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Genomic insights into Cushing syndrome

L'apport de la génomique dans le syndrome de Cushing

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Abstract

In the setting of Cushing syndrome, genomic analyses can be performed either in tumors responsible for endogenous Cushing, or in patients exposed to glucocorticoid excess. Genomics of tumors identified several new genes – including *ZNRF3* in adrenocortical carcinomas, *PRKACA* in cortisol-producing adrenal adenomas, *ARMC5* in primary macronodular adrenal hyperplasia and *USP8* in pituitary corticotroph adenomas. These genes shed new lights on the mechanisms responsible for these tumors. Integrated genomic studies of adrenal carcinomas identified distinct molecular classes, with remarkably different prognostic outcome. Beyond the mechanistic novelties, a new generation of prognostic markers emerges, with potentially important impact on patients care. For the future, genomic efforts should be pursued, focusing on poorly characterized tumors responsible for Cushing syndrome – including endocrine tumors secreting ACTH. In addition, epigenomics is emerging as an outstanding set of tools for characterizing tumors, unraveling unprecedented aspects of tumorigenesis. Applying these tools to endocrine tumors responsible for Cushing syndrome may also lead to important discoveries. Genomics of patients exposed to glucocorticoid excess is an emerging research field. Proof of principle studies have been performed, identifying molecular markers of glucocorticoid excess in blood. Research efforts should now concentrate on markers of mild glucocorticoid excesses – endogenous or exogenous –, owing to their high prevalence in general population. In addition, markers of individual susceptibility to each type of glucocorticoid complication are needed. It remains to be determined whether genomics can identify such markers.

Keywords: Genomic; Cushing syndrome; Glucocorticoid

Résumé

Dans le cadre du syndrome de Cushing, des études basées sur la génomique ont été réalisées pour caractériser soit les tumeurs responsables du syndrome de Cushing endogène, soit les patients exposés à un excès de glucocorticoïdes. La génomique des tumeurs a identifié plusieurs nouveaux gènes – *ZNRF3* dans les carcinomes surrénaliens, *PRKACA* dans les adénomes surrénaliens produisant du cortisol, *ARMC5* dans l'hyperplasie macronodulaire primitive des surrénales, et *USP8* dans les adénomes hypophysaires corticotropes. Ces gènes apportent un nouvel éclairage sur les mécanismes responsables de ces tumeurs. Les études génomiques intégrées des carcinomes surrénaliens ont identifié des classes moléculaires distinctes, associées à un pronostic remarquablement différent. Au-delà des nouveautés en termes de mécanismes, une nouvelle génération de marqueurs pronostiques en ressort, avec un impact potentiellement important sur la prise en charge des patients. Dans l'avenir, les efforts génomiques doivent être poursuivis, en se concentrant sur les tumeurs responsables de syndrome de Cushing mal caractérisées – notamment les tumeurs endocrines sécrétant de l'ACTH. De plus, l'épigénomique apparaît comme un ensemble d'outils remarquables pour caractériser les tumeurs, révélant des aspects originaux de la tumorigenèse. L'application de ces outils aux tumeurs endocrines responsables du syndrome de Cushing peut également conduire à des découvertes importantes. La génomique des patients exposés à un excès de glucocorticoïdes est un domaine

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de recherche émergent. Des études préliminaires ont été réalisées, identifiant des marqueurs moléculaires de l'excès de glucocorticoïdes dans le sang. Les efforts de recherche devraient maintenant se concentrer sur les marqueurs d'excès de glucocorticoïdes a minima – endogènes ou exogènes – en raison de leur prévalence élevée dans la population générale. En outre, des marqueurs de susceptibilité individuelle à chaque type de complications secondaires à l'exposition aux glucocorticoïde sont nécessaires. Il reste à déterminer si la génomique peut identifier de tels marqueurs.

