Editorial

Haploid genetic screens identify key regulators of protein phenotypes and signaling transduction

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Genome-wide genetic screen has been a powerful system to study the regulation of a variety of signaling pathways, expression and essentiality of genes, and targeted therapy resistance mechanisms in human cells, especially cancer cells (1-5). However, the direct connection between genomic mutations and the regulation of protein states remains elusive. In a recent paper published in Nature, Brockmann and colleagues utilized gene-trap mutagenesis approach coupled with antibody staining for proteins of interest to execute a series of genetic screens in human haploid HAP1 cells, aiming to investigate the direct link between genomic perturbations and specific protein phenotypes (6).

Technically, the gene-trap mutagenized HAP1 cells (5) were treated to induce desired signaling pathways or directly harvested, followed by fixation, permeabilization, staining with primary antibody specific to protein of interest and fluorophore conjugated secondary antibody, and fluorescence-activated cell sorting (FACS) to obtain two cell populations with low signal and high signal respectively. Then the positive/negative regulators of examined protein state can be identified by comparing the disruptive integrations in either the high- or low-query populations. With the availability of specific antibodies, the authors were able to assess a variety of protein states related to a suite of cellular processes, including signaling transduction (Wnt/β-catenin and interferon signaling pathways), post-transcriptional (splicing) and post-translational modifications (protein phosphorylation, methylation, crotonylation, and glycosylation), and successfully captured known and novel modifiers of the protein states. Interestingly, the same screening approach was recently employed to identify the regulators of the programmed death-1 (PD-1) ligand 1 (PD-L1) protein (7), which is exploited by cancer cells to evade T-cell-mediated immunosurveillance. Importantly, the authors made all processed screen results accessible in an interactive database (https://phenosaurus.nki.nl), thus providing very useful resources to researchers with broad interest.

Subsequently, the authors performed RNA sequencing (RNA-seq) to assess the gene expression levels in HAP1 cells, and observed a positive correlation between the gene's transcription level and its likelihood of regulating protein phenotypes. Comprehensive genetic network analysis further revealed that chromatin-modifying enzymes, such as PRC2 complex, contribute to the regulation of a variety of protein phenotypes, whereas most other regulators are contributors to the specific query trait.

Having proved the power of the genetic wiring screen system, the authors were particularly interested in further understanding the AKT signaling cascade, which is a fundamental pathway for regulating cell proliferation, survival, among others (8), by assessing the phosphorylation of AKT at S473 site (pAKT-S473). The success of the screen was first confirmed by the identification of many known negative (for example, PTEN, INPPL1, and INPP4A) and positive (for example, AKT2, AKT3, RICTOR, and MAPKAP1) factors affecting pAKT-S473. Among the previously unrevealed regulators of pAKT-S473, the authors identified the cullin E3 ligase CUL3 as well as its substrate adaptor KCTD5 as potent negative regulators

in HAP1 cells, the phenotype of which can be further validated in additional cell line models by assessing the pAKT-S473 levels. Interestingly, the increased pAKT-S473 level in KCTD5-deficient cells is not attributed to the alteration in the canonical AKT cascade components such as PTEN and RICTOR (9), suggesting an alternative route controlling the AKT signaling. To further understand the mechanism of action of KCTD5, the authors applied the same screen strategy in KCTD5-deficient HAP1 cells, and were able to identify a class of genes as potent positive regulators of pAKT-S473 exclusively in KCTD5-deficient cells, including G-protein β and γ subunits (GNB1, GNB2, and GNG5), their chaperone protein PDCL which is required to generate Gβγ dimers, as well as their downstream effector PI3KCB. This observation suggested a Gβγ signaling dependent regulatory mechanism of pAKT-S473 by KCTD5.

To demonstrate the potential KCTD5-Gβγ-pAKT-S473 axis, the authors employed the unbiased quantitative proteomics approaches to identify proteins whose abundance and ubiquitination can be increased and reduced, respectively, upon loss of KCTD5, leading to the identification of GNB1, GNB2, and GNG5, encoding Gβγ subunits. To further determine the cellular context in which KCTD5 regulates the degradation of Gβγ, the authors again applied their haploid genetic screen strategy in both wild type (WT) and KCTD5-deficienct cells with GNB1 as the readout, revealing the Gα subunits and the corresponding chaperone protein RIC8A (10,11) as positive regulators of GNB1 level in a KCTD5-dependent manner. Using co-immunoprecipitation assays, the authors confirmed that KCTD5 competes with Gα subunits to bind Gβγ dimers, leading to the degradation. Interestingly, multiple mutations in Gβ proteins GNB1 and GNB2 have been recently identified in a variety of cancers, resulting in the loss of interaction with Gα subunits and concomitant activation of AKT pathway (12). The authors were able to demonstrate that these mutated Gβ proteins also lost their capability to bind KCTD5.

Altogether in this study, Brockmann and colleagues have successfully adapted a powerful haploid genetic screen approach to assess specific protein phenotypes, expanding the understanding of regulation of a variety of signaling cascades. The closely related genetic suppressor screen in cells deficient in specific gene provides a robust platform to investigate the underlying mechanism associated with specific protein phenotypes. However, the success of this screening approach largely relies on the availability, quality, and specificity of antibodies recognizing proteins of interest, limiting its broader application. An alternative route to bypass this issue could be the utilization of CRISPR-Cas9-mediated gene tagging, such as fluorescent protein like GFP, to the genomic locus of interest (13). The identification of KCTD5 as an off-switch for GPCR signaling expanded the understanding of how GPCR signaling can be tightly regulated. Although the authors established the KCTD5-Gβγ-AKT regulatory axis, whether KCTD5 has any implications in diseases or cancers still awaits further investigation.

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None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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