Genome-wide CRISPR-Cas9 screening identify key nodes regulating the NLRP3 Inflammasome

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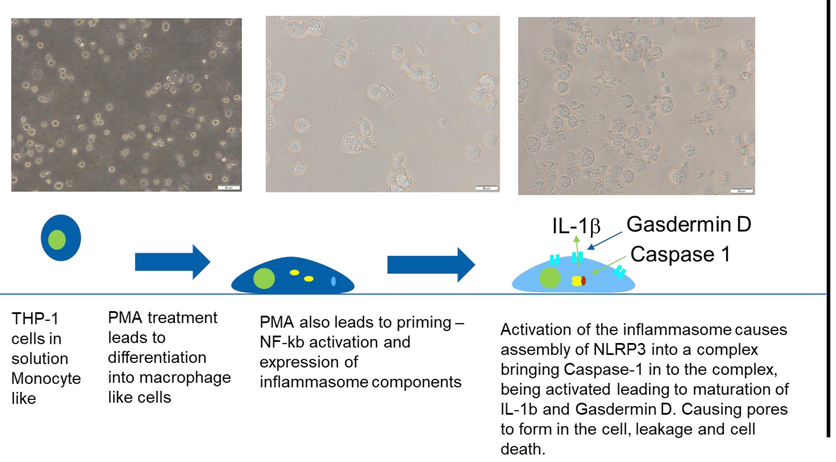
Abstract
CRISPR-Cas9 technology offers an unprecedented opportunity to use phenotypic screens to identify the genetic nodes regulating the activity of a biological pathway. This report describes the application of this technology to screen for genes regulating nigericin induced pyroptosis mediated by the NLRP3 inflammasome in THP-1 cells (a human cell line, resembling macrophages).

Inflammasomes are multiprotein complexes that sense danger or damage-associated molecular patterns, DAMPS, as part of the innate immune system. This recognition leads to the release of cytokines and other signaling molecules that can then lead to cell death. Typically the term inflammasome refers to the complex of proteins including PYCARD (ASC), NLRP3 (NLRC4 or AIM2) and pro-caspase-1. Upon activation by various DAMPS this multi-protein complex promotes activation of caspase-1, which then leads to a cascade of events which cause release of intercellular signals such as IL-1b and IL-18. These danger signals, as well as released intracellular components, can then further activate inflammasomes present in surrounding cells. In addition, the activation of the inflammasome and caspase-1 can also lead to cell death due to the activation of membrane pores such as Gasdermin D. Mutations in components of the inflammasome have demonstrated this as a key pathway regulating autoimmune diseases; e.g. cryopyrin-associated periodic syndrome (CAPS), pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) and Familia Mediterranean Fever (FMF). A detailed understanding of the constituents of the inflammasome, and the pathway leading to its activation, will have utility in designing treatments for a range of diseases associated with inflammation.
This report describes the development of an assay monitoring the induction of inflammasome mediated cell death (pyroptosis). Development of this assay allowed a genome-wide CRISPR-Cas9 screen to identify genes which regulate assembly and activation of the inflammasome. The assay utilized nigericin induction of the NLRP3 inflammasome mediated cell death in PMA differentiated THP-1 cells.

The screen successfully identified known components of the inflammasome as well as a number of genes which have not been previously implicated in inflammasome induced cell death. The use of a genome-wide screen allowed a comprehensive evaluation of the pathways controlling inflammasome assembly and activation. The top 1000 genes were identified for the creation of a focused mini-pool library of potential targets. Retesting of this mini-pool of potential targets confirmed the activity of many of these genes as modulating the inflammasome. So a further selection of genes was then made and these genes were knocked out individually using CRISPR-Cas9.
This presentation will discuss a number of the challenges faced in validation of genes using this system as well as discussing potential means to address these issues.

Background & Motivation

Assay Overview



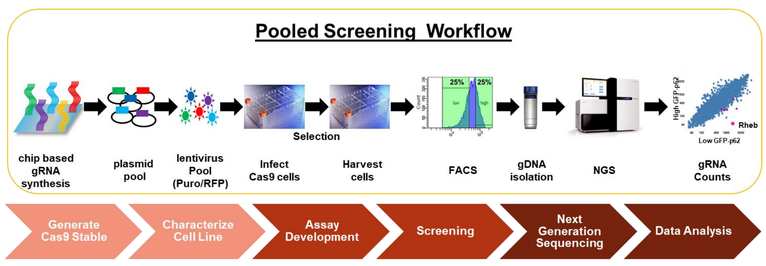
The discovery of truly novel pharmaceutical agents requires assays that monitor novel aspects of biology. The NLRP3 inflammasome (and it's activation subsequently leading to the production of IL-1b) has been implicated in a number of diseases such as neuroinflammation, atherosclerosis, macular degeneration and diabetes). The human cell line THP-1 has been shown to recapitulate the activation of the NLRP3 inflammasome requiring first the cells to be differentiated into macrophage like cells (with the subsequent "priming" of the cells by activation of the NF-kb pathway leading to expression of the inflammasome components). These primed cells can then be activated by a number of secondary stimuli such as nigericin (which forms membrane pores), ATP and crystals such as MSU and CPPD. These signals all cause to changes in the intracellular environment that leads to the assembly of the NLRP3 inflammasome a multi-protein complex that leads to the activation of Caspase-1; which subsequently leads to the generation of mature IL-1b and IL-18; pro-inflammatory cytokines and cell death by pyroptosis.

