

Review Article

From Resistant to Aggressive and Malignant Prolactinomas: A Translational Approach

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Received: September 08, 2014; **Accepted:** October 08, 2014; **Published:** October 10, 2014

Abstract

Prolactinomas are the most frequent pituitary adenomas. Most are successfully treated with Dopamine-Agonists (DA) and Cabergoline (CAB) is recommended as a first line therapeutic option. However, up to 20% may present primary or secondary DA/CAB resistance. Primary resistance is more frequent in macro- and/or invasive prolactinomas, in males and in the presence of inherited genetic predisposition to pituitary adenomas. Secondary resistance develops during follow-up, possibly indicating a change in tumour behaviour. Whereas partial resistance can be frequently overcome by increasing the weekly CAB dose above the labelled dose, severe resistance is typically associated with more aggressive features, often requiring a multimodal approach. Surgery may be indicated to improve neurological symptoms, before pregnancy, or to reduce pharmacological requirement. Because highly aggressive and malignant prolactinomas are life-threatening diseases, a panel of clinical, pathological and molecular features may be considered in order to achieve an early diagnosis and plan an adequate follow-up and treatment. In addition to surgery and/or radiotherapy, Temozolomide (TMZ) currently represents the best option for highly aggressive/malignant prolactinomas. However, up to 30-40% of these tumours may not respond satisfactorily to TMZ and require innovative and personalized therapeutic approaches, such as molecular or radionuclide therapies targeted upon further characterization of the tumour. Increasing knowledge about the pathways involved in severe DA resistance and the aggressive behaviour of prolactinomas should help improve the clinical outcome of such patients.

Keywords: Prolactinoma; Dopamine-Agonists; Pharmacological resistance; Pituitary carcinoma; Target therapy

Introduction

Prolactinomas are the most frequent Pituitary Adenomas (PA) and a large majority are successfully treated with Dopamine-Agonists (DA), in particular Cabergoline (CAB), which has become the first line drug due its excellent efficacy and tolerability [1]. However, a minority develop aggressive features, which may be present at diagnosis or develop during follow-up. Resistance to DA is more frequent in invasive prolactinomas. Aggressive prolactinomas represent an ill-defined group of invasive tumours characterized by uncontrolled growth/recurrences and increasing Prolactin (PRL) secretion despite increasing doses of DA, often requiring repeated surgery and/or radiotherapy. Malignant prolactinomas are defined by the presence of metastasis and are typically resistant to high dose DA. The large majority of them arise from invasive macroprolactinomas and, until the last decade, their outcome was poor despite multimodal approaches including conventional chemotherapy [2,3]. The introduction of Temozolomide (TMZ) has greatly improved the treatment of aggressive and malignant prolactinomas, rapidly becoming the first line chemotherapy in these patients [1,4,5]. Based on increasing knowledge about abnormal pathways involved in the pathogenesis of aggressive/malignant prolactinomas, molecular target therapies represent promising additional tools [4,5]. The aim of this review is to summarize current knowledge and prospective views on this challenging topic, with special reference to the frequent

relationship observed between DA resistance and aggressive behaviour in prolactinomas.

Definition and Epidemiology

The prevalence of prolactinomas has been variably appreciated in the literature, due to variations in diagnostic criteria and recruitment bias. Since 2006, case-finding studies have reported an overall clinical prevalence of PA around 1/1000 inhabitants, with prolactinomas accounting for 57-66% [6-8]. Among these, about 20% were macroprolactinomas (maximal diameter > 10 mm). A minority of prolactinomas (1-4%) are giant (> 40 mm) [9,10]. Although prolactinomas are particularly frequent in young females, the male-to-female ratio increases with age (1:1 after the age of 50). Macro- and giant prolactinomas are more frequent in males, regardless of patient's age [11]. Prolactinomas should not be missed in the perimenopausal age as they can present later as large tumours [12-14]. Because of the relationship between tumour volume and PRL secretion in prolactinomas [11], huge tumours are typically associated with very high PRL levels, which may be missed in sandwich immunometric assays unless the serum is appropriately diluted (e.g. 1:100). This so-called "hook effect" [15] should be considered in all patients with large pituitary tumours, regardless of age and gender, in order to avoid inappropriate surgical approaches.

