Sandia National Laboratories

SAND2019-13956C

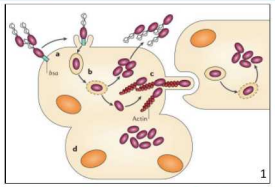
Host-directed CRISPR based countermeasures for Burkholderia

Colleen Courtney[1], Anupama Sinha[2], Debjit Ray[2], Jessica Techel[1], Kunal Poorey[2], Nicole Collette[3], Ashlee Phillips[3], Sahar El-Etr[3], Magdalena Franco[3] and Steven Branda[4]

[1]Biotechnology and Bioengineering, Sandia National Laboratories, Livermore CA [2]Systems Biology, Sandia National Laboratories, Livermore CA [3]Lawrence Livermore National Laboratories, Livermore CA [4]Bioenergy and Defense Technologies, Sandia National Laboratories, Albuquerque NM.

Motivation and Background

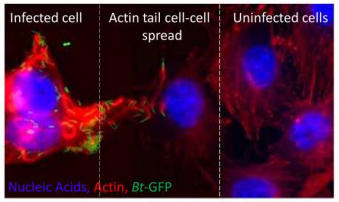
Why *Burkholderia*?



- *Burkholderia pseudomallei* (*Bp*) and *B. mallei* (*Bm*) are Select Agent bacterial pathogens that are intrinsically resistant to most antibiotics and for which no vaccine is currently available.
- Highly virulent, especially via airway.
- Readily isolated from environment.
- High-risk biothreat agent.

Why Host-Directed?

- Bolstering host defense, rather than attacking the pathogen directly, could provide broad-spectrum protection without encouraging development of resistance.

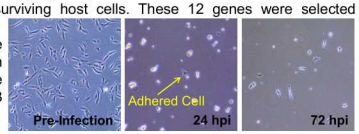


Our approach:

- CRISPR-based genome-wide screen for host gene knock-outs (KOs) that protect human airway epithelial cells (A549) from infection by *B. thailandensis* (*Bt*), a surrogate for *Bp* and *Bm*.
- Develop assays to confirm their protective action and elucidate genetic mechanisms in pathogenesis. Confirmation of protective genes will offer new host drug targets.
- Transition validated, protective genes to primary cell and murine *in vivo* models.

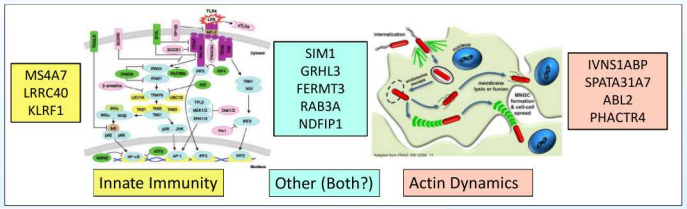
CRISPR-Based Genome-Wide Screen

A library of sgRNAs targeting every gene in the human genome[2] (6 sgRNAs per gene, ~20,000 genes) was introduced into A549 cells that constitutively express Cas9, generating a population of host cells each bearing a single gene KO mutation. This population was challenged with *Bt* at high dose (500-1000 MOI), and the rare host cells that survived were recovered for sgRNA sequencing; enabling identification of both the sgRNAs and gene associated with survival during *Bt* infection. Through three independent genome-wide screens, each featuring eight replicate infection cultures, we identified 12 genes disproportionately targeted for KO in surviving host cells. These 12 genes were selected because they were on the diagonal of results to maximize the number of sgRNA enriched for the individual gene (i.e. 5 out of 6 sgRNA returned in 3 infections) and the number of infections the sgRNA were enriched in the screen (i.e. 3 sgRNA returned in 7 infections) (table below).

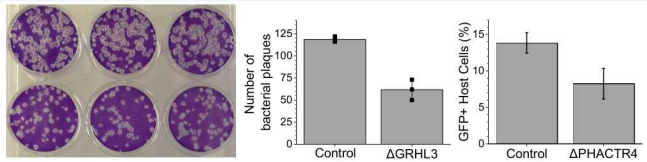


sgRNAs for Protein Coding Gene	Protein Coding Genes	Average Number of Infections from which sgRNA was Recovered							
		1	2	3	4	5	6	7	8
1	**5325**	3580	325	184	169	184	246	225	412
2	**817**	370	101	91	122	93	21	11	8
3	**79**	32	11	27	5	2	0	2	0
4	**5**	3	1	1	0	0	0	0	0
5	**1**	0	0	1	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0

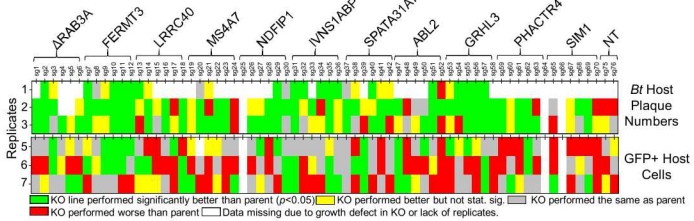
Host Defense Targets for Confirmation



Variability in Finite Time Point Assays



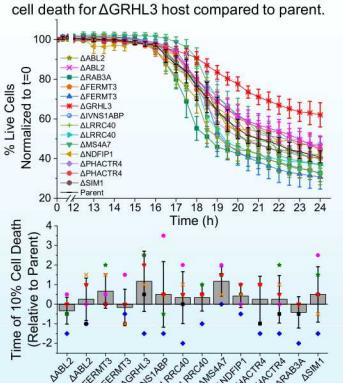
A KO library consisting of 68 individual cell lines (12 KO genes of interest with 6 unique sgRNA) was generated in A549 airway epithelial cells and tested through a series of assays to identify priority targets for in-depth study. *Bt* forms plaques in host cell lawns (top, left) that can be quantified by counting to evaluate infection (top, middle). We performed plaque assays across KO cell lines (MOI 2-5) and observed quantifiable differences in a number of cells lines. We additionally developed a flow cytometry assay (MOI 2-5) to quantify GFP intensity in host cells from *Bt*-GFP internalization (top, right). The multi-assay approach aimed to evaluate KO line performance in different infection stages: bacterial internalization (early), *Bt* spread (middle), and infection progression to host cell death (late).