This study utilized a THP-1 cell expressing Cas9 to screen for genes that would inhibit activation of the NLRP3 mediated inflammasome and subsequent cell death in a genome-wide pooled screening approach. The screen identified most genes known to be involved in NLRP3 mediated pyroptosis as well a number of novel genes. One of these genes was studied further and was shown to have a role in nigericin induced pyroptsis in iPS derived macrophages.

Assay Development

Pooled CRISPR screening

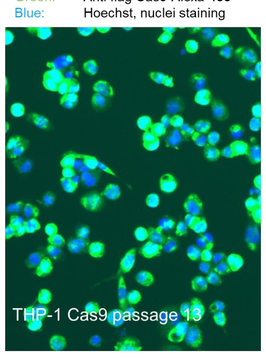
Outline of Workflow



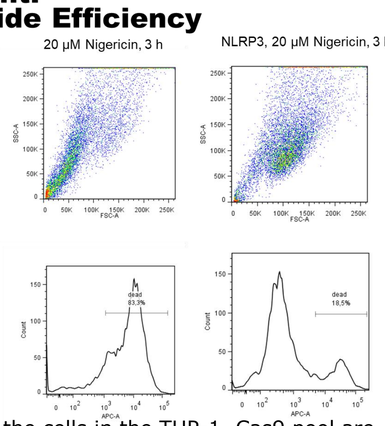
This Figure shows the results of the genome wide CRISPR screen showing genes over (or under represented) in the surviving cell pool after nigericin treatment

Assay Development: Cell line QC & Guide Efficiency

Green: Anti flag Cas9-Alexa 488
Blue: Hoechst, nuclei staining



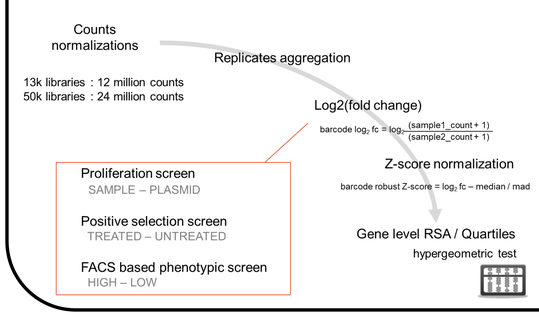
20 µM Nigericin, 3 h | NLRP3, 20 µM Nigericin, 3 h



These figures show, first that the cells in the THP-1, Cas9 pool are expressing Cas9 (as shown by the green fluorescence). The plots from FACS analysis of the cell pools treated with nigericin after knock out of NLRP3 shows cell protection from apoptosis.

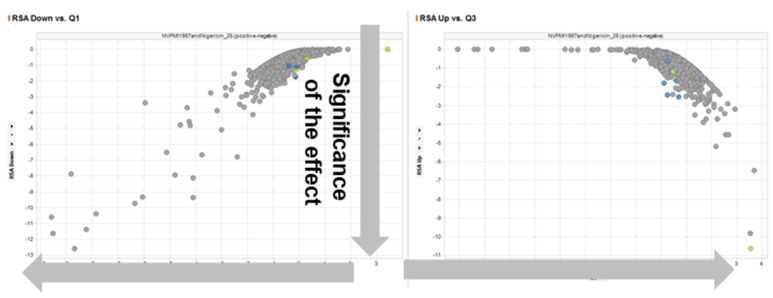
CRISPR Informatics

From counts to genes



In addition to development of an assay reporting on the desired biology a robust data analysis pathway was required, an overview is described on the left.

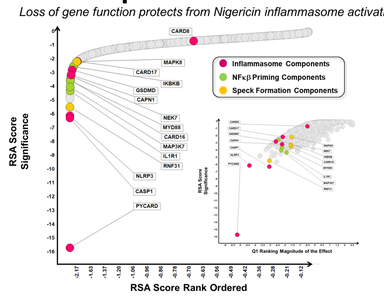
Screening



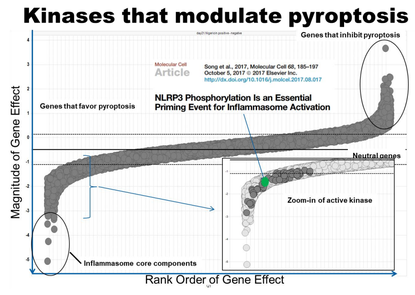
This Figure shows the results of the genome wide CRISPR screen showing genes over (or under represented) in the surviving cell pool after nigericin treatment.

