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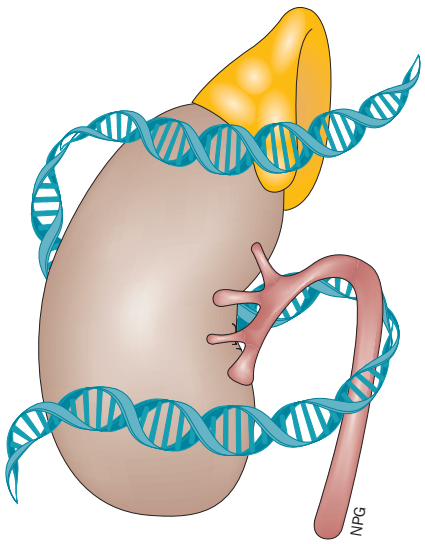
NEUROENDOCRINE CANCER

An activating hotspot mutation in *PRKACA* provides clues for adrenal Cushing syndrome therapeutics

New genetic findings might have important implications for developing therapies against adrenal Cushing syndrome. In a new study published in *Science*, researchers have identified a hotspot mutation in the *PRKACA* gene (which encodes the catalytic subunit of protein kinase A, PKA C) in sporadic adrenocortical adenomas (ACAs) that drives increased catalytic protein kinase A (PKA) function and induces expression of genes associated with tumour growth and adrenal steroidogenesis.

Adrenal Cushing syndrome most frequently results from excess production of cortisol by adrenocortical tumours (ACTs), and occurs independently from adrenocorticotropic hormone signalling. "Comprehensive understanding of the genetic deficiency in adrenal tumours is crucial for the development of novel diagnostic and therapeutic approaches," explains Guang Ning, study leader and Chairman of the Shanghai Clinic for Endocrine and Metabolic Diseases, Rui-Jin Hospital, Shanghai, China.

The researchers explored the landscape of somatic mutations in ACTs by performing whole-exome sequencing of DNA from 49 cortisol-producing ACTs and matched blood pairs. The histological subtypes of the ACTs were confirmed by pathological analysis and included 39 ACAs, seven adrenocorticotropin-independent macronodular adrenocortical hyperplasias (AIMAHs) and three adrenocortical oncocytomas (ADOs).



In all, over 400 candidate somatic mutations were discovered in the study, a subset of which were confirmed by Sanger sequencing. Highly expressed genes with mutations were verified with RNA-seq analysis. Ning and his team identified 12 genes with potential gain-of-function mutations in the study; however, *PRKACA* was the only gene that was significantly mutated ($q = 3.77 \times 10^{-11}$).

The c.T617G/p.L205R hotspot mutation in *PRKACA* was present in 27 of the 49 ACTs (55.1%) and in a staggering 27 of the 39 (69.2%) ACAs analysed. The prevalence of the same mutation in multiple adrenal tumours makes this discovery particularly exciting. According to Ning, "finding a recurrent mutation in the exact same amino acid in nearly 70% of tumours is highly unusual". Interestingly, the L205R mutation in *PRKACA* was found almost exclusively in tumours from adult female patients and was not found in any of the AIMAHs or ADOs that were included in the study.

RNA-seq analysis of tumour tissues revealed 232 differentially expressed genes in ACAs in which the *PRKACA* gene was mutated compared with tumours that retained the wild-type gene. Pathway analysis of the differentially expressed genes showed that Gene Ontology terms associated with steroid and cholesterol synthesis and metabolism and with a response to chemical stimulus were enriched in the tumours with the L205R mutation.

Ning and his team also sought to determine the mechanisms by which the L205R hotspot mutation could contribute to tumour growth. They analysed available crystal structures of the wildtype PKA C protein and mapped the L205 residue to the P+1 loop of the catalytic subunit, which is a critical site for mediating specific binding between the kinase and its substrates. The computational modelling analysis suggested that the tumorigenic effects of the L205R mutation might be driven by altering the dynamics of substrate binding to PKA and by modifying the catalytic activity of the PKA C subunit.

Cell culture studies in which either the mutated *PRKACA* gene or the wild-type gene were overexpressed supported the computational findings. Overexpression of mutant PKA C protein enhanced activation of the PKA catalytic subunit compared with overexpression of wild-type PKA C, and led to increased phosphorylation of PKA substrates, such as cAMP response element binding protein (CREB), and increased transcription of CREB target genes. CREB target genes were also found upregulated in the ACAs harbouring the L205R mutation.

"These data confirm our hypothesis ... that all benign adrenocortical lesions have something to do with aberrant cAMP signalling," says Constantine Stratakis, from the National Institute of Child Health and Human Development, NIH, USA, who was not involved in the study.

"The importance of cAMP/PKA signaling is known in adrenal hyperplasia," agrees Ning, "however, the activating mutations in onocogenes are particularly valuable for designing cancer-specific molecularly targeted therapies."

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