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Treatment Strategies for Dopamine Agonist-Resistant and Aggressive Prolactinomas: A Comprehensive Analysis of the Literature

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ABSTRACT

Despite most of the prolactinomas can be treated with endocrine therapy and/or surgery, a significant percentage of these tumors can be resistant to endocrine treatments and/or recur with prominent invasion into the surrounding anatomical structures. Hence, clinical, pathological, and molecular definitions of aggressive prolactinomas are important to guide for classical and novel treatment modalities. In this review, we aimed to define molecular endocrinological features of dopamine agonist-resistant and aggressive prolactinomas for designing future multimodality treatments. Besides surgery, temozolomide chemotherapy and radiotherapy, peptide receptor radionuclide therapy, estrogen pathway modulators, progesterone antagonists or agonists, mTOR/akt inhibitors, pasireotide, gefitinib/lapatinib, everolimus, and metformin are tested in preclinical models, anecdotal cases, and in small case series. Moreover, chorionic gonadotropin, gonadotropin releasing hormone, TGFβ and PRDM2 may seem like possible future targets for managing aggressive prolactinomas. Lastly, we discussed our management of a unique prolactinoma case by asking which tumors' proliferative index (Ki67) increased from 5–6 % to 26 % in two subsequent surgeries performed in a 2-year period, exerted massive invasive growth, and secreted huge levels of prolactin leading up to levels of 1 605 671 ng/dl in blood.

Introduction

Approximately 10–15 % of all manifest intracranial tumors originate from the hypophysis (pituitary gland) [1]. Prolactinomas are benign neoplasms (adenoma) that produce prolactin [2]. Prolactin can be secreted by various pituitary neuroendocrine tumors deriving either from somatotrophs or mammo-somatotroph cells besides the lactotroph cells [3]. Similarly, densely or sparsely granulated lactotroph tumors, poorly differentiated Pit1-lineage tumors and acidophil stem cell tumors can also cause hyperprolactinemia [3]. Prolactinomas – originating from lactotroph cells – are the most frequently hormone-secreting pituitary tumors (approximately 30–40 % of all pituitary tumors), and its prevalence is about 94 per 100 000 inhabitants [2]. Prolactinomas are much more frequently diagnosed in female population with a sex ratio around 10:1 before the fifth decade, but after this age, the prolactinoma frequency is about the same in both gender [3]. This shows that female hormones may propagate prolactinoma growth; and indeed, there exist substantial evidence supporting this hypothesis, which will be discussed below. But seemingly paradoxical at the first glance, prolactinomas appearing in boys, at a young age (< 20 years), and/or with accompaniment of a genetic predisposition have worse prognosis [4]. The most frequent clinical signs of prolactinomas are gonadal and sexual dysfunction and subsequent infertility in both sexes [2]. Dopamine agonists, such as bromocriptine and cabergoline, are employed for treatment in prolactinomas since prolactinomas express high levels of dopamine receptors (D2R) [2]. When the patients cannot tolerate dopamine agonists or are refractory to medical therapy, they undergo surgical treatment and/or radiotherapy [5, 6]. Ten to 15 % of patients are resistant to medical treatment. Twenty-five percent of the patients receiving bromocriptine treatment fail to normalize prolactin whereas it is only 10–15 % in those receiving cabergoline [6]. Almost half of tumor volume reduction is encountered in 33 % percent of those receiving bromocriptine and 10–15 % of those treated with cabergoline [6]. Resistance to cabergoline is defined by the absence of prolactin normalization or lack of the tumor to reduce in size by 50 %, and these refractory prolactinomas tend to exert higher angiogenesis, cell proliferation and atypia and invasiveness [5]. Quinagolide is a non-ergot-derived D2R-selective agonist, which could provide significant reduction in tumor size and prolactin levels in around 90 % of patients [3]. Quinagolide is usually given at the average dose of 150 – 300 µg/day and compared to bromocriptine, more prominent reduction in dizziness, nausea, vomiting, and drowsiness is generally witnessed [3].

Most patients who respond well to dopamine agonists with reduction of prolactin levels exert evident decreases in tumor volumes, but not all do. On the contrary, some patients may experience almost total reductions in tumor volumes unaccompanied with normalizing prolactin levels [6]. Resistance mechanisms include lowered D2R (dopamine receptor D2) gene transcription, lowered receptor activity which regulate D2R expression, and lowered inhibitory G protein-expression which couples the D2R to adenylyl cyclase [5, 6]. Prolactinomas are classified and treated according to their size, that is, microprolactinomas (< 1 cm) do not generally invade the adjacent anatomic structures, while macroprolactinomas (> 1 cm) tend to locally invade and compress surroundings. Surgical cure rates for invasive macroprolactinomas are meager, and even if resected, larger prolactinomas tend to recur [2]. Here, data regarding molecular endocrinology of treatment resistant and aggressive prolactinomas are reviewed. Prolactinomas developing at a young age are correlated to higher proliferation and invasion. Other features, including the expression of growth factors such as VEGF (vascular endothelial growth factor) and EGF (epidermal growth factor), adhesion molecules (E-cadherin), the genes regulating proliferation, invasion and differentiation, matrix metalloproteinase 9, and chromosome abnormalities (chromosomes 1, 11, and 19) also correlate with aggressiveness [4].

Evidence Acquisition and Synthesis

To obtain data on the pathogenesis and treatment of aggressive prolactinomas, the following keywords were searched in PubMed database: prolactinoma AND (invasive OR aggressive OR malignant) AND (chemotherapy OR radiotherapy OR molecular OR gene OR genetic), which yielded 2288 results (Last Check: 11th May, 2021). Based on the obtained results from PubMed data search, we mostly focused on molecular endocrinological features of these tumors since they are clinically targetable. The role of conventional treatments including temozolomide chemotherapy and radiotherapy is also analyzed. At first, we will define the entities of “invasive”, “aggressive” and “malignant” prolactinomas and pituitary carcinomas, their anatomical spread mechanisms and conventional treatment modalities. Then, we will discuss molecular endocrinological features of these tumors as potential treatment targets.

Are the Invasive Prolactinomas, Aggressive Prolactinomas, Malignant Prolactinomas, and Pituitary Carcinomas Different Entities? Anatomy and Immunohistopathology