The prevalence of aggressive prolactinomas has not been

specifically addressed, probably due to the absence of specific diagnostic criteria and the frequent need for a sufficient follow-up to disclose tumour aggressiveness. For example, giant prolactinomas have reached a sufficiently aggressive potential at diagnosis to grow outside the sella and invade surrounding structures. However, most of them are slowly growing and will respond to DA, with PRL normalization in 60-80% and significant tumour shrinkage since the first weeks/months of treatment [9,10]. These latter cases may therefore exit the subgroup of clinically aggressive/challenging prolactinomas. In contrast, a subset of prolactinomas will show an aggressive, uncontrolled growth despite increasing doses of DA/CAB and seldom evolve towards malignancy. The large majority of them present as macroprolactinomas. We found 77/92 prolactinomas resistant to standard doses of CAB to be macroadenomas at diagnosis (83.7%), out of which 15 were giant [16]. Overall, 7.6% developed highly aggressive or malignant features, 4.8% died from neurological complications or metastasis [16]. Thus, in clinical practice, resistance to DA may be a stronger negative prognostic factor than initial macroscopic characteristics and severe DA resistance is a serious concern.

Pathological and molecular markers are being searched for in order to optimize the early identification and treatment of aggressive prolactinomas, hopefully reducing in the future the risk of uncontrolled growth or malignant evolution. The WHO 2004 classification defined as "atypical adenomas" a subset of PA characterized by active proliferation (Ki67 >3%, high mitotic activity), p53 immunoreactivity and cellular atypia [17]. Atypical prolactinomas may represent 2.9-11% of surgically treated cases [18,19]. The prognostic value of this classification was recently investigated [20], confirming a higher rate of recurrence in atypical PA. However, atypical PA are largely represented by invasive macroadenomas [19,20], which are the most likely to recur. A recent classification also takes into account the presence of invasive characteristics, defined by pre- and intra-operative criteria [21]. With a mean post-operative follow-up of 8 years, this model showed that in prolactinomas, the presence of invasive features dramatically increased the risk of recurrence, with proliferative characteristics alone being associated with a mild increase only [21]. Limits in the use of Ki67 and p53 consist of tumour heterogeneity and methodological issues which may contribute to apparently conflicting data on their clinical significance. However, high Ki67 values with convincing p53 immunostaining are sufficiently negative prognostic factors to deserve special clinical attention [4,5,22]. An unresolved issue remains the impact of pre-operative DA, which induces significant morphological changes in prolactinomas [23], on such parameters. Due to the anti-proliferative effects of DA, lower Ki67 values can be observed in treated patients [24,25], though this has not been unequivocally reported [18,25]. This may reflect differences in DA sensitivity, since higher Ki67 values were found in bromocriptine-resistant tumours [26]. Therefore, a medium/high proliferative activity is likely to have a stronger negative prognostic value in prolactinomas treated with DA before surgery than in untreated cases.

Pituitary carcinomas are defined by the presence of histologically proven metastatic dissemination in the Central Nervous System (CNS) or outside the CNS and represent < 0.4% of pituitary tumours [2-4]. No pathological feature is specific of pituitary carcinomas at the primary

site [2-5]. More than 30% of pituitary carcinomas are PRL-secreting [2], with a mean time interval between the diagnosis of prolactinoma and metastasis around 7 years and large individual variations (1 month-20 years) [27]. Up to 40% of malignant prolactinomas initially present as atypical adenomas, but active proliferation and pleomorphism are inconstant even in metastatic tissues [27]. Metastasis may be suspected in the presence of unexplained raising PRL levels or local compression symptoms, in particular for cranio-spinal localisations [2-4,27], or be revealed by incidental imaging or at autopsy. Diagnostic pitfalls are mainly represented by co-existing solid tumours of extra-pituitary origin. Although pituitary carcinomas are increasingly reported, it is likely that in the absence of a gold standard technique for advanced functional imaging, able to detect metastases at an early stage, their prevalence remains underestimated. Scintigraphy with isotopic ligands of the dopamine receptor D2R such as ¹²³I-IBZM and ¹²³I-epidepride, can be used [27-29], although it may not be sensitive enough in the presence of poor D2R expression [29,30]. Alternatively, somatostatin receptor imaging [31] and Positron Emission Tomography (PET) for D2R (¹¹C-raclopride) and markers of metabolic activity (¹¹C-L-methionine, ¹⁸F-fluorodeoxyglucose/FGD) [32] may be proposed. Except for ¹⁸F-FDG, these techniques are poorly available and there is limited experience in malignant prolactinomas [27,33].

Pathogenesis

The pathogenesis of PA is a complex multistep and multifactorial process, which includes early initiating events, growth promotion by a variety of extracellular growth factors, abnormal transduction and proliferative pathways [34,35]. Genetic and epigenetic events may be involved in the initiation of PA and contribute to tumour progression, invasiveness and exceptionally metastasis. These include gene promoter methylation, histone modifications and an abnormal expression of non-coding RNAs, in particular microRNAs [34,35].

