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RESEARCH HIGHLIGHT

# Cushing's disease: towards precision medicine

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**The pathogenesis of Cushing's disease is poorly understood; two recent reports identifying somatic mutations in *USP8* in pituitary corticotroph tumors provide exciting advances in this field. These mutations alter EGFR trafficking and signaling, raising the prospect that EGFR inhibitors may move the treatment of this disease into the era of precision medicine.**

Cushing syndrome (CS), a condition of chronic exposure to excess glucocorticoids, poses great clinical challenges. The diagnosis is hindered by overlapping clinical features and biochemical test results with other more common disorders, such as obesity. Moreover, this disorder can be severe and potentially fatal, leading to cardiovascular disease, metabolic syndrome, susceptibility to infections, and mood disorders [1].

Treatment with supraphysiologic doses of exogenous glucocorticoids is the most common cause of CS. Endogenous hypercortisolism is rare and may result from autonomous adrenal cortisol production or from excessive adrenocorticotropic hormone (ACTH) stimulation of the adrenals. Excess ACTH may derive from tumors of pituitary corticotroph cells, referred to as Cushing's disease (CD), or from ectopic sources. Corticotroph tumors comprise the majority of cases of endogenous hypercortisolism. The diagnosis of CD is challenging — the search for an underlying pituitary tumor can be difficult and differentiation from an ectopic ACTH-secreting tumor may require multiple invasive procedures.

The causes of CD are largely unknown. While generally not familial, CD has been reported in association with multiple endocrine neoplasia type 1 due to mutations in *MEN1*, familial isolated pituitary adenoma (*AIP*), McCune-Albright syndrome (*GNAS*), Carney complex (*PRKAR1A*), and MEN4 (*CDKN1B*) [2]. However, these mutations account for only rare cases of CD. Moreover, somatic mutations have only rarely been reported in association with corticotroph adenomas.

In this landscape of a dearth of insight into the pathogenesis of CD, recent reports from two independent groups demonstrating the association of somatic mutations in *USP8* with corticotroph tumors, identified by whole-exome sequencing of tumor tissue DNA, represent an exciting leap forward in our understanding of the pathogenesis of this disorder and may contribute to important advances in targeted therapy [3, 4]. One of these reports identified mutations in 6 of 16 ACTH-secreting adenomas (ACTH adenomas) [3], while the other identified mutations in an impressive 75 of 120 ACTH adenomas [4]. Interestingly, somatic mutations in *USP8* demonstrated remarkable specificity for CD, with no mutations found in any of 64 somatotroph adenomas, 60 prolactinomas, or 60 nonfunctioning pituitary adenomas screened in these studies, and only rare somatic mutations reported in other malignancies. These data indicate that *USP8* mutations are common and unique genetic alterations in ACTH adenomas [4]. Patients harboring ACTH adenomas with *USP8* mutations were more commonly female, had smaller tumors, and although ACTH and cortisol levels tended to be the same or even lower than in patients without *USP8* mutations, they had proportionally more ACTH production when accounting for tumor size [3, 4].

USP8 is one of ~90 deubiquitinases (DUBs) encoded by the human genome [5]. Ubiquitination is a reversible post-translational protein modification that regulates the fate and function of proteins in eukaryotic cells, typically targeting them for lysosomal degradation, although other cell fates can also ensue [6]. USP8 is a deubiquitinating enzyme that protects growth factor receptors from degradation, including EGFR. In all cases, *USP8* mutations reported in ACTH adenomas were heterozygous, suggesting a gain-of-function effect. Strikingly, all mutations identified were in or adjacent to a 14-3-3 protein binding motif (RSYSS) of the USP8 protein, a domain highly conserved across species. Among several mutations tested, all inhibited 14-3-3 protein binding to USP8 and resulted in reduced EGFR ubiquitination. Further exploration of the mechanism of these effects revealed that the DUB activity of USP8 is activated by cleavage immediately upstream of the RSYSS sequence into 90 and 40 kD moieties, with the 40 kD fragment possessing the DUB activity. The mutations identified in USP8 disrupt 14-3-3 binding, which is postulated to allow a protease to access the RSYSS sequence, leading to pathologically increased generation of the DUB active fragment. The nature of this putative protease is yet to be identified.

EGF binds to and activates EGFR, causing its endocytosis into endosomes and lysosomes. The 40 kD, DUB active fragment of USP8 deubiquitinates EGFR, allowing the receptor to be recycled back to the cell surface [3, 4]. EGFR deubiquitination is increased by the identified USP8 mutants, leading

to inhibition of degradation, thereby preventing downregulation of ligand-activated EGFR and resulting in augmented and more sustained EGF signaling (Figure 1).