Invasive prolactinomas are prolactin secreting tumors invading adjacent anatomical structures, while aggressivity determines not only the invasion but also medical treatment-resistance and high tendency for recurrence. It is recommended the term “aggressive” should not be used synonymously with “invasive” as aggressivity encompasses a broader biologically ominious behavior including drug resistance and occasionally atypical histology besides invasiveness [5]. In general, invasiveness is mostly a radiological and aggressiveness a clinical definition [7]. Invasive prolactinomas are tumors with proven invasion into adjacent structures, including cavernous and sphenoid sinuses and bony structures, which can be defined radiologically with preoperative MR investigations, during operation, or with histopathological demonstration of tumor spread to the bone, dura, or nasal mucosa [7]. A relatively recent study demonstrated that prolactinomas constitute most invasive pituitary tumors [7]. In our index case, which will be discussed below, extensive cavernous sinus, prepontine area and pontocerebellar edge invasion and encasement of internal carotid artery, CV, CVII, and CVIII nerves were detected. According to World Health Organization’s (WHO) recent criteria (2016), increased mitotic index, Ki67 labeling index greater than 3 % percent and robust p53 expression indicate the aggressivity of pituitary adenomas [8]. But it shall be also emphasized that there exist conflicting results between Ki67 indices with invasiveness of pituitary tumors [7]. The tumor suppressor *TP53* gene encodes the transcription factor p53,

which is a genomic "gate keeper" that initially blocks cell cycle and succeedingly induces DNA repair following genomic damage. However, when the DNA injury is irreversible, it induces apoptosis of the cells, thereby eliminating the spread of mutations into the cell progeny [9]. Wild type p53 protein exists in benign cells, but due to its very relatively half-life, p53 is present in diminutive amounts, even undetectable by immunohistochemistry (IHC), whereas accumulated mutant p53 protein are detectable by IHC. Therefore, positivity of p53 expression is associated with p53 mutations [9]. It was shown that non-invasive and invasive adenomas and pituitary carcinomas exerted expression of p53 in 0, 15.2, and 100 % of cases, respectively, which correlated with invasion in a recent study [7]. In addition, it was shown that non-functioning pituitary adenomas are non-reactive for p53, while functional pituitary adenomas express p53, and functioning adenomas have more aggressive features than nonfunctioning adenomas including immunoreactivity for p53, S100, prolactin and MGMT (methylguanine methyltransferase) [7]. Basaran et al. defined the fraction of MGMT-immunopositive tumor cells among pituitary adenomas according to the following score: –, no positive tumor cells; + , < 10 % positive tumor cells; ++ , 10–50 % positive tumor cells; +++ , > 50 % positive tumor cells, regardless of intensity and showed that MGMT expression correlated with invasiveness [7].

There exists a spectrum of medication resistance and it is considered that most treatment-resistant prolactinomas are not carcinomas [5]. The single criterion to define a prolactinoma as "malignant" is the presence of distance metastasis according to the WHO 2016 classification. Peculiarly, it is not necessary that "malignant prolactinomas" exert histopathological features associated with malignancy (high mitotic rates, necrosis etc.) and prolactinomas with completely benign morphological appearance can also metastasize [10]. Therefore, the definition for malignancy of pituitary tumors is a debated issue as histological features, immunohistochemistry, or even electron microscopical features cannot distinguish a malignant pituitary adenoma unless metastases develop [10]. Nonetheless, some researchers suggest that p53 immunopositivity indicating *TP53* gene mutation tend to be more frequent among malignant prolactinomas [11]. Malignant prolactinomas may metastasize to bony skeleton, lymph nodes, lung, liver, and ovaries [11]. Pituitary carcinomas invade of adjacent structures and exhibit prominent cell proliferation and are defined by the existence of craniospinal and/or systemic metastases. Moreover, besides these features, they also exert histopathological features of malignancy. Pituitary carcinomas account nearly 0.1–0.2 % of all pituitary tumors with an average survival period less than 4 years [8]. Pituitary carcinomas possess a higher Ki-67 labeling index (higher than 11 %). Unexpectedly, there are reports of pituitary carcinomas with lower Ki67 indices, indicating that there exist certainly other factors that contribute to malignant potential features [5]. The existence of nuclear pleomorphism and high rates of mitosis should raise suspicion for pituitary carcinoma [5].

Anatomical Mechanisms of Prolactinoma Spread

For the anatomical mechanisms of metastasis, several mechanisms are considered: spread through dural venous channels, metastases through blood and lymphatic metastases [10]. Subarachnoid dissemination during surgery or spontaneously are also presumed as responsible mechanisms. Some authors prefer the term "metastases" only for extracranial spread, while they accept the term "seeding deposits" for cerebrospinal metastases [10]. Nonetheless, some early studies suggested that the disease course does not support such a distinction, because hematogenous and cerebrospinal fluidborne metastases exert equally malignant behavior [10]. It is also assumed that intraoperative dispersion of neoplastic cells into the subarachnoid space may occur in patients operated by transcranial or even transsphenoidal approach for pituitary macroadenoma. Nonetheless, metastases develop rarely and approximately 50 % of cases with systemic metastases had no surgical treatment and the same may be relevant for intraoperative dispersion of tumor cells via the blood stream [10]. Taking the high percentage of locally invasive tumors invading venous structure bone and dura (ranging between 10 to 42 % percent) into account, it might be considered that tumor cells enter the circulation in more patients than in those who do develop metastases [10]. Conceivably, it is more logical to assume that factors that enhance survival and implantation of the tumor cells play a greater role in metastatic spread [10].

Classical Treatment Modalities of Dopamine Agonist-Resistant Prolactinomas

Surgical Treatment

Before the development of dopamine agonists, surgery was the main treatment for the management of a prolactinoma [3]. Currently, the main indications of surgery are resistance to medical treatment, pituitary apoplexy, and the patients personal choice. Even in patients with visual defects due to a macroprolactinoma, dopamine agonists constitute the standard of care as compared to surgery [3]. In highly trained hands, adenomectomy normalizes prolactin levels in 75–90 % of microprolactinomas. In invasive macroprolactinomas, the surgical cure is achieved in about 40 % of cases with recurrence rates about 20 % for 10 years [3]. Even in an expert pituitary center, surgery for macroprolactinomas can be complicated by anterior pituitary deficiencies in 1–15 %, cerebrospinal fluid leak in 2–10 %, and diabetes insipidus in around 5 % of cases, respectively [3].

Radiotherapy

Radiotherapy is generally employed in conjunction with surgical treatment to manage recurring and/or aggressive pituitary adenomas [12]. Radiotherapy aims to slower or block growth of tumors and normalize prolactin levels which include stereotactic radiosurgery (SRS) and external beam radiation therapy (EBRT) [12]. Both approaches are almost equivalent in reducing prolactin levels (34.1 % for EBRT vs. 31.4 % for SRS). SRS is preferably applied as its three dimensional approach enables faster correction of prolactin oversecretion and reduced risks of carotid stenosis and radiotherapy-associated secondary malignancies [3]. Amongst SRS, GKRS (gamma knife radiosurgery) provides a highly selective conformal intervention in a single application using a linear particle accelerator or multiheaded cobalt unit and performed with image guidance [3]. On the contrary, the conformal radiotherapy is applied with

several fractions over time (general administration on a daily basis) [3]. According to some observers, prolactinomas' radiation-sensitivity is not high, with one study demonstrating only an 18 % remission rate at 4 years for prolactinomas treated with SRS [12]. On the other hand, another study revealed that Gamma Knife SRS normalized hyperprolactinemia in 50 % of cases with medication-refractory prolactinomas, yet cavernous sinus invasion was a predictor of treatment failure to normalize prolactin levels [12]. A more recent study reported good outcomes with GKRS in treatment of prolactinomas [13]. Indications for GKRS were (i) dopamine agonist resistance (17 patients), (ii) intolerance to dopamine agonists (5 patients), or (iii) attempts to reduce the length of treatment and/or the dosage of dopamine-agonist treatment (6 patients). After GKRS, normal prolactin level was achieved in about 82 % of patients, out of which hormonal remission (normal prolactin levels after discontinuation of dopamine agonists) was achieved in 13 (46.4 %), and endocrine control (normal prolactin levels while taking dopamine agonists) in 10 (35.7 %) patients [13]. GKRS blocked adenoma growth or reduced adenoma size in all cases.

