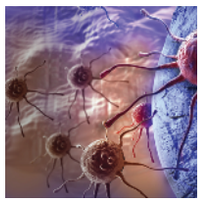
Metastasis

Major finding: An *in vivo* genome-wide CRISPR/Cas9 screen in mice identifies genes that control lung metastasis.

Concept: The primary tumor growth advantage conferred by loss-of-function mutations correlates with metastasis.

Impact: CRISPR/Cas9 screens can be used to assess the role of genes in cancer-related phenotypes *in vivo*.

AN *IN VIVO* GENOME-SCALE CRISPR SCREEN DEFINES REGULATORS OF METASTASIS



The genomic landscape of cancer cells is complex, harboring a mixture of driver and passenger mutations. Genetic screens have been essential in determining causal links between genetic alterations and cancer phenotypes, including tumor growth and metastasis. To identify loss-of-function mutations that promote primary tumor growth and metastasis *in vivo*, Chen, Sanjana, and colleagues performed a genome-wide CRISPR/Cas9–mediated screen using a nonmetastatic mouse non–small cell lung cancer (NSCLC) line. Introduction of a pooled genome-scale mouse CRISPR knockout library containing 67,405 single-guide RNAs (sgRNA) targeting 20,611 protein-coding genes and 1,175 microRNAs enhanced primary tumor growth and the formation of lung metastasis compared with uninfected cells. Deep sequencing of early- and late-stage primary tumors and lung metastases revealed dynamic changes in sgRNA representation during tumor evolution; less than half of sgRNAs were retained in early primary tumors, with only 8% of these sgRNAs detected in late-stage tumors and ≤1.1% of these sgRNAs selected for in corresponding lung metastases. Importantly, late primary tumors and metastases were enriched in sgRNAs targeting proapoptotic genes and tumor suppressors that are mutated or downregulated in human NSCLC. Furthermore, lung metastases were enriched in only a small number of sgRNAs, which were highly correlated with the sgRNAs enriched in late primary tumors, suggesting that mutant cells with a proliferative advantage in late-stage tumors preferentially seed metastases. *In vivo* validation of top-scoring genes using individual sgRNAs or sgRNA minipools revealed that loss-of-function mutations in genes including *Nf2*, *Pten*, *Trim72*, *Cdkn2a*, *Fga*, *miR-152*, and *miR-345* were sufficient to enhance lung metastasis and that accelerated primary tumor formation correlated with increased metastatic incidence. Together, this work identifies specific loss-of-function mutations that drive tumor growth and metastasis and highlights the use of CRISPR/Cas9 screening technology to characterize the functional role of genes in cancer cell phenotypes *in vivo*. ■

Chen S, Sanjana NE, Zheng K, Shalem O, Lee K, Shi X, et al. Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis. Cell 2015;160:1246–60.

Transcription Factors

Major finding: The biologic activity of MYC is largely dependent on binding of WDR5 to the MYC MbIIIb motif.

Concept: Disrupting the MYC–WDR5 interaction broadly reduces MYC chromatin binding and blocks tumor growth.

Impact: Small molecules that target the MYC–WDR5 interface may be effective in MYC-dependent cancers.

WDR5 IS A MYC COFACTOR REQUIRED FOR MYC-DRIVEN TUMORIGENESIS

Binding of MYC to DNA is dependent on its heterodimerization with MAX, but other determinants of MYC localization to target genes likely exist. Because little is known about the function of the central portion of the MYC protein, Thomas and colleagues screened for proteins that bind this region and identified WD repeat domain 5 (WDR5), a component of several chromatin modifying complexes, as a MYC binding partner that specifically interacts with the highly evolutionarily conserved MYC box IIIb (MbIIIb) motif within the MYC central region. Chromatin immunoprecipitation sequencing revealed a significant overlap between MYC and WDR5 binding sites, and mutation of the MbIIIb residues found to interact with WDR5 in a crystal structure prevented MYC binding to approximately 80% of its target sites across the genome, implicating WDR5 as a key cofactor of MYC that is broadly required for its binding to chromatin. Consistent with these findings suggesting that the interaction with WDR5 is required for the biologic activity of MYC, WDR5 binding–deficient MYC mutants were incapable of reprogramming mouse embryonic fibroblasts to induced pluripotent stem cells when expressed with OCT3/4, SOX2, and KLF4 and showed a dramatically reduced capacity to induce tumor growth *in vivo* compared with wild-type MYC. In addition to identifying WDR5 as a MYC binding partner and a role for the MbIIIb motif as a WDR5 interaction module, these findings suggest that targeting the interaction between MYC and WDR5 may be a tractable and effective strategy to directly block MYC transcriptional activity and suppress MYC-driven tumor growth. ■

Thomas LR, Wang Q, Grieb BC, Phan J, Foshage AM, Sun Q, et al. Interaction with WDR5 promotes target gene recognition and tumorigenesis by MYC. Mol Cell 2015 Mar 26 [Epub ahead of print].