Most prolactinomas are believed to arise from the sparsely granulated PRL-secreting cells, which actively release PRL, rather than from the densely granulated cells, considered as resting storage cells [36]. Indeed, densely granulated prolactinomas are rare [18,37]. A minority may also arise from GH/PRL-secreting cells [36,37], which is clinically relevant since it may impact tumour treatment and patient management. Hence, mixed GH/PRL-secreting adenomas should be recognized even in the absence of typical signs and symptoms of GH/IGF1 hypersecretion, especially in macroadenomas. Elucidating the molecular mechanisms of tumorigenesis in prolactinomas is hampered by their first line pharmacological approach, which not only limits the amount of samples available for molecular studies but potentially represents a confounding factor. Nonetheless, a subset of somatic alterations and abnormal signalling have long been reported in prolactinomas [36,38,39]. The development of powerful methodological approaches (genomics/epigenomics/transcriptomics), able to explore hundred of genes simultaneously, has become an essential tool for the identification of new players in prolactinoma pathogenesis [40-44]. Because these studies are performed on a limited number of cases (including single tumours or pooled samples), results must then be validated by studies of gene/protein expression on larger series. Elucidating the biological role of candidate genes/proteins may be challenging. Prolactinomas are

characterized by a very low rate of progression from microadenomas (< 10 mm) to macroadenomas, suggesting the presence of differential molecular mechanisms since an early stage of tumour development. Recent evidence for a pituitary niche of stem cells in the adult pituitary raises the possibility of tumour formation from incompletely differentiated cells, with a more aggressive potential than tumour arising from mature cells [45]. This would also explain considerable overlap between pathways involved in tumour aggressiveness and in DA resistance. Interestingly, some developmental signaling molecules are overexpressed in prolactinomas (e.g. BMP4, Notch3) [Table 1]. Alternatively, progressive tumour de-differentiation may occur. Most prolactinomas are believed to be monoclonal in origin but somatic initiating events are poorly understood. Animal models represent essential tools to unravel the capacity of single gene abnormalities to drive prolactinoma pathogenesis [46,47]. In human prolactinomas, multiple somatic abnormalities have been reported, none of which being identified as an initiating event. Among cytogenetic abnormalities, trisomies involving chromosome 5,8 and 12 have been observed [48] and their molecular implications are being increasingly unravelled. For example, polysomy of chromosome 12, as well as rearrangements in 12q14-15, contribute to the frequent overexpression of the *HMGA2* oncogene in prolactinomas [47] which may in turn be responsible for *Pit-1* upregulation [36,43,49]. Among the several abnormalities in chromosome 11 reported in PA, allelic loss in 11p and in 11q were observed in aggressive and malignant prolactinomas, respectively, with transcriptomic and proteomic analysis identifying a subgroup of dysregulated genes in 11p [50].

Allelic loss of the whole chromosome 11 was reported in aggressive and malignant prolactinomas [33]. In contrast, classical somatic mutations of oncogenes and inactivating mutations of Tumor Suppressor Genes (TSGs) are rare [36,38,39]. Recently, *H-Ras* and *PIK3CA* mutations have been reported in invasive prolactinomas [51] and an activating *GNAS1* mutation (*Gsp*) was found in an aggressive prolactinoma shifting to a mixed GH/PRL-secreting tumour [52]. Rather, overexpression of oncogenic proteins and/or downregulation of TSGs occur [Table 1], with accumulating evidence for underlying epigenetic changes [34,35]. This may translate into the identification of immunohistochemical markers of aggressiveness, as proposed for nuclear PTTG [42] or strong galectin-3 immunostaining [53]. A set of prognostic biomarkers have been proposed [54], though they may not invariably apply to all functional phenotypes. Increased apoptosis has been reported in invasive/aggressive and especially in malignant prolactinomas [54,55].

Extracellular signalling also plays an essential role in the control of PRL secretion and cell proliferation, the development of invasive/aggressive features and angiogenesis. The best characterized model is represented by estrogen-induced prolactinomas developing in some strains of rats [56,57], which illustrates the complex cascade of events induced by a single molecule (e.g. 17βestradiol): (i) estrogens exert direct transcriptional effects on a panel of genes including PRL, molecules involved in cell cycle progression (e.g. *PTTG*, *c-myc*, *E2F1*), Growth Factors (GFs)/neuropeptides and their receptors, (ii) some of these factors will contribute to stimulate cell proliferation (e.g.

