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The Molecular Pathology of Cushing Disease: Are We Nearly There?

Ashley B. Grossman

Oxford Centre for Diabetes, Endocrinology, and Metabolism, University of Oxford, Oxford, OX3 7LE, United Kingdom

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Pituitary tumors are not uncommon: minuscule microadenomas can be found in up to one quarter of autopsy specimens, whereas clinically significant tumors are present in ~1/1000 of the general population [1]. They may present as local space-occupying lesions, especially when nonfunctioning, in which case they are often several centimeters in diameter impinging on local structures such as the optic apparatus. However, when they retain hormone-secretory characteristics of their cell of origin, such tumors may cause profound and occasionally devastating clinical effects, even when relatively small. This is especially the case with corticotroph tumors, whose mean diameter is of the order of 6 mm. Making some simple assumptions regarding mean cell size, this leads to the conclusion that such tumors comprise, to an order of magnitude, ~10 billion cells, a large number, but still among the smallest of neoplasms. Such tumors must have grown to be their present size, but in many cases further growth does not seem to occur, at least in the medium term, and progression to a pituitary carcinoma is—fortunately—exceedingly rare. Such pituitary tumors, and there is an increasing move to use this term in place of "adenoma" or even "neoplasm," should offer an ideal model system to study the earliest stages of oncogenesis, or tumorigenesis if one wishes to avoid any cancer connotation. This is especially the case for corticotroph tumors, as in contrast to microprolactinomas they are usually treated surgically in the first instance, thus providing histopathological material. There is evidence that they are monoclonal, at least for the most part, although data supporting oligoclonality are also scattered throughout the published literature [2]. If we accept the multistep theory of tumorigenesis, whereby a single cell mutates through a sequence of genes to gradually inherit the cardinal features of a metastatic malignancy [3, 4], then it could be speculated that these tiny corticotrophinomas should harbor the fewest number of genetic mutations, possibly just a single critical change—a driver mutation—which would allow it to undergo selective proliferation. In this Darwinian scheme, a new steady state of tumor size with increased hormone secretion should be reached, exactly as is seen in pituitary-dependent Cushing syndrome, Cushing disease.

1. Molecular Pathology: Classic Approaches

To establish the nature of such a genetic aberration, various approaches have been used in the past. These have included the exploration of gene abnormalities seen in inherited disease states associated with Cushing disease to see whether these could be extrapolated to sporadic tumors: essentially, are these known germline genes responsible for somatic mutations? An alternative but complementary approach has been to postulate some form of

Abbreviations: CDKI, cyclin-dependent kinase inhibitor; EGF, epidermal growth factor; EGF-R, EGF receptor; USP8, ubiquitin-specific protease-8.

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abnormal feedback, such that a mutated cell would not see the correct cortisol level, would secrete excessive ACTH as a compensatory mechanism, and this would in turn suppress surrounding corticotrophs. This resetting would parallel exactly the situation seen in Cushing disease, with a resetting of the feedback point for cortisol (resistance to low doses but sensitivity to high doses of dexamethasone); this would include mutations of the glucocorticoid and ACTH receptors on corticotrophs, as well as of their DNA-regulatory agents. Unfortunately for this theory, and also for the many germline syndromes investigated, there is little evidence favoring mutations in such candidate genes in sporadic tumors [5–7].

Another approach has been to explore possible derangements in known pathways and processes involved in cell division and proliferation. Our knowledge of such pathways, especially in terms of signaling, has grown in an exponential manner over the last few years, and this knowledge has been applied to pituitary tumors by many groups. Changes in various tumor suppressors and, less commonly, oncogenes have been described in pituitary tumors compared with normal tissue (the problem of obtaining and interpreting normal tissue has bedeviled this work), especially of cyclins and cyclin-dependent kinase inhibitors (CDKIs). Corticotroph tumors express a very low level of the CDKI p27 [8, 9], leading to upregulation of cyclin E and thus, in theory at least, enhanced proliferation [10, 11]. However, these changes seem a response to enhanced phosphorylation of p27, increasing its degradation and preventing its import into the nucleus; this in turn may be secondary to upregulation of the serine-threonine kinase Akt. Further studies have also demonstrated increased expression of parts of the mitogen-activated protein kinase pathway [12]. Thus, the intra- and extranuclear aberrations in cyclins and CDKIs may represent a reprogramming of the regulators of cell division by cytoplasmic pathways. Such pathways diverge outward from the cell surface, from where a panoply of growth factor receptors activate and coordinate these pathways. Aberrant splicing of the fibroblast growth factor receptor 4 has been postulated as one such dysregulation [13]. Nevertheless, whereas changes in the expression of such surface receptors have been documented, specific mutations are not a common feature. One relevant finding has been the increased expression of the epidermal growth factor (EGF) receptor (EGF-R) on many tumors [14]. Specifically, it has been known for nearly 40 years that EGF is stimulatory to the hypothalamo-pituitary-adrenal axis [15, 16], but the studies from the Munich group identified EGF-R expression on pituitary tumors, being expressed most frequently (83%) on corticotroph tumors [14].

