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The role of tyrosine kinase inhibitors in the treatment of pituitary tumors: from bench to bedside

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Abstract

Purpose of review—Treatment of aggressive pituitary tumors often yields suboptimal control of the tumor and confers significant morbidity. Lactotroph and corticotroph derived tumors express ErbB receptors and ligands, and mutations in USP8, which alters EGFR degradation, have been implicated in Cushing disease (CD) pathogenesis. EGFR tyrosine kinase inhibitor (TKI) therapy has emerged as a potential new therapeutic approach for patients with aggressive prolactinomas and CD.

Recent findings—Using EGFR or HER2 driven PRL promoters, transgenic mice develop large tumors that respond to TKI inhibition. In human corticotroph primary cultures, treatment with the pan-ErbB TKI canertinib as well as the EGFR TKI gefitinib suppresses POMC mRNA, and USP8 mutations, detected in up to two-thirds of CD, may underlie the increase in EGFR signaling in these tumors. Human prolactinomas have differential ErbB receptor expression associated with aggressive behavior and data from an ongoing clinical trial suggest that resistant prolactinomas may respond to the EGFR TKI lapatinib.

Summary—Preclinical and clinical models substantiate the role of the EGFR pathway in corticotroph and lactotroph adenomas. Although further study is needed, results to date suggest targeting the ErbB pathway may be an effective therapeutic approach for patients with aggressive pituitary tumors.

Keywords

tyrosine kinase inhibitor; EGFR; HER2; Cushing disease; prolactinoma

Introduction

Pituitary tumors are invariably benign slow growing lesions, and regardless of whether they clinically secrete hormone can manifest aggressive behavior and extensive growth and, rarely, metastasize [1]. Current treatment options for aggressive pituitary tumors are insufficient to control tumor growth and often confer significant morbidity. Therapeutic targeting of molecular pathway shown to be involved in pituitary tumorigenesis may offer alternative treatments for these patients. One potential target is the EGFR pathway. EGFR

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belongs to the ErbB family of membrane receptor kinases, including EGFR (also called ErbB1 or HER1), ErbB2 (Neu or HER2), ErbB3 (HER3), and ErbB4 (HER4) [2]. Pituitary expression of the more comprehensively studied receptors and ligands in the ErbB family is presented in Table 1 and has been comprehensively reviewed by Cooper et al [2]. Although EGFR and HER2 are the most well studied, ErbB3 expression has also been demonstrated in prolactinomas and increased expression was noted in a prolactin-secreting carcinoma [3], and ErbB4 expression has been noted in corticotroph adenomas [4].

To date, three EGFR TKIs, gefitinib, lapatinib, and canertinib have been evaluated in the treatment of pituitary tumors. Gefitinib binds the EGFR ATP-binding site, selectively inhibiting EGFR activity, while lapatinib binds the ATP-binding pocket of the EGFR/HER2 protein kinase domain and prevents their activation. Canertinib is an experimental pan-ErbB receptor TKI [5]. We will review the preclinical evidence supporting the use of these TKIs in pituitary tumors and summarize available clinical evidence validating this novel therapeutic approach.

Targeting the ErbB Pathway in Corticotroph Adenomas

Although all of the ErbB pathway receptors are expressed in corticotrophs, EGFR is far more common and is expressed in 75% of human corticotroph tumors (reviewed in [2]), and treatment of human primary pituitary corticotroph tumor cultures with gefitinib dose-dependently reduced POMC mRNA by 95% [6], underscoring its potential value as a therapeutic target. Reduction of both POMC mRNA and ACTH was also observed in the culture medium of gefitinib-treated canine primary pituitary corticotroph tumor cultures. When AtT20 mouse corticotroph tumor cells were transfected to overexpress EGFR, activation of both ERK and Akt pathways was observed and increased cell proliferation was evident, all of which were inhibited by gefitinib treatment. In addition, features of hypercortisolemia in mice harboring xenografts of EGFR-transfected AtT20 cells were reversed by gefitinib treatment [6].

