Role of Stochasticity in Mammalian Drug Resistance

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***Short Abstract* — How cell-to-cell expression variability or noise contributes to chemotherapy survival and resistance independently from mean expression in cancer is unclear. As a model to this end, we built two synthetic noise-controlling gene circuits based on feedback in Chinese Hamster Ovary (CHO) cells. To decouple noise from mean expression, we chemically controlled the noise of a puromycin resistance gene at a similar mean. Noise delayed the adaptation time, ranging from initial cell death to regrowth, under low drug concentrations, while the opposite was true under high drug concentrations. Mathematical modeling explained these evolutionary dynamics based on the severity of cell drug-sensitivity.**

I. Purpose

Chemotherapy resistance can arise from genetic mutations, but the role of nongenetic processes, including cell-to-cell gene expression variability or noise [1], in survival and evolution is unclear. Gene expression noise can aid yeast cells to survive drug treatment [2], but little evidence exists for subsequent evolutionary effects, especially in genetically-identical mammalian cells. To properly address this question, one must decouple noise (typically quantified as the standard deviation divided by the mean) by experimentally manipulating noise for a drug resistance gene at a similar mean.

The field of synthetic biology rationally designs and builds gene regulatory networks from the ground-up. Synthetic gene circuits can then be engineered to manipulate noise in bacteria [3], yeast [4], and mammalian cells [5] using, for example, positive feedback to amplify noise [6] and negative feedback to minimize noise [7]. However, synthetic gene circuits that can control decoupled gene expression noise in genetically-identical mammalian cells are lacking.

Here, we constructed two synthetic gene circuits that amplify or minimize gene expression noise in Chinese Hamster Ovary (CHO) cells based on positive or negative feedback. We controlled gene expression noise of a puromycin drug resistance gene while maintaining comparable means, thereby allowing a controlled study of noise and mean expression in mammalian drug resistance.

II. Results

With time-lapse microscopy, we measured the adaptation time, spanning from initial cell death to the start of exponential regrowth. Noise delayed the adaptation time under low drug levels, while the reverse was true under high drug levels. A modified population genetics model [8] explained these drug dose- and expression noise-dependent evolutionary dynamics. We found that the evolutionary dynamics depends on the severity of cellular drug-sensitivity. Most of the regrowing cells maintained their resistance after drug withdrawal. In rare cases, cells reverted to their pre-treatment expression levels and regained drug sensitivity.

III. Conclusion

Gene expression noise contributes independently of the mean to the timing of adaptive drug resistance in mammalian cells. Cells with higher levels of noise that are transiently protected from drug may then acquire mutations leading to resistance. This synthetic system indicates that gene expression noise should in general contribute to cancer chemotherapy resistance.

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