Nearly complete, clone isolation-free CRISPR/Cas9-editing reveals novel functions of lipid-metabolizing enzymes

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Gene disruption is one of the most powerful strategies to study the functions of proteins. The emergence of genome-editing tools, such as CRISPR/Cas9, enabled to knockout genes very efficiently in cell culture and in organisms. In cell culture, however, one pitfall of this strategy in is that due to its incompleteness (editing efficiency does not reach 100% in bulk cells), individual clones have to be isolated to obtain a fully edited cell population. It is known that different clones might have individual characteristics that are unrelated to the genotype, and this might impede the discovery of gene functions. Indeed, we found in a lipidomics study using enzyme-mutated cell clones that the impact of this "clonal diversity" can be higher than the changes caused by the disrupted gene. To overcome this problem, we established a strategy to enrich genome-edited cells without isolating clones. This was based on the optimization of a co-targeting strategy, where a drug-selectable gene is mutated together with the genes of interests. We found multiple parameters that affect the efficacy of this strategy, and after several optimization steps, we established a protocol to obtain polyclonal genome-edited cell populations containing little or no detectable wild type cells, which worked for all the targets we selected. Since this strategy did not involve clone isolation, multiple single-gene knockout cells could be easily generated in parallel. The efficiency to disrupt multiple genes was also drastically improved with this strategy. We found that this strategy eliminates the issue of "clonal diversity" and enables us to detect changes in lipidomics that are related to the disrupted gene. Using this strategy, we found that the regulated metabolism of one class of lipids, the sphingolipids, is important to maintain the composition of another class of lipids, the glycerophospholipids. We also applied this method to analyze the functions of disease-related enzymes and found a previously unknown function of the lipodystrophy-associated enzyme AGPAT2/LPAAT2. We propose that the “clone isolation-free” nature of this new strategy increases the reliability of the results, and is especially useful for quantitative biology. Therefore, this method will be useful not only for lipid research, but potentially all cell biology researches requiring gene disruption.