Temozolomide

Temozolomide is an alkylating chemotherapeutic, which is a dacarbazine derivative with lipophilic properties enhancing its traversal through the blood-brain barrier [14]. Temozolomide efficacy was first documented in glioblastomas before its employment in treatment of neuroendocrine neoplasias and melanomas. Chen et al. described a 17-year-old male patient admitted with an aggressive prolactinoma that progressed despite surgery, gamma-knife, and dopamine agonists, which responded well to temozolomide treatment with a marked reduction of tumor mass, decrease of prolactin secretion and progressive clinical improvement [8]. The authors also underlined the presence of other aggressive prolactinoma cases which responded to temozolomide treatment and indicated that MGMT expression status is important in response to temozolomide like the situation encountered for high grade glial tumors [8]. In our case, which will be discussed below, MGMT expression was encountered which may associate with temozolomide resistance. Tang et al. reported a prolactinoma patient who was refractory to cabergoline treatment even at high doses, exerting a continuous enhancement in both the prolactin levels and the tumor volume [15]. The patient was treated with two consecutive transsphenoidal surgeries and the pathological examination revealed that the Ki67 index increased from 3 % to 30 %, and the expression levels of DRD2 and MGMT were low. The increase of Ki67 index is exactly similar to what we have observed in our index case discussed below. Following six cycles of temozolomide chemotherapy, the tumor first shrank and then vanished completely. During the 6-month follow-up, the tumor did not recur, and the prolactin level did not rise [15]. Halevy and Whitelaw reported that temozolomide might be a suitable option in aggressive pituitary adenomas and carcinomas [16]. They reviewed the published case series and concluded that 42 % of patient responded on radiographs, and 27 % of patients stabilized succeeding temozolomide. Prolactinomas and corticotroph adenomas responded to temozolomide with an approximately a 50 % response rate, but non-functioning tumors responded only half as frequently [16].

Strowd et al. reported a prolactinoma case, which showed clinical and radiographic progression, despite treatment with bromocriptine, transphenoidal surgical resection, radiation therapy, and cabergoline [1]. Temozolomide treatment was began 6 years after diagnosis and after three cycles of treatment, dramatic radiological and clinical responses were witnessed with 99.3 % reduction in prolactin levels [1]. After 3 years of follow-up, the patient again developed radiological progression and increase of prolactin levels. The patient was rechallenged with temozolomide, and following four cycles, radiological, hormonal, and clinical responses were observed with a 92.2 % decline in prolactin levels [1]. Development of temozolomide resistance was defined during transition of an atypical prolactinoma to a prolactin-producing pituitary carcinoma, which was associated with loss of DNA mismatch repair protein MSH6 in carcinoma [17]. European Society of Endocrinology Clinical Practice Guidelines advised the employment of temozolomide as first-line chemotherapy for aggressive pituitary adenomas and carcinomas in 2017 [18]. However, it is also possible that pituitary adenomas develop acquired temozolomide resistance following initial responses to temozolomide treatment [18]. We witnessed a similar situation in our index case which will be mentioned below.

Peptide Receptor Radionuclide Therapy

Little data exists on peptide receptor radionuclide therapy (PRRT) for the management of aggressive pituitary tumors [19]. Giuffrida et al. analyzed the safety, efficacy and long-term outcome of PRRT in three patients with aggressive pituitary tumors and also reviewed the available literature [19]. First patient (female, giant prolactinoma) was treated with five cycles of ^{111}In-DTPA (diethylenetriamine pentaacetate)octreotide (total dose 37 GBq) for 23 months, following inefficient surgery and long-term treatment with dopamine-agonist [19]. Second patient (male, giant prolactinoma) was treated with two cycles of ^{177}Lu-DOTATOC (DOTA^{0}-Phe^{1}-Tyr^{3}) octreotide (12.6 GBq) following multiple surgeries, radiosurgery and temozolomide [19]. In the third patient (female, non-functioning pituitary tumor), five cycles of ^{177}Lu-DOTATOC (29.8 GBq) was applied after five surgeries, radiation treatment and temozolomide. First patients tumor shrank and neurological and visual amelioration was witnessed over 8-year follow-up, while the other two pituitary tumors continuously grew causing amaurosis and neuro-cognitive disorders [19]. PRRT was not associated with any adverse systemic effects. The investigators found eleven other cases of PRRT-treated aggressive pituitary tumors from the literature. When they included the patients from the literature, 4 of 13 patients exerted tumor shrinkage and biochemical or clinical amelioration of symptoms after PRRT. Responses did not associate with age or gender, neither with the employed peptide/radionuclide, but PRRT failure was associated with failure of previous temozolomide chemotherapy [19]. Adverse effects were noted only in two patients. The authors concluded that PRRT is a safe option following failure of multimodal treatment [19].

Molecular Pathogenesis of Prolactinomas Important to Design Future Treatments

Estrogen

The major regulators of lactotroph functions are estradiol and dopamine (DA) which interact in controlling cell proliferation and prolactin secretion [20]. In prolactinomas, the main related transcription factors are estrogen receptor-α (ERα) and pituitary transcription factor 1 (PIT1) [21]. Estradiol exerts its cellular functions via specific nuclear receptors, ERα and ERβ, via genomic and nongenomic pathways [21]. ERα66 is the most commonly encountered type of Erα and a variant of ERα, known as ERα36, is formed from the promoter residing within the first intron of ERα66. Unlike ERα66, ERα36 does not harbor AF1 and AF2 transcriptional activation domains for binding to estradiol and coactivators [21]. Dimerization and DNA-binding domains and a certain part of the ligand-binding domain still exists in ERα36. In opposite to ERα66, ERα36 mainly localizes in both the cytoplasm and plasma membrane and exerts nongenomic estrogen signaling [21]. Mahboobifard examined the expression of Ki67, p53, ERα36 and ERα66 by immunohistochemistry in 62 patients with prolactinoma patients and in normal pituitaries [21]. A salient expression of ERα36 was determined in normal pituitaries. The median scores of ERα66 and ERα36 expression were 6 and 8 in normal pituitaries and 0 and 4 in tumors, respectively. Low expression of ERα36 was associated with higher Ki67 indices and more prominent tumor invasion [21]. Low ERα66 expression was also associated with tumor invasion, increased tumor volumes and dopamine-agonist resistance. After controlling for sex, the low ERα36/low ERα66 phenotype was 6.24 times more prevalent in invasive prolactinomas than in noninvasive prolactinomas [21]. The authors have underlined that these associations are relevant mostly for macroprolactinomas and could differ for microprolactinomas. They attributed the downregulation of estrogen receptors (ERs) to two possible and opposite mechanisms. At first, increased estrogen activity may subsequently downregulate ERs; second, decreased ERs may associate with reduced estrogen-induced apoptosis in lactotroph cells [21]. Li et al. defined a somatic mutation in $SF3B1^{R625H}$ (splicing factor 3 subunit B1) in about 20 % of prolactinomas which associates with higher prolactin levels and shorter progression free survival [22]. Importantly, $SF3B1^{R625H}$ mutation leads to erroneous splicing of estrogen related receptor gamma (ESRRG), a steroid hormone receptor which binds to tamoxifen metabolite 4-hydroxytamoxifen (an estrogen antagonist) and diethylstilbestrol (nonsteroidal estrogen) [22]. It remains to be elucidated whether estrogenic pathways associate with perturbed signaling cascades induced by SF3B1 mutations.