Table 1: Genes dysregulated in prolactinomas.

Function	Upregulated genes	Note	Ref	Downregulated genes	Note	Ref
Pituitary development	<i>Pit1 (POUF1)</i>		40,43	<i>Pitx1</i> <i>Frizzled homolog 7</i> <i>ID2</i>	Aggressive	42 43 41
	<i>OCT2 (POUF2)</i>		43			
	<i>ASH1</i>		41,43			
	<i>TLE4</i>		41			
	<i>Notch3</i>		43			
	<i>BMP4</i>		rev in 34,35			
	<i>PTTG</i>		42			
Cell cycle	<i>CCNB1 (Cyclin B1)</i>	Aggressive (?)	42	<i>PTTG</i> <i>P16</i> <i>GADD45β/GADD45G</i>		41 40 43
	<i>AURKB</i>	Aggressive	42			
	<i>ASK</i>	Aggressive	42			
	<i>CENPE</i>	Aggressive	42			
	<i>HMGA2</i>	Aggressive	rev in 47			
	<i>HMGA1</i>		rev in 47			
Growth factors/receptors	<i>EGFR/ErbB1</i>	Aggressive/K	rev in 34,35	<i>TGFβ1, TGFβR3</i> <i>VEGF</i> <i>NGF</i>	Resistant (°)	43 41 87
	<i>HER2/ErbB2</i>		60			
	<i>ErbB3</i>		59			
	<i>Angiopoietin1</i>		41,43			
	<i>VGF</i>		41			
	<i>FGF4 (hst)/ptdFGFR4</i>		rev in 34,35			
cAMP signalling	<i>D2R</i>		43, rev in 38, 83	<i>D2R°</i> <i>Gi2</i>	Resistant (°)	rev in 38, 83
	<i>GNAS1</i>		43, rev in 38, 83			
Extracellular matrix	<i>ADAMTS6</i>	Invasive/Aggressive ?	42	E-cadherin/β-catenin N-cadherin	Invasive	rev in 35
	<i>MMP-9</i>					
Miscellaneous	<i>PIK3CA</i>	Invasive/Aggressive	51	<i>LGALS-6</i> <i>IGFBP3</i>		41 43
	<i>LGALS-3/Galectin-3</i>		41, 53			
	<i>LAPTM4B</i>		40			
	<i>Ras-induced senescence 1 (RIS1)</i>		43			
	<i>Bcl-2-associated anathogen (BAG1)</i>		40,43			
	<i>DNAJB5</i>					
	<i>ATM</i>		43			
	<i>RAB-25</i>		41			

Legend: This table presents a non exhaustive list of dysregulated genes in prolactinomas; most are issued from transcriptomic studies. References for genes identified by specific studies can be retrieved from reviews indicated by "rev in." ° as compared with DA responders.

downregulation of *TGFβ1/2*) and angiogenesis (e.g. upregulation of *FGF2*, *VEGFA*). Angiogenesis, which results from an imbalance between angiogenic and anti-angiogenic factors, plays a peculiar role in the progression of prolactinomas [58]. As it increases with tumour volume and invasiveness, evaluation of Microvascular Density (MVD) with endothelial cell markers may be proposed as a marker of aggressiveness in these tumours [33,39] and for the design of anti-angiogenetic treatments. Among GFs/GFRs involved in prolactinoma pathogenesis [34,35], the EGF/EGFR family has also received much attention [59,60].

Inherited predisposition to prolactinomas

A subgroup of patients may develop prolactinomas in a genetic setting [61-63]. *MEN1* and *AIP*-related prolactinomas are frequently more aggressive than their sporadic counterpart and their identification may have significant implications for patient's management and/or genetic counselling. In contrast, prolactinomas represent a very minority of PA associated with pheochromocytomas/parangliomas, a new syndrome linked to germline *SDHx* genes mutations [64]. Although hyperprolactinemia frequently develops in patients with McCune Albright syndrome or Carney complex, characterized by mutations leading to a constitutive activation of the cAMP pathway and frequent somatotroph hyperplasia [65,66], PA develop in about 15% and are typically associated with acromegaly. Thus, no genetic testing for these rare conditions is justified in prolactinoma patients unless a specific clinical context is present.