The recent discovery of gain-of-function mutations in ubiquitin specific protease 8 (USP8) provided the first molecular mechanism to explain the aberrant EGFR signaling in corticotrophs [7-9].

Upon ligand binding, variable lysine residues on EGFR are either mono- or poly-ubiquitinated, promoting EGFR trafficking to degradation [10]. USP8 is a deubiquitinase that catalyzes removal of a ubiquitin residue from EGFR, thereby protecting EGFR from trafficking to the lysosome for degradation [11]. In humans, 14-3-3 proteins bind UPS8 at phosphorylated Ser718, which resides in the 14-3-3 binding motif RSYpSSP located at amino acids 715-720, and catalytically repress USP8 deubiquitination activity [12].

In an elegant and comprehensive analysis of corticotroph adenoma, Reincke et al demonstrated that heterozygous somatic USP8 single nucleotide mutation or deletions at or adjacent to the 14-3-3 protein binding domain make USP8 resistant to 14-3-3 protein binding and more prone to proteolytic cleavage, which, in turn, leads to higher rate of USP8-induced EGFR deubiquitination activity upon binding of EGF to its receptor. Levels of

deubiquitinated EGFR recycled to the cell membrane as well as its membrane expression were shown to increase in cells harboring USP8 mutations. Moreover, in USP8 mutant-harboring cells, EGF binding stimulated ERK1/2 phosphorylation to a greater extent than in cells harboring WT USP8, resulting in increased *Pomc* promoter activity and ACTH synthesis [8**].

USP8 mutations were present in 6 of 17 corticotroph adenomas analyzed (35%), while no mutation was observed in other tumor subtypes (two ACTH-secreting Nelson's syndrome tumors, 14 growth-hormone-secreting adenomas, 10 prolactinomas, or 10 non-functioning pituitary adenomas). Patients harboring the mutations were female, had smaller adenomas, lower plasma ACTH levels, and had lower serum cortisol after 1 mg dexamethasone suppression test than did patients with Cushing disease (CD) not harboring USP8 mutations [8**]. Hayashi et al found similar characteristics in CD patients with USP8 mutations [13*]. Among 60 tumors studied, patients with mutated tumors were all female, and showed lower ACTH levels, smaller tumor size, and a higher likelihood of surgical remission compared to WT tumors. *POMC* mRNA expression was higher in mutated tumors and positively correlated with *USP8* mRNA expression. However, *EGFR* mRNA and protein expression did not differ between WT and mutant tumors, although EGFR protein expression was higher in those with aggressive Crooke's cell adenomas [13*].

Perez-Rivas et al extended the analysis to 134 CD adenomas and 11 silent corticotroph adenomas. Forty-eight of the 134 (35.8%) adenomas from patients with CD had USP8 mutations vs none of the tumors from patients with silent corticotroph adenoma [9]. USP8-Ser718 and USP8-Pro720 were mutated in 52% and 48% of mutation-positive adenomas, respectively. USP8 mutation was more prevalent in females and adults and was associated with less post-operative adrenal insufficiency. There was no difference in baseline plasma ACTH levels or serum cortisol levels after dexamethasone suppression test between patient harboring the mutant USP8 vs the WT USP8. Of note, when microadenomas were analyzed separately, USP8 mutant-harboring adenomas were smaller than those with WT USP8 [9].

Finally, Ma et al showed 17 somatic USP8 mutations in ACTH-secreting pituitary adenomas from 67 of 108 (62%) Chinese patients; 77.6% of these mutations were at Ser718 and Pro720 [7]. Immunohistochemistry showed higher EGFR expression in half and more intense ERK1/2 phosphorylation in 80% of USP8-mutated tumors examined vs tumors harboring WT USP8. *POMC* mRNA levels were also higher in USP8-mutated tumors. When overexpressed in HeLa cells, USP8 mutations were associated with reduced EGFR ubiquitination and degradation as compared to WT controls. USP8 knockdown as well as treatment with gefitinib reduced ACTH secretion in primary cultures of USP8-mutated ACTH-secreting pituitary tumors [7].