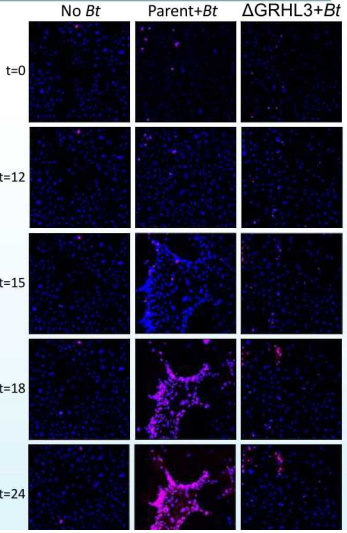


The 68 host cell KO lines performed non-reproducibly across replicates in our developed assays. Shown above are two examples of these assays (plaque assay (top) and GFP+ flow cytometry of *Bt*-GFP internalization (bottom). We hypothesized that finite time point assays were not capturing infection dynamics and sought to develop live cell, kinetic assays for infection characterization. To limit the cell lines carried into the next round, we down selected based on the most consistent/best performing lines: ΔABL2 (sg51, sg50), ΔFERMT3 (sg11, sg12), ΔGRHL3 (sg57), ΔIVNS1ABP (sg35), ΔLRRC40 (sg14, sg16), ΔMS4A7 (sg22), ΔNDFIP1 (sg28), ΔPHACTR4 (sg62, sg59), ΔRAB3A (sg2), and ΔSIM1 (sg69).

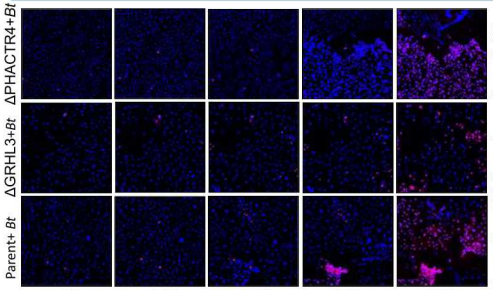
Live-Cell High-Content Imaging Analysis for Infected Host Cell Death

We developed a live-cell high-content imaging assay that tracks cells by nuclei (Hoechst, blue) and identifies dead cells by counterstaining nuclei with Propidium Iodide (PI, red). As cells lose membrane integrity, PI stains the nuclei. This assay mimicked our original screen with a high MOI of *Bt* (500-1000). We first quantified cell death in the presence of *Bt* and observed significant reduction in cell death for ΔGRHL3 host compared to parent.

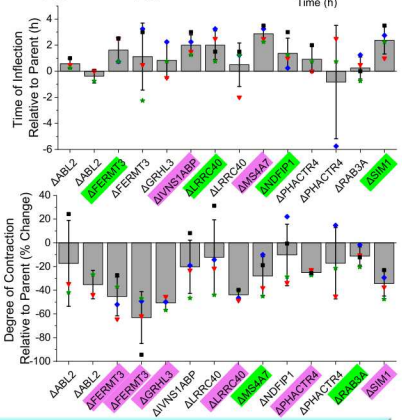




Nearest Neighbor Kinetic Analysis of Multinucleated Giant Cell Formation



After quantifying live dead kinetics, we noticed visual differences between host lines in nuclei migration indicating altered multinucleated giant cell (MNGC) formation and/or different cell-to-cell spread of *Bt* (top). Using the same image sets as the live/dead assay, we converted each nuclei centroid to cartesian coordinates. We then performed *k*-nearest neighbor (k=1) analysis over time for six images per host cell line with three wells for each biological replicate and compared to parent host line. We observed significant differences in the time nuclei contraction starts for ΔFERMT3, ΔIVNS1AB, ΔLRRC40, ΔMS4A7, ΔNDFIP1, and ΔSIM1 (right, top). Additionally, we observed significant differences in the distance/degree of contraction for ΔFERMT3, ΔGRHL3, ΔLRRC40, ΔMS4A7, ΔPHACTR4, ΔRAB3A and ΔSIM1 (right, bottom). Differences with $p<0.05$ highlighted green and $p<0.01$ highlighted purple.

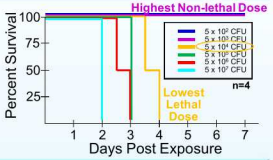


Conclusions

- We identified and validated nine genes that alter host response during infection with *Bt* providing viable targets for host directed antibiotics against *Bt*.
- GRHL3 knockout offers host protection and increased host cell survival during *Bt* infection.
- FERMT3, GRHL3, IVNS1AB, LRRC40, MS4A7, NDFIP1, PHACTR4, RAB3A, and SIM1 knockout alters dynamics in host cell MNGC formation and/or bacterial cell to cell spread during *Bt* infection.

Future Work

- Further investigate mechanism of action for validated host knockout genes.
- Test identified gene KOs for host protection to *Bp*, *Bm*, and other respiratory pathogens.
- Work towards gene editing model that allows gene of interest testing in developed mouse models of *Bt* (shown right), *Burkholderia cepacia* complex, and *Bp*.



References and Acknowledgements

1.Wiersinga, W.J. et al. 2006 Nature Reviews Microbiology
2.Shalem, O et al. 2014 Science

This work was funded under Sandia National Laboratories Lab Directed Research and Development.