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The death gaze of MEDUSA

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Chemogenetic profiling can reveal genetic determinants that coordinate phenotypic responses to therapeutics, along with predicting potential pathways of resistance. A new analytical method for evaluating chemogenetic profiles reveals contributions from death-regulatory genes.

The pursuit of effective therapies remains a crucial component of cancer research, and promising drug candidates are initially examined and validated through cell-based studies. In addition, functional genetic screens can identify pathways that regulate a cellular process, and in the case of drug discovery, which genes influence cellular responses upon exposure. When these approaches are combined, the resulting strategy – referred to as chemogenetic profiling – identifies the cellular contexts that determine drug sensitivity and, by extension, contexts that exhibit chemoresistance. Although the goal of conventional and targeted chemotherapeutics is to selectively induce death in cancer cells, many chemogenetic profiling workflows measure changes in cell proliferation, and therefore the results of these screens may conflate the biology that regulates proliferation and cell death outcomes. In this issue of *Nature Chemical Biology*, Honeywell et al.[1] developed an analytical approach for scoring chemogenetic profiles that integrates experimental data with computational models to deconvolute drug-induced changes to both cell proliferation and cell death rates.

A common method for performing functional genetic or chemogenetic screens is to transduce a population of cells with a pooled CRISPR library (Fig. 1, right). Individual cells receive different guide RNAs (gRNAs) and therefore different genes are targeted within each cell. The cells are then exposed to a challenge (for example, drug treatment) and cultured for several doublings before being pooled and sequenced to identify the relative abundance of the gRNAs. The enrichment or depletion of a specific gRNA indicates that the genetic perturbation conferred either an increased or decreased fitness to the drug challenge, respectively[2,3].

Fundamentally, these techniques measure clonal abundance or relative viability, which reflect changes to the proliferation rate of a population, but do not measure or differentiate cytotoxic responses. For example, a smaller population could be the result of: 1) a reduced proliferation rate only; 2) substantial cell death, but an unchanged proliferation rate; or 3) a combination of altered proliferation and cell death. By relying

