USP8 Status Affects the Immune Landscape of Corticotroph Pituitary Adenomas

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Research Article

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Abstract

Purpose

Activating somatic mutations in ubiquitin-specific protease-8 (*USP8*), encoding a deubiquitinating protein, are found in approximately 30% of corticotroph-derived pituitary adenomas (CPA). *USP8* has immunomodulating properties that were demonstrated in non-tumoral diseases. Our study aims to assess the influence of USP8 mutation status on the immune tumor microenvironment (iTME) of CPAs.

Methods

We analyzed 20 PCAs by RNA sequencing. In six of them, *USP8* mutations were detected. We assessed the immune landscape of tumors by quantifying 22 immune cell types based on the CIBERSORT transcriptome signature-recognition algorithms. Also, we performed a pathway analysis for genes that were differentially expressed between groups using the Wikipathways 2019 and Reactome 2016 databases and using the EnrichR platform results.

Results

CPA with activating *USP8* mutations were associated with "cold" iTME compared with wild type *USP8* CPA. This "cold" iTME was reflected by lower fractions of B cells, CD4, regulatory and gamma/delta T cells, natural killer cells, M0 and M1 macrophages, dendritic cells and eosinophils ($p < 0.05$ for all comparisons). Pathways altered by the presence of *USP8* mutation, based on the most differentially expressed genes (3,061 genes) included Microglia Pathogen Phagocytosis and multiple toll-like receptor signaling pathways ($p < 0.0001$).

Conclusion

USP8 status affects the immune landscape of corticotroph pituitary adenomas, with *USP8* mutation associated with "cold" iTME.

Introduction

The prevalence of pituitary gland adenomas (PA) ranges between 1:865 to 1:2688 persons [1]. About two-thirds of them are functional, causing clinical syndrome due to hormonal over-secretion. The minority of PAs (2-6%) show autonomous secretion of adrenocorticotrophic hormone (ACTH), causing Cushing disease (CD) [1]. Cushing disease is the most common etiology of endogenous autonomous cortisol secretion. The derived multisystemic Cushing syndrome is associated metabolic, cardiovascular, cognitive, musculoskeletal, hematopoietic, and immune implications [2]. Patients with CD typically present at their 3rd-4th decade of life with female predominance in a ratio of 3-5:1 [2]. Transsphenoidal

surgery, the first-line intervention, leads to remission in 65–90% of the patients [3,4], but with a recurrence rate reaching 30% in long-term follow-up [4–7]. Second-line interventions are either medical, by blocking cortisol action at its receptor with a glucocorticoid antagonist or blocking cortisol synthesis with a steroidogenesis inhibitor; surgical, by repeating transsphenoidal surgery when feasible; radiation therapy for extrasellar non-resectable disease; or bilateral adrenalectomy as a last resort in patients with otherwise uncontrollable hypercortisolism [1,8,9]. A subset of all corticotroph adenomas (20%) are silent (SCAs), defined by clinically nonfunctioning adenomas which are positive for ACTH on immunohistochemical staining [10,11].

Ubiquitination is a post-translational protein modification that marks proteins for degradation in the lysosome. Ubiquitination is a tightly regulated process [12] and has a major part in regulating various physiological processes. Dysregulated ubiquitination may lead to various diseases, such as malignant, metabolic, neurodegenerative, autoimmune and inflammatory disorders [13]. Ubiquitination and its opposing process, deubiquitination, have crucial roles in immune system development and in immune response through differential activation of specific immune cells [14].

Ubiquitin-specific protease-8 (USP8), encoded by the *USP8* gene, is a deubiquitinating protein that participates in the endosomal sorting of transmembrane proteins. *USP8* gain of function (GOF) somatic variants were recognized as common causative factors in ACTH-producing PAs [15], detected in about 30% of ACTH-secreting corticotroph adenomas [16] and in 20% of silent corticotroph adenomas [17]. USP8 overactivation enhances its proteolytic cleavage and catalytic activity, causing excessive endothelial growth factor (EGFR) deubiquitination and, therefore, activation of the EGFR signaling pathway [3]. The presence of somatic *USP8* variants in CD affects the clinical, morphological, and prognostic course. *USP8*-mutated adenomas are diagnosed earlier [18], show female predisposition [16,18], and are smaller in size compared to wild-type (WT) *USP8* PAs [15]. Moreover, ACTH secreting adenomas with mutated *USP8* are associated with better prognosis reflected by higher remission rates after resection [16,19,20].

USP8 was also studied in the context of several other tumors. In breast cancer, high expression of *USP8* was associated with better outcomes [21]. However, expression of *USP8* in lung adenocarcinoma [22] and cervical squamous cell carcinoma [23] was associated with poorer outcomes compared with *USP8*-negative tumors.

USP8 has an intrinsic immunomodulating role in T cells by regulating the T cell receptor signaling. T cell-specific *USP8* deletion in mice demonstrated its essential role in thymocyte maturation and in T cell hemostasis [24]. In microglia, *USP8* overexpression in neuroinflammatory states suppressed the production of several pro-inflammatory mediators, including nitric oxide (NO), cyclooxygenase-2 (COX-2), and prostaglandin E₂ (PGE₂) [25]. *USP8* downregulation led to increased production of the above-mentioned pro-inflammatory factors, including induction of NO synthase. Following this finding, USP8 was suggested as a novel therapeutic target for neuroinflammatory diseases.