Using a corticotrope-derived cell line transiently transfected with EGFR as a model, pro-opiomelanocortin (*POMC* — the gene encoding ACTH) promoter activity and ACTH secretion were increased by the USP8 mutants [3]. Furthermore, it was observed that ACTH adenomas expressing mutant USP8 had higher EGFR levels, expressed more *POMC* mRNA, and had higher ACTH production than those with wild-type USP8. *USP8* knockdown in primary ACTH-secreting tumor cells reduced ACTH secretion and EGFR levels, suggesting that inhibition of USP8 activity may be an effective treatment strategy [4]. Moreover, gefitinib, an EGFR inhibitor, inhibited ACTH secretion and corticotroph tumor cell proliferation, and increased apoptosis in corticotroph cells and in mouse models, suggesting that EGFR inhibitors may be a particularly beneficial therapeutic strategy for those ACTH adenomas with *USP8* mutations [3, 4, 7]. USP8 likely regulates other receptor tyrosine kinases and additional pathways that may also drive ACTH production and secretion, and these additional pathways may also represent potential therapeutic targets. The protease that cleaves USP8, yet unknown, is another potential target for therapy in CD.

In the normal anterior pituitary gland, USP8 is localized primarily to the cytoplasm. ACTH adenomas harboring *USP8* mutations displayed increased USP8 immunoreactivity, predominantly nuclear in distribution. While *USP8* mutation analysis may be useful in the diagnosis of CD, immunostaining for subcellular localization may be an even simpler screen, potentially as a predictor for increased responsiveness to EGFR inhibitors. Interestingly, even in some ACTH adenomas without *USP8* mutations, USP8 expression was increased and subcellular distribution was predominantly nuclear, suggesting that other mechanisms might contribute to increased USP8 activation and CD in these patients.

USP8 also colocalizes with other anterior pituitary hormones, raising questions about why mutations are causative specifically for corticotroph adenomas and not other pituitary tumor subtypes. The selective association of somatic *USP8* mutations with ACTH adenomas, in contrast with the preferential association of *AIP* and *GNAS* mutations with GH and PRL adenomas, points to genetic heterogeneity of pituitary adenomas and highlights the fact that ACTH-secreting pituitary adenomas are pathogenetically distinct. It is not yet known whether USP8 plays a role in 'silent' ACTH pituitary adenomas (which express ACTH but are not associated with excess cortisol production) or in ectopic ACTH secretion by other tumors; if this were the case, there could also be a role for EGFR inhibitors in these disorders.

Taken together, these two studies provide the first identification of a specific driver of corticotroph adenomas, and provide a novel mechanism by which the EGFR pathway is constitutively activated in human tumors. The identification of *USP8* mutations as contributors to the pathogenesis of ACTH-secreting pituitary adenomas represents an exciting advance in our understanding of Cushing's disease and a movement of this disease and its potential treatment into the era of precision medicine.

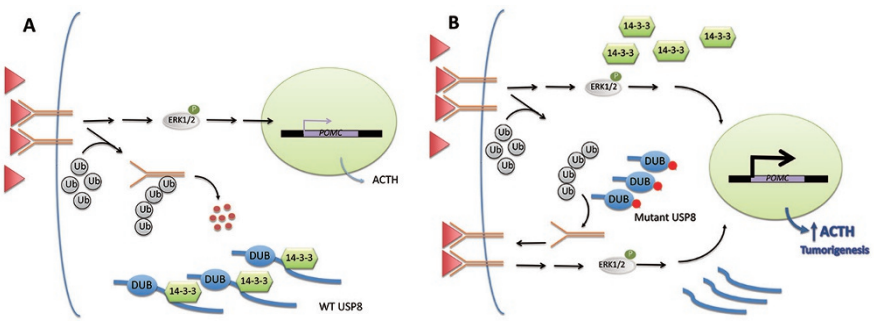


**Figure 1** Model of proposed mechanism of action of mutant USP8 on ACTH secretion in corticotrophs. **(A)** Normal corticotroph. EGF binds to and activates its receptor, leading to ERK1/2 phosphorylation, which in turn leads to increased *POMC* transcription and ACTH secretion. The activated EGFR is internalized, ubiquitinated, and degraded. **(B)** Corticotroph adenoma. In the presence of mutant USP8, 14-3-3 protein is less able to bind, allowing cleavage of USP8 and activation of its deubiquitinase activity. The cleaved, activated USP8 is then able to deubiquitinate EGFR, leading to its increased recycling and expression on the plasma membrane, which in turn leads to increased EGFR signaling, POMC expression and ACTH secretion.

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