto K, Asa SL, Yamada S, Muzisawa S, et al. Tumor-specific downregulation and methylation of the CDH1 (E-cadherin) and
CDH1 (E-cadherin) gene correlate with aggressiveness of human pituitary adenomas. Mod Pathol (2007) 20:1269–77. doi: 10.1038/modpathol.3800965
57. Sano T, Tong QZ, Kagawa N, Yamada S. Down-Regulation of E-Cadherin and Catenis in Human Pituitary Growth Hormone-Producing Adenomas. In: Molecular Pathology of the Pituitary. Basel: KARGER. (2004) p. 127–32. doi: 10.1159/000079094
58. Xu B, Sano T, Yoshimoto K, Yamada S. Downregulation of E-Cadherin and Its Undercoat Proteins in Pituitary Growth Hormone Cell Adenomas with Prominent Fibrous Bodies. Endocr Pathol (2002) 13:341–52. doi: 10.1385/EP:13:4:341
59. Orciani M, Caffarini M, Sorgettoni G, Ricciuti RA, Arnaldi G, Di Primo R. Effects of somatostatin and its analogues on progenitor mesenchymal cells isolated from human pituitary adenomas. Pituitary (2017) 20:251–60. doi: 10.1007/j11210-016-0770-x
60. Pai K, Baliga P, Shrestha BL. E-cadherin expression: a diagnostic utility for differentiating breast carcinomas with ductal and lobular morphologies. J Clin Diagn Res (2013) 7:840–4. doi: 10.7860/JCDR/2013/7575.2954
61. Mete O, Asa SL. Structure, Function, and Morphology in the Classification of Pituitary Neuroendocrine Tumors: the Importance of Routine Analysis of Pituitary Transcription Factors. Endocr Pathol (2020) 4. doi: 10.1007/s12022-020-00946-x
62. Warzecha CC, Jiang P, Amirikian K, Dittmar KA, Lu H, Shen S, et al. An ESRP-regulated splicing programme is abrogated during the epithelial-mesenchymal transition. EMBO J (2010) 29:3286–300. doi: 10.1002/embj.201019195
63. Luque RM, Ibáñez-Costa A, Neto LV, Taboada GF, Hormaeche-Aguilla D, Kasuki L, et al. Truncated somatostatin receptor variant sstSTMD4 confers aggressive features (proliferation, invasion and reduced octreotide response) to somatotropinomas. Cancer Lett (2015) 359:299–306. doi: 10.1016/j.canlet.2015.01.037
64. Ibáñez-Costa A, Gahete MD, Rivero-Cortés E, Rincon-Fernández D, Nelson R, Beltrán M, et al. In1-ghrelin splicing variant is overexpressed in pituitary adenomas and increases their aggressive features. Sci Rep (2015) 5:8714. doi: 10.1038/srep08714
65. Lekva T, Berg JP, Lyle R, Heck A, Bollerslev J, Ueland T. Alternative splicing of placental lactogen (CSTH) in somatotroph pituitary adenomas. Neuro Endocrinol Lett (2015) 36:136–42.
66. Gil I, Marqués-Pamies M, Jordà M, Fajardo-Montañana C, García-Martínez A, Sampedro M, et al. Molecular determinants of enhanced response to somatostatin receptor ligands after debulking in large GH producing adenomas. Clin Endocrinol (Oxf) (2020). doi: 10.1111/cen.14339
67. Sun B, Fang Y, Li Z, Chen Z, Xiang J. Role of cellular cytoskeleton in epithelial-mesenchymal transition. Cytoskeleton (2016) 73:235–43. doi: 10.1080/0090769X.2015.1032824
68. Gay O, Gilquin B, Nakamura F, Jenkins ZA, McCartney R, Krakow D, et al. In1-ghrelin splicing variant is overexpressed in pituitary adenomas. Mod Pathol (2017) 30:603–10. doi: 10.1038/modpathol.3800965
69. Yue P, Zhang X, Paladino D, Sengupta B, Ahmad S, Holloway RW, et al. Hyperactive EGF receptor, Jaks and Stat3 signaling promote enhanced colony-forming ability, motility and migration of cisplatin-resistant ovarian cancer cells. Oncogene (2012) 31:2309–22. doi: 10.1038/onc.2011.409
70. Colomière M, Ward AC, Riley C, Treneerry MK, Cameron-Smith D, Findlay J, et al. Cross talk of signals between EGFR and IL-6R through JAK2/STAT3 mediate epithelial–mesenchymal transition in ovarian cancers. Br J Cancer (2009) 100:134–44. doi: 10.1038/sj.bjc.6607494
71. Ashizawa T, Akiyama Y, Miyata H, Iizuka A, Komiyama M, Kume A, et al. Effect of the Stat3 inhibitor STX-0119 on the proliferation of a temozolomide-resistant glioblastoma cell line. Int J Oncol (2014) 45:111–8. doi: 10.3892/ijo.2014.2439
72. Zhou C, Jiao Y, Wang R, Ren S-G, Wawrowsky K, Melmed S. STAT3 upregulation in pituitary somatotroph adenomas induces growth hormone hypersecretion. J Clin Invest (2015) 125:1692–702. doi: 10.1172/JCI78173
73. Brittain AL, Basu R, Qian Y, Kopchick JJ. Growth Hormone and the Epithelial-to-Mesenchymal Transition. J Clin Endocrinol Metab (2017) 102:3662–73. doi: 10.1210/jc.2017-01000
74. Chesnokova V, Melmed S. Growth hormone in the tumor microenvironment. Arch Endocrinol Metab (2020) 65:683–705. doi: 10.20954/2359-3997000000186
75. Chesnokova V, Zonis S, Zhou C, Recouvreur MV, Ben-Shlomo A, Araki T, et al. Growth hormone is permissive for neoplastic colon growth. Proc Natl Acad Sci U.S.A. (2016) 113:E3250–9. doi: 10.1073/pnas.160561113
76. Tan TZ, Mow QH, Miki Y, Noda T, Mori S, Huang CY, et al. Epithelial–mesenchymal transition spectrum quantification and its efficacy in deciphering survival and drug responses of cancer patients. EMBO Mol Med (2014) 6:1279–93. doi: 10.15252/emmm.201404208
77. Lesko LJ. Personalized Medicine: Elusive Dream or Imminent Reality? Clin Pharmacol Ther (2007) 81:807–16. doi: 10.1097/CPT.0b013e3280160204
78. Kasuki L, Wildembberg LE, Gadelha MR. MANAGEMENT OF ENDOCRINE DISEASE: Personalized medicine in the treatment of acromegaly. Eur J Endocrinol (2018) 178:R89–R100. doi: 10.1530/EJE-17-1006
79. Puig Domingo M. Treatment of acromegaly in the era of personalized and predictive medicine. Clin Endocrinol (Oxf) (2015) 83:3–14. doi: 10.1111/cen.12731
80. Gadelha MR. A paradigm shift in the medical treatment of acromegaly: from a “trial and error” to a personalized therapeutically decision-making process. Clin Endocrinol (Oxf) (2015) 83:1–2. doi: 10.1111/cen.12797

Conflict of Interest: MP-D declares to have received funding from Novartis through the REMAH consortium for research purposes.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Gil, Jordà, Soldevila and Puig-Domingo. This is an open-access article distributed under the terms of the Creative Commons Attribution License CC BY. The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.