Considering the immunomodulating capacity of *USP8*, in the current analysis, we aimed to study the impact of *USP8* alterations on the immune landscape of pituitary corticotroph adenomas.

Methods

The current analysis was based on a dataset of twenty ACTH-producing adenomas (GEO accession number GSE132982)[17], with data on *USP8* gene mutation status. The sequencing methods have been detailed previously [26]. In brief, formalin-fixed paraffin-embedded (FFPE) samples positively stained for ACTH of patients with Cushing disease (CD, n=12) or "silent" (non-functional, n=8) corticotroph adenomas were processed for RNA sequencing. Differential gene expression analysis had been performed using DEseq2. Matrix including the genes and their normalized read counts per sample, together with the samples metadata, were retrieved and processed in the current analysis. To quantitatively assess the immune landscape of the tumors, we used the web-based tool CIBERSORT [27], with transcriptomic signatures of 22 immune cell types, to delineate the fraction of each immune cell per sample. All values were normalized by extracting quintiles per each variable. Plots were produced using ggplot2 [28], and statistical comparisons were added using ggpubr [29]. Immune cell subtype fractions between the groups were compared using the Mann-Whitney U test. In Addition, we performed a pathway analysis for genes that were differentially expressed between groups (false discovery rate [FDR] adjusted p-value <0.05), based on the Wikipathways 2019 and Reactome 2016 databases [30,31] and using the EnrichR platform [32].

Results

This is a secondary analysis of twenty ACTH-positive pituitary adenoma tissue samples' transcriptome data [17]. The sample consists of six adenomas with a gain of function mutations in *USP8* and 14 tumors with *wild-type* (WT) *USP8* alleles. Comparison of 22 immune cell types by *USP8* mutation status revealed an overall "cold" iTME in tumors with mutated USP8, whereas WT-USP8 samples had a "hot" iTME (**Figure 1**). The "cold" iTME was reflected by lower fractions of immune cells, including B cells, CD4, regulatory and gamma/delta T cells, natural killer cells, M0 and M1 macrophages, dendritic cells, and eosinophils (p<0.05 for all comparisons).

To assess the pathways altered by the presence of *USP8* mutation, we identified the most differentially expressed genes (3,061 genes) between WT-*USP8* and mutated *USP8* pituitary adenomas. Pathway enrichment analysis based on the WikiPathways 2019 Human database identified two pathways: Microglia Pathogen Phagocytosis Pathway (WP3937, FDR-adjusted p-value = 0.00008) and regulation of toll-like receptor (TLR) signaling pathway (WP1449, p=0.00001). A similar analysis based on the Reactome database identified enrichment of multiple TLR-based pathways, including TLR2 and TLR4 signaling related to M1/M2 macrophage polarization, in Addition to MyD88 and IRAK4-related pathways that have previously been associated with USP8 immunomodulation (**Figure 2**).

Discussion

In the current study, we assessed the impact of *USP8* on the immune tumor microenvironment (iTME) of corticotroph pituitary adenomas (CPA) using an advanced transcriptome-based deconvolution algorithm. Our analysis demonstrated for the first time that activating *USP8* mutations are associated with a "cold" iTME and that the differentially expressed genes in the *USP8*-mutated tumors were enriched mainly to immune-related pathways.

The impact of *USP8* on the iTME may be induced directly by the USP8 immunomodulatory effect or indirectly through EGFR activation. Directly, the Rhodanese domain in USP8 stabilizes neuregulin receptor degradation protein 1 (Nrpd1) [33], which suppresses pro-inflammatory cytokine secretion and induces alternative polarization of macrophages to M2 macrophages through toll-like receptors [34–36]. We demonstrated the enrichment of TLR-related pathways in CPA by *USP8* mutations status and higher M2 macrophages fractions during USP8 activation in CPAs with *USP8* upregulation. Additional pathway enriched in mutated CPAs was associated with MyD88 [34] and IRAK4 [37], that interact with and are affected by TLR interactions, respectively. Altogether, these findings strongly suggest a direct effect of USP8 activity on the iTME. However, USP8 may also affect the iTME through the activation of the EGFR signaling pathway, as reported in lung cancer [38]. EGFR signaling may modulate the immune response by several mechanisms, including upregulation of the immune checkpoint programmed cell death ligand 1 (PD-L1) [39–41], repression of MHC class I and II expression [42], and prevention of T cell infiltration [43].

In contrast to most tumors in which immunodeficient tumor environment is associated with less favorable tumor behavior [44], CTAs with USP8 mutation and "cold" iTME are smaller in size [16] and have high remission rates after surgery [17, 20, 21]. Our findings suggest that the favorable outcome of patients with *USP8*-mutated corticotroph adenomas is not immune-response dependent.

The main limitation of our study is the small sample size which is explained by the lack of transcriptome data on these rare tumor types. Nevertheless, it enabled us to characterize the immune landscape of these neoplasms compared to their somatic alterations.

In conclusion, our data demonstrate for the first time a distinct immune landscape of corticotroph adenomas by *USP8* status.

Declarations

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Availability of data and material- Not applicable

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Authors' contributions- Not applicable

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Figures

Figure 1

Immune cell quantification compared by USP8 mutation status in corticotroph pituitary adenomas (n=20).

Figure 2

Pathway enrichment analysis based on the WikiPathways Human Database 2019 and Reactome 2016 database, based on 3,061 genes differentially expressed between corticotroph pituitary adenomas with wild-type vs. mutant USP8 gene.