Multiple endocrine neoplasia type 1 (MEN1) and related conditions

Prolactinomas are the most frequent phenotype encountered in patients affected by MEN1, a highly penetrant condition [67]. PA develop in about 40% of MEN1 patients, 15% as a first manifestation of the syndrome. In a multicenter study in which MEN1 patients were matched for age with sporadic PA patients, MEN1 prolactinomas were larger and more frequently invasive at diagnosis than their sporadic counterpart, suggesting an earlier onset [68]. Accordingly, *MEN1* gene abnormalities have been recently reported in up to 6% of sporadic macroprolactinomas in young patients, which is twice the reported prevalence in unselected PA [69]. Similarly, we found clinical MEN1 in 5/92 resistant prolactinoma patients (5.4%), 4/5 with a *MEN1* mutation [16], which is consistent with a more frequent DA resistance in MEN1 prolactinomas [68]. Malignant transformation has been rarely reported in MEN1 [68]. The complex molecular effects of men in, the *MEN1* gene product and its role in prolactinomas have been reviewed elsewhere [70]. Thus, MEN1 should be taken in mind in apparently sporadic patients with macroprolactinomas, in particular if invasive, resistant or in young patients [62,69]. Prolactinomas may also be encountered in MEN1-like conditions associated with mutations in genes encoding Cyclin Kinase Inhibitors (CKI), in particular MEN4, due to a *CDKN2B/p27* inactivating mutations, but these are very rare conditions [71,72].

The aryl hydrocarbon receptor Interacting protein (AIP) gene

AIP is another pituitary tumour suppressor gene located in 11q13 [73]. Although the large majority of PA developing in patients with germline *AIP* mutations (*AIP^{mut}*) are somatotropinomas (75%),

nearly 10% are mixed GH/PRL-secreting PA and pure prolactinomas account for almost 15% [74]. *AIP^{mut}* prolactinomas may occur in the setting of Familial Isolated Pituitary Adenomas (FIPA) or present as apparently sporadic cases. In FIPA, *AIP^{mut}* prolactinomas occur in heterogeneous kindreds, i.e. in association with any other PA phenotype (mainly GH-secreting) [74]. Because *AIP* mutations account for only 15% of heterogeneous FIPA kindreds, additional genes should be involved, in particular in homogeneous prolactinoma kindreds. *AIP^{mut}* prolactinomas account for only 4.5% of unselected prolactinomas [75], but this proportion increases in young macroprolactinoma and pediatric cases [74]. Somatic *AIP* downregulation is frequent in prolactinomas, regardless of tumour aggressiveness [76]. *AIP^{mut}* prolactinomas are also frequently resistant to DA [74]. Recognizing *AIP^{mut}* prolactinoma simplifies familial screening, although disease penetrance is incomplete [74]. A challenging issue may be presented by the identification of variants of uncertain biological significance, which pathogenicity is generally estimated by combining data from *in silico* analysis and familial screening. Further search for *AIP* mutations in at-risk PA patients should help define guidelines for genetic counselling according to *AIP* sequence abnormalities.

Resistance to DA

Current definition and clinical significance

The proportion of resistant prolactinomas has been variably appreciated [77-79]. There are many reasons for that: (i) in clinical practice, prolactinoma show a spectrum of DA sensitivity which translates into different doses of treatment required to normalize PRL secretion and obtain "significant" tumour shrinkage, so that any threshold based on the percentage of PRL decrease or tumour reduction is somewhat arbitrary; (ii) the duration of treatment may be critical to evaluate tumour shrinkage; (iii) former studies reported on bromocriptine resistance; (iv) the maximal tolerated dose of DA may be lower than the efficient dose. Due to its greater efficacy and tolerability, current guidelines recommend the use of CAB as a first line treatment, or switching to CAB when other DA drugs fail [1]. DA resistance is therefore currently defined as CAB resistance. Because 82% of 122 consecutive prolactinoma patients achieved PRL normalization with a median maximal weekly dose of CAB ($Cab^{max/w}$) <1.5 mg, this threshold was proposed to define resistant patients, who therefore accounted for 18% [80]. We defined CAB resistance on the basis of persistent hyperprolactinemia on the maximal labelled dose of CAB ($Cab^{max/w}$ > 2.0 mg/week) [16], which may be unable to normalize PRL in up to 18% of macroprolactinoma patients [81]. Whatever the definition retained for DA resistance, it is more frequent in macroadenomas and in males. An important clue in defining DA resistance on the basis of a moderate persisting hyperprolactinemia is to exclude the presence of macroprolactinemia in asymptomatic patients [82]. Other pitfalls are represented by unrecognized GH/PRL-secreting adenomas or by an incorrect diagnosis of macroprolactinoma – the so-called "pseudo-prolactinoma" due to functional hyperprolactinemia in the presence of any tumour of the sellar region due to a reduced dopaminergic tone-. In this latter case, however, PRL secretion typically normalizes on low dose CAB with no tumour shrinkage, highlighting the need for neuro-radiological follow-up on DA therapy for hyperprolactinemia.

