KromaTiD

Case Study

DNA Amplification of Synthetic Gene Circuit for Chemoresistance Studies

dGH for Detecting Amplification Events

KromaTiD

Adaptive DNA Amplification of Synthetic Gene Circuit Opens a Way to Overcome Cancer Chemoresistance

Introduction

Drug resistance is still an ongoing challenge for successful cancer treatment. Innovative approaches to understand and counteract the mechanisms of drug resistance are needed.

Gábor Balázsi at Stony Brook University lead a team that used genetically engineered cell lines to create a model system that evolved Puromycin resistance to study the problem.

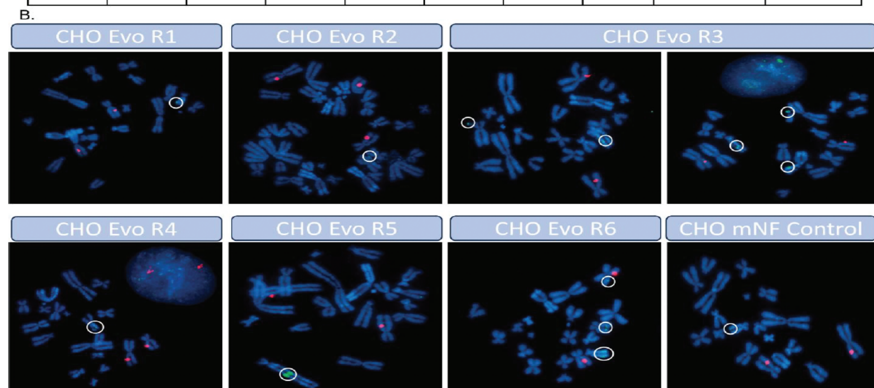
KromaTiD dGH Tech Contribution

Use of qPCR indicated that DNA amplification was involved in the majority of drug-resistant cell lines. KromaTiD's directional Genomic Hybridization (dGH™) in-Site™ assay was used to generate single-cell data to count and characterize synthetic gene circuit amplification events. This included tandem amplifications observed to be present in both chromosomes and ecDNA both implicated in the adaptive overexpression of genes conferring drug resistance.

A.

	Cell with N Inserts							Subsets of Total Inserts	
Sample	1	2	3	4	5	6	Total Inserts	HSR/Tandem	Signal on ecDNA
CHO EVO R1	17	11	1	0	1	0	47	0	1
CHO EVO R2	31	6	1	2	0	0	54	0	1
CHO EVO R3	16	16	10	6	0	0	102	50	7
CHO EVO R4	8	11	15	11	4	1	145	59	0
CHO EVO R5	19	16	8	0	1	0	80	39	0
CHO EVO R6	20	16	3	4	0	1	83	57	0
CHO NF Ctrl	31	5	1	0	0	0	44	1	0

B.



DNA in-situ hybridization confirms circuit DNA amplification.

(A) Analytical summary of total detected target inserts in each CHO EVO sample as well as control Anc5 using dGH in-Site probe. Custom probes were designed to hybridize with a mplified circuit DNA region, while genomic control probes were simultaneously applied for quality control. For each sample, 50 qualified images were analyzed for insert events quantification. Each counted insert was a visible fluorescence event in the chromosome, but actual DNA amplification level varies between inserts. HSR: Homogeneously Staining Regions; ecDNA: Extrachromosomal DNA.

(B) Representative DNA hybridization images for each CHO EVO and control Anc5 sample. Custom probes targeting amplified circuit DNA (Green) were mostly found in the tandem repeat/HSR format while some ecDNA events were also observed in EVO1-3 samples. Genomic control probes (Pink) were used for image quality control.

Conclusion

By combining triplex-forming oligonucleotide (TFO) treatment with the original drug the team was able to overcome the adaptive overexpression response and suppress growth of the CHO cell lines as well as 2 human cancer cell lines, counteracting the drug resistance conferred by DNA amplification. This work raises the possibility of a new treatment approach in combating cancer chemoresistance.

Abstract

Drug resistance continues to impede the success of cancer treatments, creating a need for experimental model systems that are broad, yet simple, to allow the identification of mechanisms and novel countermeasures applicable to many cancer types. To address these needs, we investigated a set of engineered mammalian cell lines with synthetic gene circuits integrated into their genome that evolved resistance to Puromycin. We identified DNA amplification as the mechanism underlying drug resistance in 4 out of 6 replicate populations. Triplex-forming oligonucleotide (TFO) treatment combined with Puromycin could efficiently suppress the growth of cell populations with DNA amplification. Similar observations in human cancer cell lines suggest that TFOs could be broadly applicable to mitigate drug resistance, one of the major difficulties in treating cancer.

PNAS Publication "Adaptive DNA amplification of synthetic gene circuit opens a way to overcome cancer chemoresistance" November 29, 2023 Yiming Wan1, Quanhua Mu1, Rafał Krzysztoń1, Joseph Cohen, Damiano Coraci, Christopher Helenek, Christopher Tompkins, Annie Lin, Kevin Farquhar, Erin Cross, Jiguang Wang, Gábor Balázsi3.