Mots clés : Génomique ; Syndrome de Cushing ; Glucocorticoïde

Genomics include a set of large-scale molecular measurements, applicable to any biological tissue. These techniques provide unbiased molecular profiles, and can be applied to Cushing syndrome in two main ways: either to tumors responsible for Cushing syndrome, or to patients exposed to glucocorticoid excess. Such studies provide insights on pathophysiological mechanisms, and markers potentially relevant for diagnosis, prognosis and prediction to treatment response.

1. Genomic profiling of tumors responsible for endogenous Cushing

Tumors responsible for Cushing syndrome include adrenal tumors – carcinomas, adenomas, dysplasia and hyperplasia –, pituitary adenomas secreting ACTH, and endocrine tumors with paraneoplasic ACTH secretion. Genomic analyses have been applied to some of these tumor types.

1.1. Exome sequencing in tumors: it's raining genes!

This is probably the most spectacular outcome of genomics. For almost all tumors responsible for Cushing syndrome, exome sequencing revealed new driver genes.

In adrenal cancer, exome sequencing identified *ZNRF3*, encoding for an ubiquitin ligase, regulating negatively Wnt/beta-catenin pathway [1,2]. These mutations are mutually exclusive from *CTNNB1* mutations – encoding beta-catenin, which is commonly mutated in these tumors as well –, and underline the importance of this pathway in adrenal cancer.

In adrenocortical adenomas, exome sequencing identified *PRKACA* mutations in up to 30% of patients, encoding the catalytic subunit of Protein-Kinase A [3–6]. In primary macronodular hyperplasia, *PRKACA* locus duplications were also identified, and seem rare [6]. *PRKACA* mutations enlarge the list of mutations affecting PKA/cAMP pathway genes–*GNAS*, *PRKAR1A*, *PDE8B* and *PDE11A*- previously identified in rare forms of benign adrenal diseases associated with overt Cushing.

In primary macronodular adrenal hyperplasia, exome sequencing identified *ARMC5* mutations in 25 to 50% of cases with overt Cushing and large multinodular adrenals, encoding for a protein of unknown function [7]. Mutations are germline, along with a somatic second hit specific to each adrenal nodule.

In corticotroph adenomas, exome sequencing identified *USP8* mutations [8,9], encoding for a deubiquitinase, in up to 30% of cases. The precise link between *USP8* and tumorigenesis remains to be elucidated.

1.2. Integrated genomics of tumors

The molecular landscape of adrenocortical carcinomas has been extensively characterized by studies combining genomic approaches [1,2]. Distinct molecular classes have emerged, based on important differences in terms of gene expression signature, DNA methylation, chromosomal alteration profiles and mutations. Remarkably, these molecular classes are associated with major differences in terms of outcome.

We recently achieved an integrated genomic analysis of benign adrenal tumors, including all types of adenomas, hyperplasias and dysplasias (unpublished). Different genomic approaches converged into molecular classification showing three distinct molecular signatures associated with cortisol secretion:

- one associated with PKA/cAMP pathway alterations, including tumors responsible for overt Cushing, irrespective of their tumor types;
- one associated with *CTNNB1* mutations, including adenomas either non-secreting or with subclinical Cushing;
- and a third class associated with *ARMC5*-mutated macronodular adrenal hyperplasia.

1.3. Genomic alterations in tumors: and so what?

Genomics should be considered as a molecular microscope. Genomic characterization of tumors provides an ultimate unbiased molecular classification of tumors. This comes along with optical microscopes from pathologists, which for a long time was the gold standard for classifying tumors. In addition, in Endocrinology, the hormonal secretion phenotype adds to tumor characterization. In addition to pathology and hormonology, genomic classifications add valuable additional insight in the characterization of endocrine tumors responsible for Cushing syndrome.

In terms of mechanisms, major pathways emerged. In adrenocortical carcinomas, altered pathways may orient towards developing specific therapeutic strategies. Especially, ZNRF3 alterations have extended the importance of the Wnt/beta-catenin dysregulation in these tumors. Therefore targeting this pathway is a promising direction. In addition, the different molecular alterations in adrenal carcinoma should now be distinguished, based on whether the alteration is common to almost all carcinomas – such as IGF2 overexpression –, or whether the alteration is specific to a molecular subgroup. For instance

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