2. Molecular Pathology: New Approaches

However, with the advent of rapid whole-exome sequencing, a new avenue for the identification of single-gene defects in pituitary tumors has opened. Using this technique, several series of nonfunctioning and somatotroph tumors have been studied in depth [17–19]. Rather surprisingly, the results have been generally disappointing: there have been no novel recurrent mutations seen in any of these tumors. Why is this? It is possible that such mutations are present in the noncoding regions of DNA, which clearly comprise the vast majority of the human genome—dark matter [3]: this is not junk DNA, but almost certainly consists of highly complex regulatory networks, including, but not exclusively, microRNA. Another possibility is that the primary mutational event occurs in a stem cell niche, and these in turn lead to secondary tumor formation mediated by the releases of local cytokines and related agents; such a process has been shown to occur in other systems, including a mouse model of craniopharyngiomas [20]. Or maybe this whole model of mutation-contingent tumorigenesis is simply wrong, but it is difficult to envisage what might replace it.

Nevertheless, this technique of whole-exome sequencing has been spectacularly successful in the study of corticotroph tumors. A collaboration from Germany and Japan originally sequenced a small group of ten corticotroph tumors, and noted a recurrent series of mutations in 4 of 10 in a restricted region in a gene transcribing the mRNA for the peptide ubiquitin-specific protease-8 (USP8): this peptide decreases the ubiquitination and hence degradation of EGF-R as it is recycled from the cell surface [21, 22]. The specific mutation sites block its

association with 14-3-3, leading to increased activity of USP8 and consequent diminished ubiquitination of the EGF-R: the surface expression of EGF-R is enhanced, leading to activation of cell signaling pathways, causing increased ACTH secretion. A recent survey has shown that some 36% of corticotroph tumors harbor this USP8 mutation, mainly those in young women [23].

3. Mechanism of Action of USP8

Now, in an attempt to confirm the pivotal causal role of USP8 in Cushing disease, and to further explore its mechanism of action, Melmed and colleagues [24] at Cedars-Sinai in Los Angeles in this issue of JES have generated a mouse transgenic that has enhanced EGF-R expression. They used a promoter and enhancer for corticotrophs, which specifically enhanced EGF-R in these cells. Immunostaining confirmed increased EGF-R in the mouse corticotrophs. The majority of these mice developed corticotroph tumors similar to an aggressive human Cushing disease phenotype, both histologically and pathologically. Such changes were antagonized by the EGF-R tyrosine kinase inhibitor gefitinib. Furthermore, this appears to be mediated by increased E2F1 and specifically its phosphorylation at Ser^{337}. Cyclin E was slightly increased, but significantly no change in p27 was seen. They suggest that the signaling pathway follows a sequence of EGF-*R*–E2F1–cyclin E, but, unlike the usual human corticotroph tumor, there is no significant role for p27. Another glucocorticoid-related feedback mediator, CABLES1, has also recently been isolated, but in this case CABLES1 is strongly correlated with loss of p27 [25]. It will be of great interest to see how this EGF-*R*–E2F1–cyclin E pathway relates to changes in CABLES1.

4. Conclusions

These exciting studies suggest that at last, in 2017, we are on the way to establishing the true cause of Cushing disease, which is responsible for a devastating clinical, metabolic, and cosmetic phenotype. Although the majority of cases can be cured by an experienced transsphenoidal surgeon, the recurrence rate is significant, and when such tumors become aggressive they can be exceedingly difficult to treat. The way forward is clearly to establish the precise molecular aberrations, and to personalize the mutation in given patients to customize treatment in patients with difficult tumors. A molecular understanding will be essential to provide the best possible therapy, with the current study adding further pieces to the molecular jigsaw. It is intriguing that the putative role of EGF in modulating the hypothalamo-pituitary-adrenal axis was noted so long ago—there is no new thing under the sun. However, the enigma remains: what of the 60%–70% of patients who do not harbor USP8 mutations? It is possible that there may be other mutational changes in the pathways leading to increased EGF-R expression, but these have not been identified by whole-exome sequencing to date. There is still much to learn.

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Address all correspondence to: Ashley B. Grossman, MD, University of Oxford, Oxford, OX3 7LE, United Kingdom. E-mail: ashley.grossman@ocdem.ox.ac.uk.

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