Dopamine inhibits proliferation of lactotroph cells, prolactin synthesis and release, acting via the D2R expressed in lactotroph cells [20]. Estrogen hormones may contribute to pathogenesis of prolactinomas, as these tumors grow faster during pregnancy and develop in transsexual men under estrogen treatment [23, 24]. Estradiol and estrogens in general decrease the effects of dopamine agonists, induce prolactin gene transcription and indirectly lower dopamine synthesis from the hypothalamus [6, 20, 25]. Estrogens also directly stimulate mitotic activity, suppress lactotroph cell apoptosis and modulate the blockage of dopamine on prolactin gene transcription via a decrease of D2Rs on the lactotroph cell membrane [6]. In mouse models, estrogen interacts with bone morphogenic protein 4 (BMP-4) and Smad4 to trigger enhanced cell growth [5]. In parallel, high levels of estrogens during gestation cause lactotroph hyperplasia, hyperprolactinemia, and growth of tumors, nonetheless; usually in the lack of simultaneous treatment with dopamine agonists [6]. In cell culture models of prolactinomas, 17β-estradiol induces calbindin-D9k (CaBP-9k) expression, proliferation, and apoptosis inhibition via interacting with ERα which can be inhibited with ERα inhibitor AZD9496 [26]. Nonetheless, it shall be also noted that prolactinomas in men exert lower estrogen receptor alpha (ERα) expression related to treatment resistance, higher tumor grades and worse prognosis [27]. Moreover, even in prolactinomas induced by estrogen treatment, pharmacological high-dose levels of estrogen can induce tumor regression, suggesting a dichotomic effect of estrogens in prolactinoma growth [4].

Increased aromatase expression (which catalyzes synthesis of estrogen from testosterone) was revealed in invasive prolactinomas in post-menopausal women, in comparison to its expression in noninvasive prolactinomas [28]. ER-β level was also significantly higher in patients resistant to bromocriptine [28]. For medically refractory prolactinomas, lowering endogenous estrogen with aromatase inhibitors or employment of selective estrogen receptor modulators (SERMs) are treatment candidates [6]. Indeed, there exist some anecdotal reports of prolactin lowering with aromatase inhibitors anastrozole and letrozole [27]. In experimental models, tamoxifen hinders the estrogen-stimulation of prolactin secretion and blocks growth of prolactinoma in vitro and in vivo with minimal interaction with bromocriptine. Moreover, few patients with ''bromocriptine-resistant'' invasive macroprolactinomas respond with reductions in tumor volumes and prolactin levels with tamoxifen treatment [6]. Raloxifene, a SERM employed to treat osteoporosis, decreased prolactin levels significantly in comparison to placebo in a pilot study conducted on healthy postmenopausal women. Hence, reducing estrogen signaling with such agents may be employed in future [6]. In another study, raloxifene decreased prolactin levels with a mean percentage of 25.9 % (8 – 55 %) in 10/14 (71 %) patients with prolactinoma who were treated with stable doses of dopamine agonists including 2 cases (14 %) who achieved normoprolactinemia [29]. Another evidence indicating the importance of estrogenic pathways comes from the studies which employed fulvestrant in treatment of prolactinomas in rats. Fulvestrant is a selective estrogen receptor degrader (SERD) which binds and destabilizes the estrogen receptor, leading the cell's inherent protein degradation cascades to degrade it. Cao et al. established prolactinomas in rats with estrogen and when they treated these rats with fulvestrant, they revealed that tumor volumes, weight and blood prolactin levels were substantially reduced in time- and dose-dependent manners [30]. Fulvestrant also downregulates Pyruvate Kinase-M2 and inhibits glycolytic energy production in prolactinoma cells and induces their apoptosis in accompaniment with reductions in XBP1, IRE1 and GRP78 levels [31, 32]. There also exists evidence that estrogen and mTOR pathways may synergize in development of prolactinomas. In rats, estrogen treatment induced prolactinomas with concomitant increase of mammalian target of rapamycin (mTOR) signaling, which was blocked by mTOR inhibitor rapamycin [33]. Pituitary knockout of either

mTOR negative regulators Tsc1 or PTEN also led development of prolactinomas, which were blocked by rapamycin [33]. Hence, it would not be illogical to presume that estrogen antagonists and mTOR inhibitors may act synergistic in inhibition of prolactinoma growth. However, it shall be also noted that clinical data regarding employment of estrogen modulators in treatment of aggressive prolactinomas is limited [27].

Intersecting Cascades of TGFβ, Progesterone, and Chorionic Gonadotrophin

TGFβ1 inhibits proliferation of lactotroph cells and secretion of prolactin, and it partly mediates the inhibitory action of dopamine [34]. TGFβ1 is secreted to the extracellular millieu as an inert complex, and its bioavailability is tightly regulated by diverse elements of the TGF-β1 system which involve latent binding proteins (LTBPs), local activating factors (matrix metalloproteases, integrins, Thrombospondin and others), and TGFβ receptors [34]. Activity of pituitary TGFβ1 and the expression of varying parts of the TGFβ1 cascades, are controlled by estradiol and dopamine. According to some investigators, prolactinomas (both in animals and humans) harbor lower TGFβ1 activity as well as decreased expression of diverse constituents of the TGFβ1 system [19]. Hence, it is assumed that the reactivation of TGFβ1 inhibitory potential would provide a new therapeutic approach to bypass medication resistance in prolactinomas [34]. On the other hand, there also exists data showing higher levels TGF-β1/Smad3 signaling pathway-related proteins in dopamine agonist-resistant prolactinoma specimens exerting high fibrosis; and the reversal of fibrotic and drug resistance pathways with TGFβ1/Smad3 signaling inhibitor SB431542 [35]. Female mice transgenically overexpressing the human chorionic gonadotrophin β subunit (hCGβ +) develop prolactinomas, whereas hCGβ + male mice do not [20]. Faraoni et al. revealed lower TGF-β1 levels, lower expression of TGFβ1 receptors and its target genes including *Ltbp1*, *Smad4*, and *Smad7* in hCGβ + female pituitary tissues [20]. Nonetheless, no differences were detected between the wild-type and transgenic male pituitaries, which were attributed to the fact that the lowered pituitary TGF-β1 activity in hCGβ + females cause the development of prolactinomas. Indeed, they also showed that an in vivo treatment employed to increase pituitary TGFβ1 activity succeeded in decreasing the development of prolactinomas and hyperprolactinemia in hCGβ + females [20]. Another finding was that the high amounts of hCG in circulation triggered luteinization in the ovary of β-hCG + females, and progesterone became the main steroid synthesized, but estradiol levels remained at normal ranges [20]. These findings concurred to the previous observations of Ahtiainen et al. [25]. The authors analyzed the endocrine pathogenesis of prolactinomas in female transgenic mice expressing the β-hCG. The LH/CG levels were increased in the mice, with subsequent stimulation of progesterone synthesis in ovaries [25]. Despite normal levels of estrogen, these mice developed large prolactinomas and progesterone involvement in prolactinoma pathogenesis was shown with several lines of evidence. The progesterone-antagonist mifepristone blocked prolactinoma growth and postgonadectomy estradiol + progesterone combined treatment was more potent than single estrogen in stimulation of prolactinoma tumorigenesis [25]. Although estrogen was not increased in hCGβ-transgenic mice model, these findings highly suggest that both female hormones may accelerate prolactinoma tumorigenesis. Further evidence for direct growth-stimulatory effect of progesterone was encountered in primary mouse pituitary cell and rat somatomammotroph GH3 cell cultures [25]. In cultured cells and tumors of the mice, progesterone stimulated the cyclin D1/cyclin-dependent kinase-4/retinoblastoma protein/transcription factor E2F1 cascades [25].

