Stony Brook University
The Graduate School

Doctoral Defense Announcement

Abstract

Novel CUL3-mediated regulation of oncoprotein NRF2 revealed by marker-based CRISPR screen

By

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Transcriptional dysregulation is a common driver mechanism of human tumorigenesis shared across nearly all types of cancer. Alterations to the function of several transcription factors (TFs) have now been identified as the source for this dysregulation in many cancers. One example of a selective oncogenic TF in lung cancer (LC) is NRF2, the master regulator of the antioxidant response program. NRF2 hyperactivation, typically as a result of loss of function mutations to its primary negative regulator KEAP1, grants cancer cells a selective advantage by supporting further proliferation despite unfavorable redox conditions. However, while NRF2 regulation is well characterized under physiological conditions, significant questions remain regarding the specific rewiring of its regulatory network during malignant hyperactivation. To better understand how NRF2 is regulated in cancer, we aim to identify novel players in the molecular mechanisms that control NRF2 function in this specific context. To accomplish this, we developed a high throughput genome-wide marker-based CRISPR/Cas9 screen capable of assessing NRF2 activity to identify novel cancer-relevant NRF2 modulators. Unexpectedly, this unbiased approach identified CUL3, the molecular scaffold protein of the CUL3-RING ubiquitin ligase (CRL3), as an activator of NRF2 activity selectively in *KEAP1*-mutant LC. We paired CRISPR knockout (KO) with transcriptomic analysis and found that *CUL3* KO dampens the NRF2 transcriptional profile uniquely in a KEAP1 deficient context. To better understand how CUL3 supports the NRF2 transcriptional program, we combined bulk KO RNA-seq experiments with proteomics to suggest that CUL3-mediated support of NRF2 activity functions through an indirect mechanism. Furthermore, *CUL3* KO followed by either NRF2 or H3K27ac ChIP-seq revealed that while CUL3 does not affect NRF2 DNA binding, it specifically diminishes the activity of many NRF2-binding motifs. Combined, our studies reveal an alternative function of CUL3 in *KEAP1*-mutant LCs that supports NRF2 activity through a potentially indirect mechanism.

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Time: 12:30 PM EST **Dissertation Advisor**: Christopher Vakoc, MD/PhD
Place: Bush Auditorium, Cold Spring Harbor Laboratory

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