It may be of clinical interest to distinguish between “partially resistant” prolactinomas those tumours which will be controlled by a $\text{Cab}^{\text{max/w}} > 2.0$ mg and “severely resistant” those unresponsive to high dose CAB (e.g. > 3.0 mg), since they are likely to represent diseases with different prognostic and therapeutic indications. We observed that the mean $\text{Cab}^{\text{max/w}}$ in resistant prolactinomas- 4.1 ± 1.7 mg [range 2.0-10.5] – significantly increased with treatment complexity: 3.4 ± 1.2 mg in patients treated by DA only, 4.3 ± 1.8 mg in patients treated with surgery and DA, and 5.5 ± 2.0 mg in patients treated with DA, surgery and postoperative radiotherapy ($P=0.003$) [16]. All the patients who developed highly aggressive or malignant prolactinomas received and were resistant to $\text{Cab}^{\text{max/w}}$ up to 3.0-8.0 mg [16]. In contrast, CAB could be progressively tapered during follow-up in a significant subset of patients who achieved PRL normalization [16]. Another issue is represented by a progressive escape from an initial response to DA. We found 8.7% of CAB-resistant patients to have secondary resistance [16]. This occurred more frequently in males, but initial tumour characteristics and the $\text{Cab}^{\text{max/w}}$ dose required to normalize PRL were similar to those with primary resistance. Close follow-up of such cases is necessary, since increasing secondary resistance is more frequently observed in aggressive/malignant prolactinomas, possibly due to tumour dedifferentiation [16,27].

Molecular basis

Inhibition of PRL transcription and cell proliferation by dopamine and DA in lactotrophs are mediated by D2R, which is expressed as a short (D2Rs) and a long (D2Rl) isoforms. D2R-deficient mice develop lactotroph hyperplasia and late-onset prolactinomas, especially in females [46]. No D2R mutations have been identified in resistant prolactinomas. Rather, underexpression of D2R, in particular D2Rs [83] or alterations in D2R signalling, such as a reduced expression of Gi2 α or the cytoskeleton-associated protein filamin A may be present [83,84]. Interestingly, the genetic D2R polymorphism NcoI T was found to be associated with DA resistance regardless of tumour volume [85]. Estrogens may induce dopamine resistance [56], with a possible increase in DA requirement during sex steroid therapy in prolactinoma patients, but no specific alterations in ER expression was found in resistant prolactinomas [86,87]. NGF exerts autocrine anti-proliferative effects on lactotrophs and loss of NGF expression has been linked to D2R downregulation and pharmacological resistance [88,89]. Whether crosstalk with other abnormal extracellular signaling pathways may contribute to DA resistance, in particular in aggressive prolactinomas, has been poorly investigated yet.

High dose CAB in resistant prolactinomas: pro and cons

Whereas increasing $\text{Cab}^{\text{max/w}}$ above 2.0 mg is useful in most partially resistant patients, experience with very high doses (e.g. > 3.5 mg) has led to conflicting results. Ono et al. reported PRL normalization in up to 73.1%, 88.5% and 96.2% of resistant cases when increasing $\text{Cab}^{\text{max/w}}$ to 6.0, 9.0 and 12 mg, respectively [81], whereas others found no significant advantage above 3.5 mg [80,90]. We observed PRL normalization in 5/19 patients receiving $\text{Cab}^{\text{max/w}} > 3.5$ mg (26.3%), with some degree of tumor shrinkage in 10/19 (52.6%). None had tumor disappearance but none had tumor progression [16]. Therefore a stepwise dose increase can be reasonably proposed in a compliant patient if: (i) each step determines a further PRL decrease; (ii) clinical

side-effects are acceptable; (iii) echocardiographic monitoring is performed. If clinical side-effects associated with high CAB doses might be transient [81], the potential serotonergic effects of CAB on the heart – long-term valvular thickening with potential regurgitation, mediated by the 5HT-2B receptor – should be considered [90]. The large majority of studies performed on prolactinoma patients receiving labelled doses of CAB showed no significant increase in valvular regurgitation, though subclinical alterations could be observed [1,78,91-95]. Since a cumulative dose-effect may be present and experience in DA-resistant prolactinomas is limited, echocardiographic monitoring should be performed [1,91]. In female patients, the risk of tumour enlargement in macroadenomas in pregnancy [96] may be even higher in uncontrolled tumours and progression from micro- to macro-adenoma may occur [16]. Because the tolerance and safety of high doses DA in pregnancy is largely unknown [97], pregnancy should be carefully planned in such patients, keeping in mind that surgery is able to significantly reduce the risks of pregnancy-related complications [1,77,96] and drug requirement [16].