Janus Faces of Progesterone in Prolactinoma Growth. Role of Membrane Progesterone Receptors (mPRs)

Above, we indicated the studies which showed progesterone stimulation of prolactinoma growth. Nonetheless, there also exists paradoxical opposite data. Membrane Progesterone Receptor-α (mPRα) is highly expressed in the rat hypophysis and primarily in lactotroph cells, mediating progesterone's inhibitory actions on secretion of prolactin [36]. Importantly, expression of nuclear PRs and mPRs is significantly lesser in tumoral pituitary tissues in comparison to benign ones and the relative proportion of mPRα and mPRβ is significantly higher in prolactinomas. A selective mPR agonist (Org OD 02-0) significantly blocked prolactinoma release in both tumoral and normal hypophysis explants, exerting a more salient efficacy in tumoral pituitary tissues [36]. As progesterone also controls prolactinoma secretion indirectly via dopaminergic neurons, mPR involvement in this action was also studied. Noteworthy, the hypothalamus highly expresses mPRs and both OrgOD 02-0 and progesterone enhanced dopamine release in hypothalamus explants. Moreover, in an in vivo experiment, the mPR agonist robustly lowered the hyperprolactinemia in transgenic females harboring prolactinoma [36]. Therefore, progesterone may exert dichotomic effects on prolactinoma growth. Hence, if progesterone antagonists or agonists will be employed in treatment of aggressive or malignant prolactinomas, each of these agents shall be tested in primary cultures of aggressive prolactinoma tissues, whether they exert risks of tumor propagation or whether they potently inhibit tumor growth.

GnRH (Gonadotropin Releasing Hormone)-Signaling Pathway

Overexpression of proto-oncogenes encoding proteins driving cell cycle-progression, growth factors or receptors were detected in prolactinomas including high mobility group A2 gene and FGF receptor-4 [5]. Zhao et al. analyzed gene expression profiling to determine differentially expressed genes (DEGs) between prolactinoma (n = 4) and normal (n = 3) samples [2]. They revealed that the DEGs were enriched in 15 Gene Ontology (GO) categories in which the GO category "developmental process" ranked first and "system development" ranked second. Two pathways including gonadotropin -releasing hormone (GnRH)-signaling pathway and neuroactive ligand-receptor interaction was determined. β-LHB (luteinizing hormone β subunit) and β-FSH (follicle stimulating hormone β subunit) were downregulated in prolactinoma, which both involve in the GnRH (Gonadotropin Releasing Hormone)-signaling pathway [2]. GnRH is a tropic hormone produced and released from GnRH neurons within the hypothalamus which is responsible for the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

Somatostatin

Immunohistochemical analysis of somatostatin receptors (SSTR) demonstrated that all SSTR types exist in prolactinomas; SSTR5 were mostly frequent, followed by $SSTR_{2A}$ and $SSTR_1$[27]. The somatostatin receptor subtype 5 (SSTR5) is the most regulator subtype in modifying prolactin secretion and among the FDA-approved somatostatin analogs, only pasireotide has prominent activity at SSTR5 [6]. In cell culture, three medication-resistant prolactinomas responded to pasireotide; and hence, pasireotide application might be tested in a patient failing all other medical modalities. There also exist anecdotal case reports demonstrating the efficacy of pasireotide to achieve reductions of tumor volumes and prolactin levels in resistant prolactinomas, sometimes with dramatic responses [27, 37, 38].

Epidermal Growth Factor Receptor (EGFR) Dependent Cascades

The epidermal growth factor receptor (EGFR) family comprises transmembrane tyrosine kinase receptors including EGFR (ErbB1, HER1), p185ErbB2/neu (ErbB2, HER2), ErbB3 (HER3), and ErbB4 (HER4) [39]. Ligand binding triggers the assembly of receptor homo- and heterodimers, activation of the intrinsic kinase domain and subsequent intracellular signaling [39]. Enhanced expression of ErbB receptors has been demonstrated in aggressive pituitary tumors and carcinomas. EGFR, ErbB receptors, p185her2/neu, ErbB3, and ErbB4 were shown to associate with tumor progression and an enhanced de-differentiated state in prolactinomas. ErbB receptors and ligands are also synthesized by nontumoral lactotrophs and stimulate prolactin secretion [39]. Mixed lacto-somatotroph tumors express ErbB receptors and ligands, and interfering with these pathways modifies prolactin secretion, and tumor size [39]. Lactotroph tumor cascades blocked by tyrosine kinase inhibitors (TKI) provide evidence that ErbB receptors stimulate prolactin secretion and growth of lactotroph cells [39]. Gefitinib binds the ATP-binding site of EGFR leading selective blockage of the EGFR activity, lapatinib binds the ATP-binding of the EGFR/HER2 protein kinase domain and hinders their activation and canertinib is a pan ErbB-inhibitor [39]. Gefitinib reduced proliferation of rat GH3 somato-lactotroph cells and prolactin mRNA expression in vitro and xenograft tumor volume and prolactin release in vivo [40]. Importantly, lapatinib reduced prolactin mRNA and protein secretion from human prolactinoma cells in vitro. Cooper et al. comprehensively assessed expression of ErB receptors in prolactinomas and evaluated their association with their clinical features [39]. Expression of EGFR was detected in 82 % of adenomas, ErbB2 in 92 %, ErbB3 in 25 %, and ErbB4 in 71 % [39]. Enhanced ErbB3 expression associated with optic chiasm compression, suprasellar extension and carotid artery encasement. Yet, medication-response rates were significantly higher in tumors with higher expression of ErbB3. Cooper et al. treated two subjects with aggressive resistant prolactinoma with lapatinib 1250 mg daily for 6 months [39]. Tumor sizes and prolactin levels were reduced with lapatinib treatment and the authors also suggested the possibility that D2R-targeting classical medications may synergy with lapatinib [39]. In 2021, the same group published their experience on lapatinib, which was applied to 4 patients with drug resistant prolactinoma; they witnessed that 3 patients had disease stabilization, with 2 exerting a 6 % enhancement and 1 exerting a 16.8 % reduction in tumor diameter [41].