Waterfall plot

Loss of gene function protects from Nigericin inflammasome activation

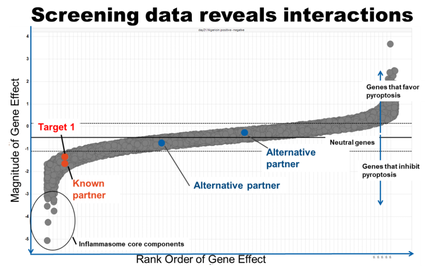


Kinases that modulate pyroptosis

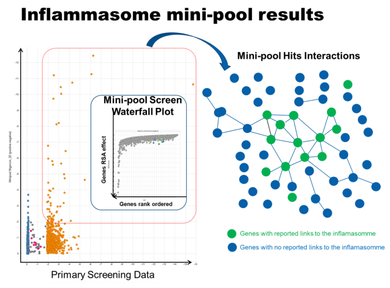


These figures represent the screening results as waterfall plots; emphasizing how the hits are enriched for genes known to be involved in the inflammsaome (in fact all the known components of the NLRP3 inflammasome were identified as hits and a number of kinases (many known to modulate the inflammasome) were also found by such an analysis.

Screening data reveals interactions



Inflammasome mini-pool results



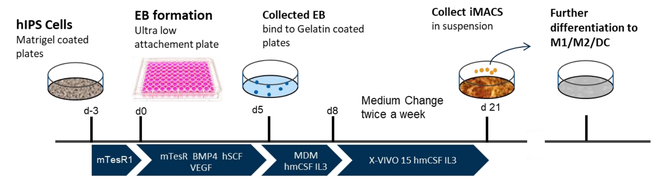
Additional analysis of the results identified previously unreported genes modulating nigericin induced pyroptosis. To further test this first as mini-pool of hits was generated and tested in the assay for initial validation. These genes were then subjected to pathway analysis using the program STRING.

Hit validation

IPS-derived macrophages for genetic target validation

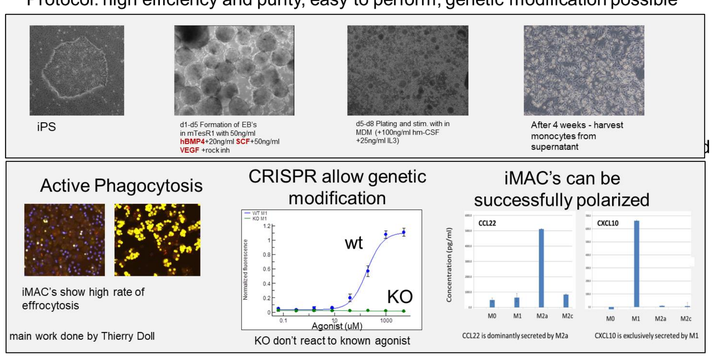
iMACS (M0 macrophages) can be differentiated from hiPS cells in a 21 day protocol using growth factors and cytokines

Further subtype differentiation is possible after the M0 state has been achieved



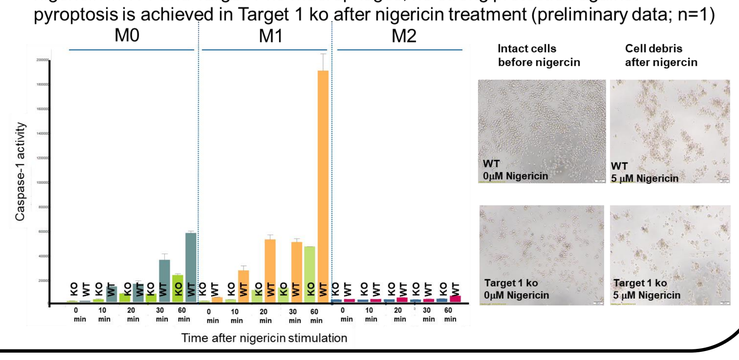
iPS derived macrophages

Protocol: high efficiency and purity, easy to perform, genetic modification possible



ko IPS macrophages show reduced Caspase-1 activation

Caspase-1 activity is reduced or delayed in Target 1 ko, cell lysis is observed after nigericin in WT and Target 1 ko macrophages; no strong protection against pyroptosis is achieved in Target 1 ko after nigericin treatment (preliminary data; n=1)



Conclusions and Summary

1) A genome-wide CRISPR-Cas9 screen was used to monitor the effect of gene knock outs, on the activation of the NLRP3 inflammasome and subsequent cell death. This screen identified most genes known to be involved in the activation of the NLRP3 inflammsome.
2) In addition a number of additional, novel, genes were identified as playing a role in nigericin induced pyroptosis.
3) One of these genes was studied further by monitoring the effect of knocking out this gene in iPS derived macrophages; where the gene had the expected effect leading to significant protection from nigericin induced pyroptosis.