Alternative endocrinological approaches

The estrogen sensitivity of prolactinomas has long been thought as a potential pharmacological target. All prolactinomas express ER α [37]. Limited clinical experience with tamoxifen showed only a mild effect on PRL secretion and new generations of selective estrogen receptor modulators have not been used in humans so far [37]. However, encouraging experimental data have been recently obtained *in vitro* [98] and *in vivo* [99] with the use of fulvestrant, a pure antiestrogen compound. Despite prolactinomas also express variable levels of Somatostatin Receptors (SSTRs), they do not significantly respond to octreotide/lanreotide. Based on a preferential expression of SSTRs1/5, SOM230/pasireotide could be more effective, but this is not always the case *in vitro* [100] and no data are available *in vivo*. Preliminary studies with chimeric D2R/SSTRs analogues *in vitro* yielded disappointing results [100]. An interesting application of SSTRs expression is radionuclide therapy, which has been successfully used in a resistant prolactinoma [101], although further experience is needed [102].

Surgery

It is currently recommended that first line surgery in prolactinomas should be limited to neurological emergencies or extensive hemorrhagic/cystic changes [1]. Transcranial surgery is rarely necessary and debulking Transsphenoidal Surgery (TSS) should be performed in a specialised center. This approach is justified by the high percentage of PRL normalization and tumour shrinkage in macroprolactinomas treated with CAB (up to 90 and 80%, respectively), with a rapid improvement of visual defects in responsive cases [1,77,91]. Despite significant improvements in TSS approach, post-surgical PRL normalization still occurs in a minority of macroprolactinomas, especially if invasive, with a limited risk of post-operative complications and frequent recurrences [103,104]. Surgery is therefore proposed in patients who do not tolerate or respond to DA, with rare indications resulting from complications of DA in large tumours, such as CSF leak or intratumoral hemorrhage/apoplexy [1,9,10]. For such reasons, patients with huge tumours should be best started with very low dose CAB [9,10,91]. In our

experience, post-operative PRL normalization was achieved in < 10% of resistant prolactinomas but debulking TSS significantly reduced the weekly dose of CAB required to control hyperprolactinemia and tumour growth, which may be relevant for the long-term safety of treatment [16]. Higher rates of post-operative PRL normalization (>40%) were obtained on a series of resistant prolactinomas including a lower proportion of macro-and/or invasive tumours, but a similar reduction on post-operative CAB requirement was observed [104]. As discussed hitherto, surgery may also be safely offered before pregnancy to women with resistant prolactinomas, especially if persistent anovulation requires pharmacological induction.

Radiotherapy

Prolactinomas have long been considered as being poorly sensitive to radiotherapy. PRL normalization has been reported in a minority of patients (about 1/3) and may require several years (up to 10-20 years), whereas *de novo* pituitary deficits develop in a large proportion of patients and increase with time [1,77,91]. Neurocognitive effects, an increased risk for delayed cerebrovascular events and radio-induced neoplasia should also be considered [105-107]. However, radiotherapy may be useful in resistant prolactinomas [1,77,91,108] and significance advances have been made in pituitary irradiation techniques [109]. Stereotactic fractionated techniques are typically employed as post-surgical tool to control the risk of tumour regrowth. Gamma-knife radiosurgery, which is suitable for small target volumes, has also been used in non-operated resistant prolactinomas, with a good tumour control, clinical improvement in a subset of patients, but a low rate of PRL normalization [110]. A precise definition of the target volume may in some cases allow a safe re-irradiation in the presence of localized tumour regrowth [109]. An advantage of multimodal therapy (DA, surgery and radiotherapy) of resistant prolactinomas is that, despite its use in more aggressive diseases, it may further reduce the requirement for high dose DA and increase the chance of CAB withdrawal [16]. Finally, radiotherapy may be used in the symptomatic treatment of metastasis.