PRDM2 (PR/SET Domain 2 / RIZ1-Retinoblastoma Interacting Zinc Finger protein-1)

PRDM2 is a tumor suppressor gene which is a member of the nuclear histone methyltransferase superfamily and encodes a zinc finger protein, which binds to ER, retinoblastoma protein, and the TPA-responsive element of the heme-oxygenase-1 gene [42]. Gao et al. analyzed the exomes of six drug-responsive prolactinomas and six drug-resistant prolactinomas by whole-exome sequencing [23]. They identified ten somatic variants that regulated metabolic cascades and DNA repair including PRDM2. Quantitative gene expression analysis with RT-qPCR revealed that PRDM2 mRNA levels were about five-fold lower in drug-refractory prolactinomas [43]. More importantly, restoration of PRDM2 expression increased D2DR levels, showed a synergistic action with bromocriptine to reduce prolactin secretion and MMQ prolactinoma cell line viability [43]. Hence, PRDM2 seems like an interesting candidate in prolactinoma treatment by being a member of the main gene-gene interaction hubs including ER.

miRNA Based Analyses Reveal the Potential for Drug Repurposal for Aggressive Prolactinomas

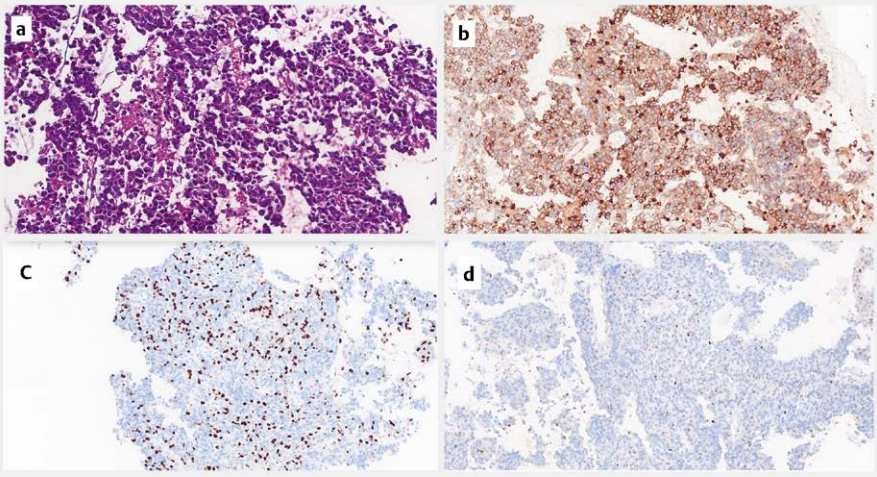
Aydin et al. analyzed the transcriptomic features of prolactinoma through mRNA and miRNA level data integration and repurposed novel drugs based on this integration [44]. They repurposed 7 drugs including 5-flourocytosine (an antifungal agent), nortriptyline (an antidepressant), neratinib (an antineoplastic used for breast cancer), puromycin (an aminonucleoside antibiotic), taxifolin (an anticancer flavonolol), vorinostat (an antineoplastic histone deacetylase inhibitor), and zileuton (an anti-asthmatic 5-lipoxygenase inhibitor) for the treatment of resistant prolactinoma [44]. They also analyzed effects of these drugs MMQ cell vitality via investigating PI3-Kinase/Akt signaling pathway and arrest of cell cycle via western blotting and flow cytometry [44].

Other Treatment Possibilities

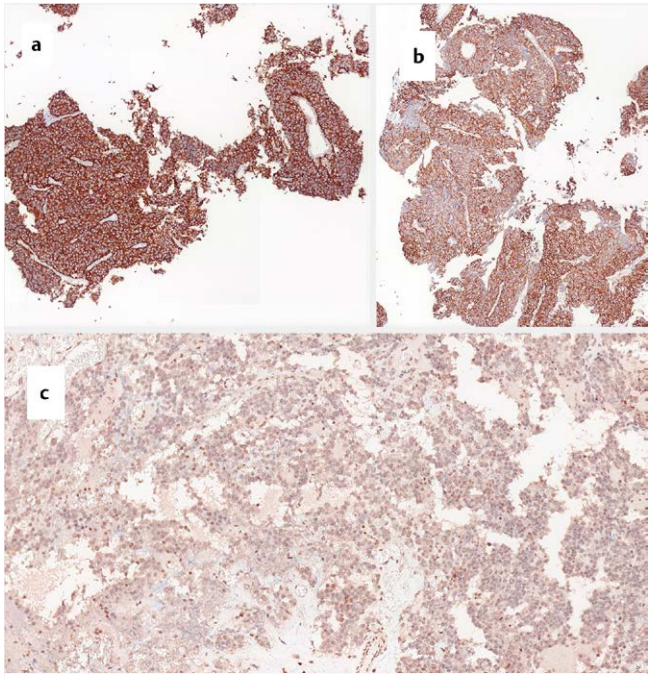
There exist anecdotal reports that metformin, an oral antidiabetic could normalize prolactin levels when it was used in combination with bromocriptine in bromocriptine-resistant prolactinomas [3]. There also exist in vitro and in vivo evidence that metformin – in combination with brocriptine – inhibits growth of prolactinomas [3]. mTOR signaling pathway is a promotor of tumor formation in pituitary tumors, more specifically in prolactinomas [45]. In vitro studies revealed that mTOR inhibitor rapamycin, could act effective in the management of prolactinomas [46]. There also exist clinical reports that mTOR/akt pathway inhibitor everolimus could normalize prolactin levels and reduce tumor volumes in dopamine agonist-resistant prolactinoma, which harbors high levels of p-AKT, p70S6K and p4EBP1 [47]. Everolimus can also inhibit growth of prolactinoma cells carrying prolactin receptor variants which overactivate akt pathway [48].

Clinical and Radiological Description of the Index Case

▶ **Figures 1, 2**, and **3** represent pathological features of the case and ▶ **Fig. 4** represents the radiological features of the case. A 51-years-old Caucasian female patient suffering from amenorrhea and galactorrhea was found to have macroprolactinemia in 2013



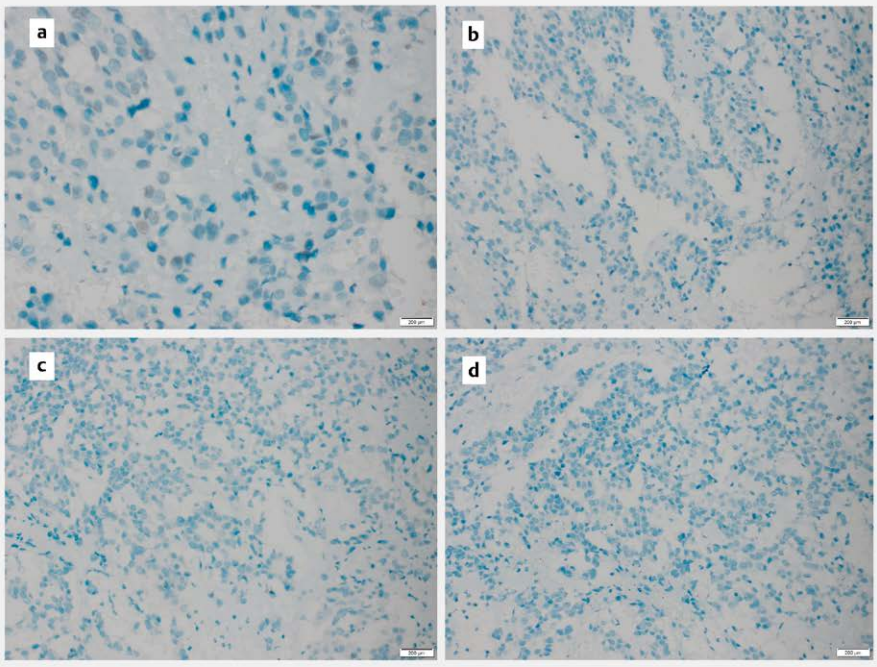
▶ **Fig. 1** Pathological features of the aggressive prolactinoma. **a**: The normal acinar structure of the pituitary gland is distorted and neoplastic cells reveal papillary configuration with prominent atypia, pleomorphism, and distinct nucleoli (100×). **b**: Neoplastic cells show a heterogeneous staining pattern with prolactin antibody (200×). Other hormonal immunohistochemical stains were negative. **c**: The Ki67 staining rate was as high as 25% (200×). **d**: Some of the cells reveal faint p53 positivity (200×).



