KIDNEY CANCER

Differential mTORC1 pathways in BHD

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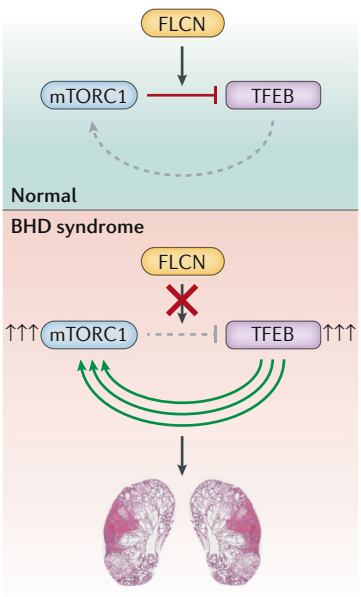
A study in *Nature* has shed light on the mechanisms underlying the pathogenesis of Birt–Hogg–Dubé (BHD) syndrome, an autosomal dominant inherited disorder caused by germline loss of function (LOF) mutations in the folliculin (*FLCN*) gene that predispose to benign and malignant renal tumours.

Napolitano and colleagues investigated the role of mechanistic target of rapamycin complex 1 (mTORC1) activity and its differential interactions with downstream substrates.

Activation of mTORC1 — which controls the cellular response to environmental cues via kinase activity on various substrates — occurs at the lysosomal membrane, where it is mediated by the small GTPase Ras homologue enriched in brain (RHEB). RHEB is induced by growth factors and inhibited by the tuberous sclerosis complex (TSC). Recruitment of mTORC1 to the lysosomal membrane occurs when Rag GTPase heterodimers (RagA or RagB (RagA/B) in complex with RagC or RagD (RagC/D)) are in their active configuration. Rag activation is mediated by the GTPase-activating proteins GATOR1 and FLCN in response to local nutrient levels.



BHD, Birt–Hogg–Dubé; FLCN, folliculin; mTORC1, mechanistic target of rapamycin complex 1. Image adapted courtesy of A. Ballabio.

TFEB, a controller of cell metabolism, is negatively regulated by mTORC1; phosphorylation of TFEB by mTORC1 promotes the cytoplasmic localization of TFEB and inhibits its nuclear translocation. The team first investigated whether TFEB behaves differently to other mTORC1 substrates, S6K and 4E-BP1, at phosphorylation by exposing these substrates to serum and amino acid deprivation. They observed that, whereas S6K and 4E-BP1 phosphorylation could be inhibited by both serum and amino acid deprivation, only amino acid deprivation affected the subcellular localization and activity of TFEB. Accordingly, siRNA silencing of *RHEB* and its homologue *RHEBL1* did not affect the phosphorylation, cytosolic localization or activity of TFEB, but did reduce the phosphorylation of S6K and 4E-BP1. By contrast, knockdown of RagC and RagD impaired TFEB activation and localization, an effect that could be reversed by re-expression of either RagC or RagD. These data suggest that phosphorylation of TFEB by mTORC1 is unaffected by alterations in the TSC–RHEB axis (which is activated by growth factors) but is sensitive to the activation of Rag GTPases.

The requirement of interaction with Rag GTPases for TFEB phosphorylation by mTORC1 is curious, as no other mTORC1 substrates interact with Rag GTPases. The TFEB protein sequence does not contain a TOR signalling (TOS) motif (as is found in S6K), leading the team to hypothesize that TFEB phosphorylation by mTORC1 might be via an alternative substrate-recruitment mechanism involving Rag GTPases. This hypothesis was supported by studies revealing direct interaction between TFEB and a Rag GTPase dimer, which seemed to be via the first 30 amino acids of the TFEB sequence. Furthermore, insertion of the S6K TOS into TFEB made TFEB phosphorylation behaviour sensitive to RHEB depletion and, therefore, similar to S6K, further supporting the hypothesis that differential substrate recruitment mechanisms are responsible for the selective phosphorylation of mTORC1 substrates.

As FLCN, a repressor of TFEB activity, is implicated in BHD syndrome, the team then investigated whether the kidney phenotype of patients with BHD syndrome is induced by TFEB activation. First, they generated mice with a kidney-specific double knockout of FLCN and TFEB and compared their phenotype to mice with kidney-specific knockout of *Flcn* alone. Whereas the *Flcn*-knockout mice had massively enlarged kidneys and died by postnatal day 30, as well as exhibiting increased nuclear localization of TFEB and enhanced mTORC1 signalling, the double knockout mice were phenotypically normal and displayed no signalling abnormalities. These data suggest that constitutive activation of TFEB caused by LOF of FLCN is implicated in the renal phenotype seen in BHD syndrome. "We showed that TFEB is the main driver of the cystic and cancer phenotype of BHD syndrome, as demonstrated by the full rescue of the kidney phenotype in FLCN/TFEB double KO mice," corresponding author Andrea Ballabio tells *Nature Reviews Urology*. "The effect of TFEB on renal cystogenesis and tumorigenesis is due, at least in part, to its ability to upregulate mTORC1. This mechanism is not limited to BHD syndrome but operates in other diseases associated with kidney cysts and cancer."

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