Predominant nuclear localization of EGFR was shown in human and canine corticotroph tumors [6], as was UPS8 in USP8-mutated human corticotroph tumors [8]. By contrast, non-mutated corticotroph expressed UPS8 in both cytoplasm and nucleus in humans [8**] and canines [14]. This suggests further association of the two molecules, and awaits further elucidation.

Although a USP8 inhibitor is readily available [15], none of the above studies utilized it in an attempt to suppress mutated USP8 activity. In mouse AtT20 pituitary corticotroph cells, a USP8 inhibitor (9-ethylxyimino-9H-indeno [1,2-b] pyrazine-2,3-dicarbonitrile) downregulated EGFR protein expression, attenuated ACTH synthesis, and promoted cell apoptosis [16]. However, it is as yet unknown whether USP8 is mutated in AtT20 cells and whether the increased deubiquitination activity of mutated USP8 can be inhibited by a USP8 inhibitor.

Studies on the role of ErbB4 in corticotroph adenomas are also underway [4]. In 14 cultured human corticotroph adenomas, ErbB4 was detected in all tumors, and canertinib, an experimental pan-ErbB receptor TKI dose dependently suppressed POMC mRNA and ACTH secretion, with a 70% reduction seen in those with higher ErbB expression compared to no response in those with low levels. Tumors with high ErbB4 expression also tended to be smaller and less invasive. These data suggest a potential role for inhibitors that target multiple ErbB receptors simultaneously.

Targeting the ErbB Pathway in Lactotroph Adenomas

The effect of ErbB pathway signaling on lactotroph tumorigenesis was demonstrated following expression of EGFR or HER2 in the pituitaries of mice using rat prolactin enhancer and promoter [17]. Enlarged pituitaries or pituitary adenomas were observed in 30% of female EGFR transgenic mice vs 8% of wild-type (WT) mice at ages 11-13 months, while 41% of female HER2 transgenic mice exhibited enlarged pituitaries or pituitary adenomas vs 8% in WT mice. Both transgenic mouse models had a 4- to 5-fold increase in circulating prolactin (PRL) levels, which was reduced by 60% in EGFR transgenic mice and by 40% in HER2 transgenic mice after treatment with lapatinib. Treatment with lapatinib did not affect circulating PRL levels in control mice. Western blot analysis of pituitary tissue demonstrated inhibition of PRL expression as well as ERK and Akt phosphorylation with lapatinib in transgenic mice but not in control mice [17].

Gene expression data confirmed that EGF and EGFR are aberrantly expressed in prolactinomas [18], and over half of prolactinomas express EGFR and 26% express Her2 (reviewed in [2]). More aggressive pituitary adenomas show higher EGFR and ErbB2 protein expression, with 40% of invasive adenomas staining positive for ErbB2 compared to 1.2% of noninvasive adenomas [19]. We demonstrated that approximately 80% of 28 human prolactinomas express ErbB receptors [20**], with EGFR positivity in 23/28 (82%), ErbB2 positivity in 24/26 (92%), ErbB3 positivity in 7/28 (25%), and ErbB4 positivity in 20/28 (71%). Higher ErbB3 expression was correlated with higher rates of optic chiasm compression, suprasellar extension, and encasement of the carotids compared to those without these radiologic findings. Higher ErbB4 was observed in tumors with sphenoid sinus invasion compared to those without invasion. Two subjects who experienced tumor shrinkage and PRL reduction on dopamine agonist therapy had tumors which were 100% percent positive for ErbB3 on their adenomas compared to 13 without tumor reduction who had 0-10% ErbB3 staining [20**].