▶ **Fig. 2** Pathological features of the aggressive prolactinoma. **a**: The tumor is synaptophysin positive; suggesting a pituitary adenoma (100×). **b**: The tumor shows cytokeratin positivity (100×). **c**: There was partial expression of MGMT (200×).

following examinations in an external center. Neurological examination was normal in her admission. Patient's laboratory and radiological examinations revealed prolactin levels above 200 ng/ml and the presence of a central/right paracentral-localizing pituitary adenoma with a size of 21 × 12 mm. Cabergoline treatment was initiated. During a 5 months follow-up there was a progressive increase in prolactin levels and cabergoline dose was substantially increased from 1 mg/week to 3.5 mg/week. Despite this treatment, prolactin levels elevated up to 5061 ng/ml and radiological investigations revealed an increased mass lesion reaching 3 × 2 × 4.5 cm. At this stage, left eye ptosis developed due to involvement of the CIII. In the January of 2014, a transsphenoidal pituitary adenomectomy was performed in an external center due to resistance to medical treatment and only 50% of the lesion could be surgically removed. Pathological examination revealed a prolactinoma (lactotroph adenoma) with a Ki67 labeling index of 5–6% and p53 staining ratio of 3% and no signs of anaplasia. Cabergoline treatment was continued. In May of 2014, gamma-knife radiosurgery was applied to the residual lesion at a dose of 19 Gy in one fraction.

Following these treatments, signs of the CIII involvement alleviated. In October of 2014, prolactin levels declined to 400 ng/dl. In her MR scans obtained in January and April of 2015, a regressing mass was observed when compared to initial lesion detected one year ago. Thereafter, the prolactin levels began to rise and cabergoline dose was gradually increased up to 8 mg/week. In June of 2015, the patient developed a traumatic intracranial hemorrhage following a fall at home. The patient was followed up conservatively. Yet the prolactin levels progressively increased to 2634 ng/dl, 3267 ng/dl and 6599 ng/dl. At this stage, cabergoline treatment was stopped and bromocriptin treatment was began, but the patient did not tolerate bromocriptine and cabergoline treatment was restarted. Cabergoline treatment was increased to 10 mg/week. Due to the aggressiveness of the lesion, no surgical options were advised by consulted centers. The patients vision deteriorated because of the right eye ophthalmoplegia due to tumor expansion, that was accompanied by high prolactin levels despite medical treatment. At this stage, the patient admitted to our neurosurgical clinic and a second transsphenoidal pituitary adenoma resection was performed in September 2016 by our neurosurgical team. Pathology revealed a sparsely granulated prolactinoma with



▶ **Fig. 3** Pathological features of the aggressive prolactinoma. **a**: The tumor was faintly positive for ER (400×). **b**: The tumor was negative for PR (200×). **c**: The tumor was negative for ErbB2 (200×). **d**: The tumor was negative for β-HCG (200×).

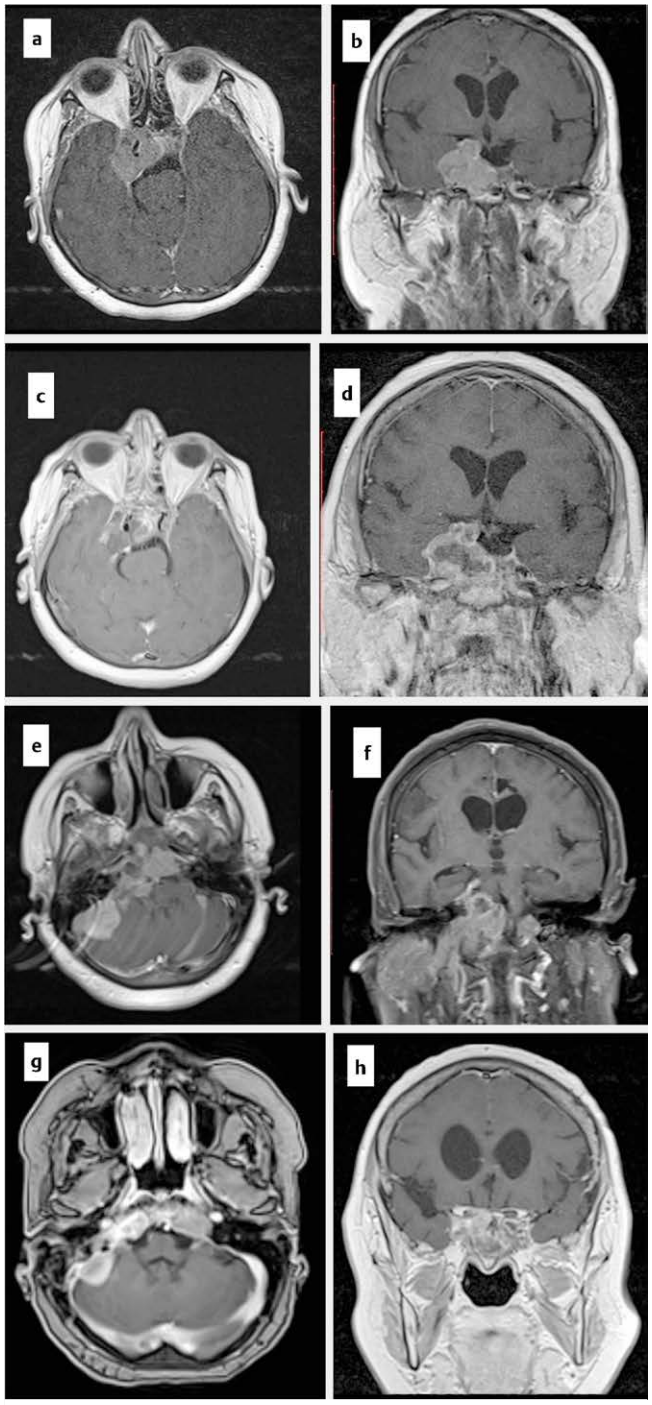
a Ki-67 labeling index of 26 %, p53 level of 2 % and positive MGMT immunoreactivity. Postoperative prolactin level was 2541 ng/ml. Gamma-knife radiosurgery was applied to the right side at a dose level of 15 Gy in one fraction in October 2016.