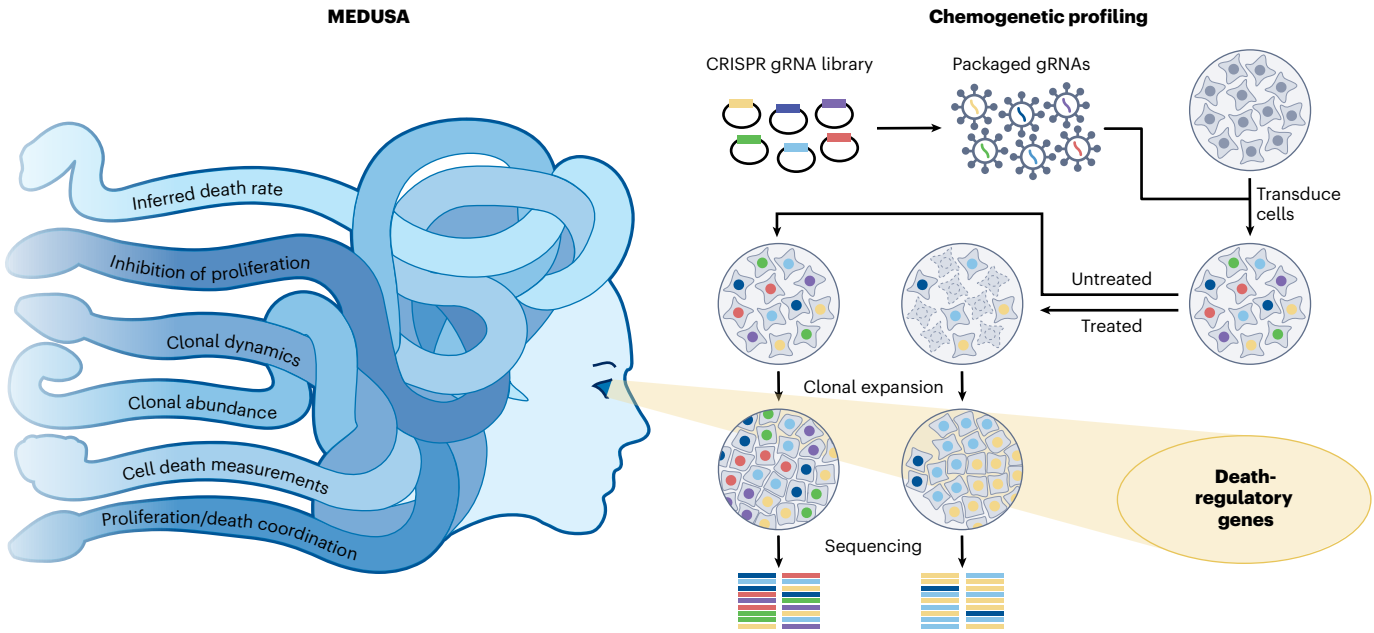

Fig. 1 | A new analytical approach identifies death-regulatory genes in chemogenetic profiling screens. Left: MEDUSA uses models of clonal dynamics derived from time-resolved measurements of drug-induced changes to proliferation and cell death. By leveraging these data and experimentally-derived models, MEDUSA can infer a death rate from measured clonal abundance and calculated proliferation inhibition data. Right: chemogenetic profiling involves transducing cells with a library of CRISPR gRNAs to generate transgenic knockout clones exhibiting gain- or loss-of-function to a drug. After a period of clonal expansion, the relative abundance of each gRNA is measured, and those that are significantly enriched or depleted indicate the genetic determinants of the drug response. Whereas conventional analyses rely only on relative abundance, analysis with MEDUSA extrapolates the death rate of each clone to specifically identify death-regulatory genes that underlie the drug response.

on relative viability as a readout for cell-based screens, quantifying drug sensitivity or assessing synergistic potential becomes more difficult due to the confounding effects of inhibition of proliferation rate[4].

Honeywell et al.[1] demonstrate that traditional chemogenetic profiles measuring relative viability are more sensitive – and even biased – towards detecting proliferation-regulatory genes while failing to identify death-regulatory genes, and this effect is compounded as population doublings eclipse the cell death response. To accurately identify death-regulatory genes using chemogenetic profiling, the authors developed a new strategy to infer the death rate of each transgenic clone, and termed this approach Method for Evaluating Death Using a Simulation-assisted Approach, or MEDUSA. MEDUSA is able to extrapolate a death rate by using simulations of drug-induced population dynamics constructed using a workflow previously developed by this group: 1) a plate-based assay for measuring and parameterizing live and dead cell populations over time[5], and 2) an analysis for characterizing the drug-induced coordination between proliferation and death rates[6]. MEDUSA uses relative abundance and the measured proliferation rate inhibition metrics to calculate the relative death rate according to the model of proliferation/death coordination (Fig. 1, left). By parameterizing both the proliferation and death rates for each gene knockout, the authors demonstrate that MEDUSA improves the accuracy of detecting genetic determinants within chemogenetic profiling.

As a proof of concept, the authors studied DNA damage-induced death in the presence or absence of *TP53*, a commonly mutated tumor suppressor gene that induces both cell cycle arrest and pro-apoptotic signaling downstream of DNA damage[7]. While loss of *TP53* resulted in decreased sensitivity to DNA damaging drugs (that is, continued proliferation), p53 deficiency also led to increased rates of drug-induced death, in apparent contradiction with prior studies measuring viability or apoptosis[8,9]. Experimental and genetic approaches revealed that p53-deficient cells switch to a non-apoptotic mechanism of DNA damage-induced cell death. MEDUSA analysis corroborated these findings, demonstrating a loss of apoptotic gene enrichment in the p53-deficient model. Furthermore, gene enrichment signatures derived from MEDUSA indicated a unique dependency upon the electron transport chain and mitochondrial oxidative phosphorylation in p53-deficient cells treated with the conventional chemotherapeutic agent, etoposide. The authors further determined that the DNA damage-induced cell death in p53-deficient cells is dependent on high NAD^+ levels, and drug-induced lethality was rescued by genetic or pharmacological reduction of the NAD^+ pool.

In this study, Honeywell et al.[1] demonstrated the bias in chemogenetic profiles based on clonal abundance and developed MEDUSA to score the death-regulatory function of genes. MEDUSA appears to be a superior method for assessing gene-dependent drug responses while avoiding false positives and false negatives scored by traditional (that is, relative abundance or viability-based) analyses. Although future investigations are needed to explore the capability of MEDUSA beyond DNA-damaging drugs, MEDUSA should be applicable to a wide variety of drug mechanisms by modifying the underlying model of proliferation/death coordination. Of note, MEDUSA determines death rates through extrapolation and does not require recovery of dead cells, which makes this analysis particularly promising for models that undergo non-apoptotic cell death (for example, ferroptosis and necroptosis). The non-apoptotic cell death literature demands additional insights for its role in both disease and therapy[10], and MEDUSA may be uniquely capable of identifying the genetic determinants to close that knowledge gap. Furthermore, future studies with MEDUSA may aid in characterizing forms of non-apoptotic cell death that remain poorly understood. Collectively, MEDUSA is a promising analytical method for chemogenetic profiling efforts with broad implications in cell death, drug discovery, and cancer research that will likely extend into broader human diseases and therapies.

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Competing interests

The authors declare no competing interests.