Her2 was further shown to have a significant role in prolactinomas, as treatment with the dual TKI lapatinib decreased PRL by 90% compared to minimal response to gefitinib in primary culture of human prolactinomas [21]. Following on these data, we initiated a 6-month proof-of-concept clinical trial with lapatinib in patients with dopamine agonist resistant prolactinomas. The first subject presented with continued growth of her prolactinoma (4 cm at the time of study) and PRL elevation despite 13 years of cabergoline 7 mg/week and surgery. With the addition of lapatinib, she demonstrated near normalization of PRL and a 22% reduction in tumor volume (Figure 1). The second subject similarly had persistent hyperprolactinemia and tumor growth on cabergoline 7 mg/week for 4 years and after surgery; with the addition of lapatinib, she achieved a 42% reduction in PRL and tumoral size stabilization [20**]. These encouraging responses to lapatinib in patients with prolactinomas are now being validated in an ongoing multi-site Phase IIa trial in patients with resistant prolactinomas.

Conclusion

ErbB pathway is overexpressed in pituitary corticotroph and lactotroph adenomas, and preclinical models demonstrate that functional regulation of tumoral growth and hormonal secretion respond to EGFR TKI inhibition. While there is a suggestion that EGFR expression correlates with more aggressive behavior in lactotroph tumors, it remains to be seen whether targeting this pathway will open an avenue of new therapy. The discovery of UPS8 mutations that increase EGFR activity in corticotroph tumors supports a role for EGFR-targeting therapeutics in patients with CD, but this approach has yet to be tested. In prolactinomas, only preliminary evidence is available of possible efficacy of a TKI in resistant tumors. Therefore, we need to await results of clinical trials before recommending TKI therapy for patients with prolactinomas.

Nevertheless, as with the increasing role of TKIs in metastatic thyroid cancer, therapies aimed at specific molecular targets are coming to the forefront of pituitary tumor management. This molecularly targeted approach has the potential to reduce morbidity and cost of unnecessary interventions, ensuring that the patients receive the appropriate therapy and potentially changing the treatment algorithm in the medical management of aggressive pituitary adenomas.

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Abbreviations

ACTH	adrenocorticotrophin
EGFR	epidermal growth factor receptor
HER2	human epidermal growth factor receptor 2

PRL	prolactin
TKI	tyrosine kinase inhibitor
WT	wild type

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Key Points

- ErbB receptors are expressed and regulate corticotroph and lactotroph tumor behavior
- Transgenic models demonstrate that EGFR and HER2 overexpression lead to larger tumors and respond to TKI therapy
- Many corticotroph tumors have USP8 mutations, which may promote increased EGFR signaling
- TKI therapy targeting ErbB receptors shows preliminary efficacy in dopamine agonist resistant prolactinomas and remains to be validated

Figure 1.

Prolactin levels and tumor volumes in lapatinib treated subject. A. Baseline MRI demonstrates homogeneously enhancing mass in right aspect of sella extending into cavernous sinus and encasing the carotid vessel, and abutting inferior aspect of right prechiasmatic optic nerve. Lesion measures $23 \times 27 \times 34$ mm. B. MRI after 6 months on lapatinib shows regression of central portion of the tumor mass. C. Graph shows PRL levels (ng/mL) and tumor volumes (mm³) at baseline (study visit 1) and at monthly intervals. Source: Modified from Cooper O, et al. Prolactinoma ErbB receptor expression and targeted therapy for aggressive tumors. *Endocrine*. 2014; 46(2):318-27.

Table 1

Expression of ErbB receptors and ligands in pituitary.

	Ligands		Receptors	
	EGFR	TGF α	EGFR	ErbB2
Normal pituitary				
Corticotroph	$\pm$	+	+	+
Gonadotroph	+	+	+	+
Lactotroph	+	+	+	+
Somatotroph	+	+	+	+
Pituitary adenomas				
Corticotroph	+	–	++	+
Gonadotroph	+	$\pm$	+	+
Lactotroph	+	NA	+	+
Somatotroph	+	NA	+	+

EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; TGF, transforming growth factor. +, present; ++, highly expressed; –, absent; NA, no data available.

Based on data from Cooper O, Vlotides G, Fukuoka H, Greene MI, Melmed S. Expression and function of ErbB receptors and ligands in the pituitary. *Endocr Relat Cancer*. 2011;18(6):R197-211.