The patient's general condition was moderate and kept under cabergoline treatment until 2018 till gait problems and blurred vision appeared. Prolactin levels progressively increased during this period, and 5 cycles of temozolomide treatment were applied which started on January of 2018. Until May of 2018, the lesion was stable and prolactin levels did not rise under temozolomide treatment. However, massive progression was detected in cranial MRI examination performed in August 2018. The patient's general condition deteriorated. Difficulty in swallowing, intense pain during chewing, tingling in tongue, difficulty in speech and enhanced balance disorder developed. MRI revealed a residual mass lesion of 2 cm size on the right side of pituitary invading the cavernous sinus and the ICA (internal carotid artery). It was accepted as a de novo macroadenoma of 3 cm in size at the clivus extending to prepontine region and accompanied with another mass lesion of 11 cm size on the left ICA surrounding the cavernous sinus. The patient was discussed in a special tumor council for alternative treatment modalities.

Following explaining the possible risks to the patient and obtaining her consent, VMAT (Volumetric Arc Therapy) was applied to the tumor lodge at a level of 45 Gray at 25 fractions in September of 2018. The prolactin level declined from 14 000 ng/dl to 4800 ng/dl following radiotherapy. MRI revealed regression of total tumor mass two months after VMAT and the patient was followed under cabergoline treatment at a dose of 6 mg/week. In January of 2019, the prolactin level rose to 1 605 671 ng/dl, MRI revealed re-progression of old lesions besides development of novel lesions. A PET-CT analysis did not demonstrate systemic metastasis. The patient's treatment was planned as 30 mg cabergoline per 28 days by the medical oncology department, her general neurological condition improved, and the pain level decreased in February of 2019 following this treatment. In June of 2019, octreotide treatment was started due to minimal progression of the lesions in MR investigations. She was again referred to radiation oncology department due to prominent progression in February of 2020 and she received another series of Cyberknife stereotactic radiosurgical treatment (3500 cGy/10 fractions) to the tumor lodge which progressed. Regression of the mass lesion in prepontine, pontocerebellar edge and petroclival areas was observed (▶ **Fig. 4**). The patient is still under follow-up by neurosurgery and medical oncology departments.

Pathological Features of the Prolactinoma

In the specimen obtained from the second operation in September 2016, the following features are observed, that is, normal acinar structure of pituitary gland is distorted and neoplastic cells reveal papillary configuration with prominent cellular atypia, pleomorphism and distinct nucleoli (400×) (▶ **Fig. 1a**). Synaptophysin [Biocare (27612)] and pancytokeratin [Scytek (5d3lp34)] stainings were positive (▶ **Fig. 2a, b**, respectively). All neuroendocrine hormonal immunoassays (GH, ACTH, LH/FSH, TSH) were negative but neoplastic cells show disperse staining pattern with prolactin antibody [Genetex (b109.1)] (200×) depicting a sparsely granulated



▶ **Fig. 4** Radiological features of the aggressive prolactinoma.**a**: T1-weighted axial view of the MRI performed in August of 2016. Hyperintense mass lesion with high heterogeneous contrast uptake filling the cella at the right paramedian area, embracing the right cavernous sinus, extending to the prepontine cistern at the medial of the 5th cranial nerve in the posterior intracranial area.**b**: In T1-weighted coronal sections, extension of the lesion to the sphenoclival area and extension of the mass lesion to the base of the 3th ventricle and sphenoclival area is observed.**c**: T1-weighted axial and **d**: coronal view of the postoperative MRI performed in September of 2016 presented the internal decompression of the mass lesion. **e**: T1-weighted axial view of the MRI performed in February of 2019. Progression of the lesion with intense contrast uptake invading cavernous sinus, prepontine area and pontocerebellar edge. **f**: T1-weighted coronal view of the MRI performed in February of 2019. A very extensive invasion of the lesion was observed involving the cavernous sinus, prepontine and petroclival areas, 5th, 7th, and 8th cranial nerves with intense contrast uptake. **g**: T1-weighted axial view of the MRI performed in July 2020. Regression of the mass lesion in prepontine and pontocerebellar edge. **h**: T1-weighted coronal view in July 2020. Regression of the mass lesion in prepontine and petroclival areas.

prolactinoma (▶ **Fig. 1b**). Ki67 [DAKO (MIB-1)] labeling index was prominently high as 25 % (200 ×) (▶ **Fig. 1c**). Some of the cells showed faint reactivity to p53 [Scytek (do/7)] positivity (200 ×) (▶ **Fig. 1d**). Disperse MGMT [Novus (mt 23,2)] immunoreactivity was detected (▶ **Fig. 2c**). Estrogen receptor [ER (Leica 6F11)] was faintly positive in some cells (▶ **Fig. 3a**), while Progesteron receptor (PR) was negative (▶ **Fig. 3b**). c-ErB-B2 ▶ **Fig. 3b** (Ventana Her-2/neu 4B5)] and human-Chorionic Gonadotropin [h-CG (Biocare)] (▶ **Fig. 3d**) stains were also negative. Temporal changes in the radiological characteristics of the tumor are shown in ▶ **Fig. 4** and its respective features are described in the associated figure legend.

Dramatically, Ki67 indices of the tumor were calculated as 5 %-6 % and 25 % in consecutive specimens obtained from surgeries performed in January of 2014 and in September 2016, respectively. Hence, a transformation involving loss of cell cycle checkpoint and/or DNA repair genes may contribute to this bizarre phenomenon and such a high level of Ki67 may inherently associate with the aggressive tumor biology in this index case considering that even the Ki67 threshold for pituitary carcinomas is 11 % [4]. In our case, the recurred tumor did not show an extensive p53 expression. In our current case, more than 10 % of tumor cells stained with MGMT. As MGMT involves in the repair of temozolomide-induced DNA damage, the temozolomide resistance in our case may associate with the positive selection of MGMT-expressing tumor cells in a time dependent manner. The tumor stained very faintly with ER in very few cells; thus, an estrogen-antagonist treatment was not considered. The tumor did not stain either with b-HCG or PR; hence, a progesterone-antagonist (such as mifepristone) was not employed for treatment.

Conclusions

Despite most of the prolactinomas are typically benign, some of these tumors may follow a poor course like a malignant tumor. Prominent cellular atypia, pleomorphism, and distinct nucleoli may be encountered in aggressive prolactinomas, whereas even metastatic malignant prolactinomas may have a paradoxically benign appearance in histopathologic examination. Both aggressive, yet still histologically benign prolactinomas and malignant prolactinomas are challenging pathologies not only for clinicians but also for pathologists. They must be managed by a professional and specified team consisted of neurosurgeons, medical/radiation oncologists, and endocrinologists. It would not be wrong to envisage that future teams dealing with these tumors would also include molecular pathologists and clinical geneticist. Only after such an exten-

sive collaboration, patient-tailored novel treatments targeting aggressive tumors may provide remissions and even cures in these patients.

Compliance with Ethical Standards

Ethical Approval

For this study, only retrospective analysis of the already present pathological specimens and radiological imaging pictures were evaluated and no further laboratorial tests or invasive procedures were performed for research purposes. The patient's identity was not disclosed. Under these circumstances, the local ethical committee did not request an ethical approval process. The patient and a witness signed an informed consent form approving the report of their pathological, radiological, and clinical condition under these conditions.

Conflict of Interest

The authors declare that they have no conflict of interest.

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