Temozolomide

Until the last decade, chemotherapy regimen (mainly based on CCNU/lomustine and 5-FU) have been used for the treatment of highly aggressive or malignant pituitary tumours, with transient beneficial effects in some patients [3,4,111]. Since the first report of its remarkable efficacy in a PRL-secreting carcinoma [112], Temozolomide (TMZ) has become the first-line chemotherapy for pituitary carcinomas [1]. TMZ has anti-proliferative, anti-secretory and pro-apoptotic effects in rodent pituitary cell lines [113]. TMZ is administered orally, side-effects are generally mild to moderate. Based on experience with glioblastoma patients, the preferred regimen is 150-200 mg/m² dose for 5 days every 28 days, although daily low doses (50-75 mg/m²) have also been proposed [114-116]. Tumour control has currently been reported in about 60% of aggressive PA and carcinomas, which represents a significant advance as compared to previous chemotherapy regimen [114-116]. PRL-secreting tumours are among the most responsive, with tumour shrinkage (>20%) reported in 66.6% of treated cases (n=15, including 7 carcinomas) [114], so that TMZ represents a valuable option for selected resistant prolactinomas [116]. Tumour shrinkage is associated with a significant reduction in PRL secretion, which may eventually normalize. Because the O⁶-methylguanine-DNA Methyltransferase (MGMT) enzyme is

able to reverse DNA abnormalities induced by TMZ, its expression may limit the efficacy of treatment [117]. Reduced MGMT expression may be driven by promoter methylation [117]. Current experience with pituitary tumours indicate that low MGMT expression, rather than MGMT promoter methylation, is significantly associated with TMZ response [118,119]. However, the negative predictive value of MGMT status is not strong enough to deny patients a 3-months TMZ trial, which is generally able to identify responders [114,120]. Accordingly, we obtained a strong and sustained response to TMZ in a highly aggressive MEN1 prolactinoma displaying strong MGMT immunostaining and a fully unmethylated MGMT promoter [121]. A remarkable response was also reported in a MEN1 malignant prolactinoma with unknown MGMT status [122]. Expression of the mismatch repair enzyme MSH6 has been recently associated with pituitary tumour response to TMZ, potentially due to increased DNA damage signalling [123,124]. Open issues with the use of TMZ are the optimal duration of treatment, long term toxicity and follow-up after drug withdrawal, and potential acquired resistance. There are no recommendation about DA treatment on TMZ; our policy is to maintain CAB unchanged until the efficacy of TMZ is proven and there after taper the dose progressively.

Target therapies

GRs, GFRs and related abnormal intracellular signalling pathways, the Raf/MEK/ERK and PI3K/Akt/mTOR pathways offer interesting opportunities for the development of molecular target therapies in resistant/aggressive/malignant prolactinomas [125,126]. Anti-VEGF therapy (bevacizumab) was effective in experimental estrogen-induced prolactinomas, including dopamine resistant tumours [127,128], but clinical experience is limited to a corticotroph carcinoma [129]. Tyrosine Kinase Inhibitors (TKI) have been proposed to target the EGFR/HER1 alone (gefitinib) or both EGFR/HER1 and Erb2/HER2 (lapatinib), with lapatinib inducing a higher reduction in PRL secretion from human prolactinomas *in vitro* [60]. Promising data also arise from experimental data using antagonism of the heregulin/HER3 pathway [59] or anti-angiogenic thymospondin-1 analogues [130]. Inhibitors of mTOR (e.g. rapamycin, RAD001/everolimus) reduce cell proliferation in GH₃ and MMQ cells [131,132] with a potential radiosensitizing effect [132], but experience with human pituitary tumours is limited to non-functioning PA *in vitro* [133]. Experimental studies based on the use of epigenetic drugs [134] and adenovirus-mediated gene therapy [135-138] remain at a very preliminary stage.

Conclusion

Up to 20% prolactinomas may present partial or severe DA resistance, which is generally disclosed since the early phase of treatment, but may develop during follow-up. Primary resistance is more frequent in genetic forms, secondary resistance may reveal a change in tumour behaviour. There is significant overlap between DA resistance and tumour aggressiveness, and in some cases the evolution may be life-threatening due to intracranial growth or metastatic dissemination—indeed, a subset of highly aggressive PA may be viewed as “cancers without metastasis” [24,33,139]. The clinical management of DA-resistant prolactinomas may be challenging and a panel of clinical, pathological and molecular features may be considered in an attempt to recognize an aggressive potential at an

early phase and plan an adequate follow-up and treatment [33,140]. Multimodal treatment is often necessary and TMZ has greatly improved the treatment of aggressive and malignant prolactinomas. Increasing knowledge about the molecular basis of tumour invasion, proliferation, metastasis and pharmacological resistance to DA and TMZ should help defining personalized strategies in aggressive and malignant prolactinomas.

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