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Identifying Novel Regulators of Necroptosis

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Necrosis had long been considered a form of dysregulated cell death caused by disease. Recent evidence has revealed that some forms of necrosis have defined molecular pathways. Necroptosis is the most studied of these pathways and involves receptor interacting protein kinase 3 (RIPK3). Necroptosis has been implicated in a variety of diseases including inflammatory bowel diseases, pancreatitis, and ischemia-reperfusion injury. Despite its suspected involvement in these maladies, little is known about the molecular pathway of necroptosis as only a few key proteins have been identified. We hypothesize that there are other genes involved in this process which have yet to be associated with necroptosis as other cell death pathways have many more molecular players. In order to discover novel regulators of this pathway we conducted a broad genetic screen, using a genome wide CRISPR/Cas9 library to search for genes whose loss led to resistance to necroptosis. We packaged the CRISPR/Cas9 machinery into a lentiviral delivery system, which was used to infect mouse embryonic fibroblasts (MEFs). The infected cells are subject to either control stimulus or necroptopic stimulus. Virtually all of the wild-type cells died when subject to necroptosis. Surviving cells likely have a resistance phenotype generated by the loss of key genes. Treated and control groups were then analyzed via PCR amplification and deep sequencing. Subsequent computational analysis of the sequencing results provided a list of genes with potential involvement in necroptosis, including those already implicated in the pathway such as *RIPK1*, *RIPK3*, *MLKL*, *CYLD*, and *TNFRSF1A*. We then selected a number of candidate genes and subjected them to further molecular analysis. We believe that this CRISPR/Cas9 based screening strategy will be a powerful tool for identifying novel regulators of necroptosis.