ELRIG ABSTRACT

Abstract Title

Recycling and re-analysis of archived CRISPRn image sets identifies novel regulators of genome integrity

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Abstract

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Target discovery projects through arrayed CRISPRn screening with imaging endpoints can generate vast collections of image data. Once phenotypic endpoints are extracted for hit selection, image sets are normally confined to archives. However CRISPRn image sets may contain valuable information on genetic perturbation far beyond the scope of the original discovery screening project. We performed a proof-of-concept study to show that repurposing of archived CRISPRn screening images can lead to new discoveries and clinically relevant findings.

The presence of micronuclei in cytoplasmic regions is a hallmark of chromosome instability and can be utilised to identify targets with potential genotoxicity for DNA Damage Response inhibitor potential. Micronuclei can be detected in images with standard DNA specific stains. Functional Genomics and Safety Innovation teams within AstraZeneca collaborated to use joint expertise and apply micronuclei quantification algorithms to image sets previously generated from historic arrayed CRISPRn screens. The screens were originally run to identify targets regulating estrogen receptor (ER) stability and mechanisms of ER degradation.

The collaboration reanalysed 2 arrayed druggable and 2 arrayed whole genome screens for micronuclei presence and identified 48 genes (36 novel) which consistently induced micronucleus formation when knocked out. A multi-endpoint genotoxic assessment CRISPRn assay was developed for hit validation and mechanism of action information. The 36 novel genes were screened for confirmation and selected hits were further validated with multiple guide sequences and siRNA.

Our repurposing work uncovered a role of a number of novel genes in maintenance of genomic stability which validated with orthogonal endpoints. It is estimated that extracting data from historic image sets saved a year of scientific work, ~$50k in costs, and over a tonne of plastic waste. This proof of concept study demonstrates that recycling and repurposing of image sets is a sustainable, innovative and cost-effective approach to harness mechanistic insights that may inform target identification.

Disclosure

The authors N.M., V.Q., R.G., J.P., P.O., D.R.T., C.W., L.R., D.G., and A.W. declare the following competing interest: they are employees of, and shareholders in, AstraZeneca.