Suppression of the FOXM1 transcriptional program via novel small molecule inhibition

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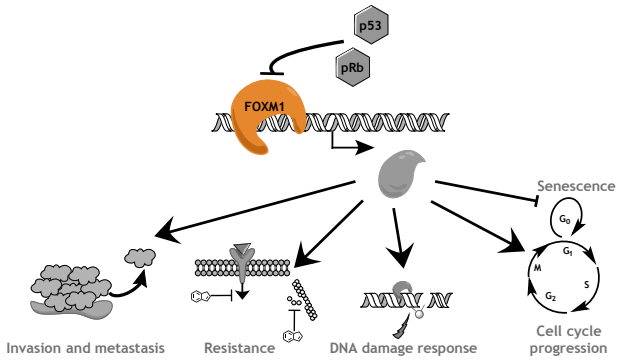
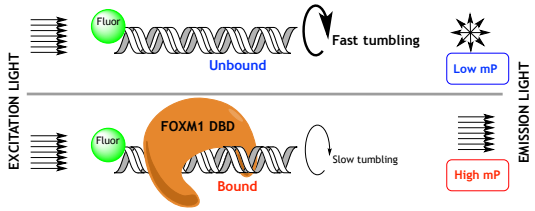
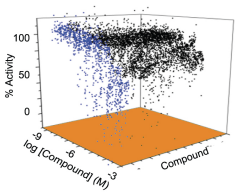
Abstract

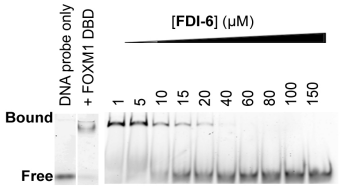
Forkhead box M1 (FOXM1) is a transcription factor of considerable importance. In a healthy, non-terminally differentiated cell, FOXM1 directs diverse cellular processes by binding consensus DNA targets in gene promoters and activating their transcription. However, aberrant overabundance of FOXM1 through mutations in upstream regulators or gene amplification has been identified in most human cancers and FOXM1 expression correlates with severity of prognoses. As such, the stratification of tumor suppressive and oncogenic roles of FOXM1 in different contexts is an area of intense interest. Thus, the chemical inhibition of FOXM1 has become both a highly sought probe and a major goal for therapeutic utilization. We designed a novel in vitro assay to detect disruption of FOXM1 DNA binding. We successfully miniaturized this assay for quantitative high-throughput screening (qHTS) and interrogated a novel collection of 54,211 compounds, which consisted of diverse drug-like molecules intended as starting points for medicinal chemistry lead development. Our screening cascade identified a novel family of Forkhead Domain Inhibitors (FDI). The lead compound, **FDI-6** is a potent inhibitor for the binding of FOXM1 to its consensus DNA binding motif and we characterized its interaction in detail by biophysical analyses. We confirmed that **FDI-6** binds directly to FOXM1 protein, and also demonstrated that this small molecule displaces FOXM1 protein from promoters of target genes in MCF-7 breast cancer cells. Finally, using next generation sequencing, we employed RNA sequencing (RNA-seq), to show that **FDI-6** selectively down-regulates the FOXM1 transcriptional program of cell cycle regulation. Importantly, **FDI-6** is specific for FOXM1 binding and has no effect on the expression of genes regulated by other related forkhead factors, which exhibit homology with the DNA binding domain of FOXM1. Our study shows that the genomic interaction of this clinically important transcription factor can be manipulated with small molecules to regulate the expression of key gene families. This finding demonstrates clear potential for the pursuit of FOXM1 as a therapeutic target in the future.



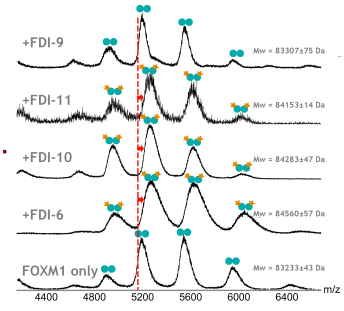
Biophysical screen for inhibitors of FOXM1

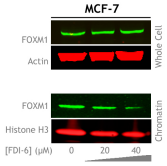


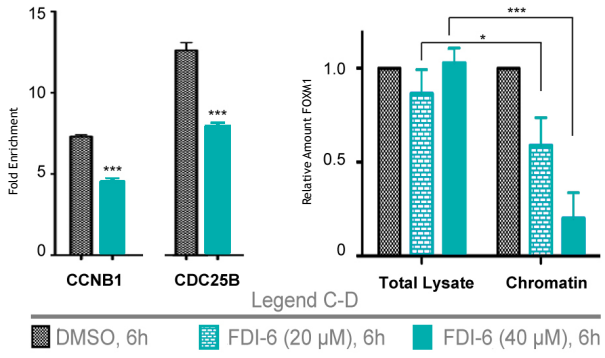




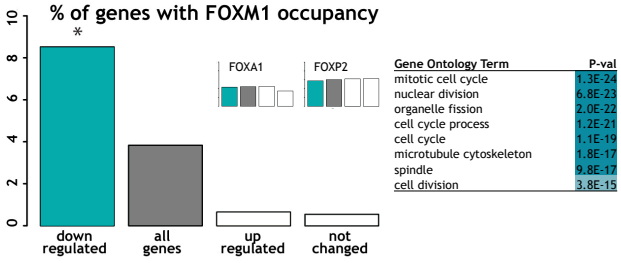
FDI-6 binds and displaces FOXM1 from chromatin



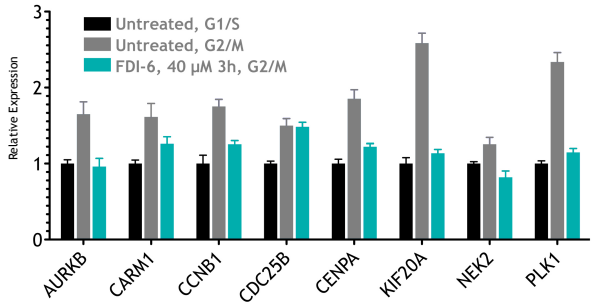




FOXM1 transcriptional program is globally suppressed by FDI-6



Gene Ontology Term	P-val
mitotic cell cycle	1.3E-24
nuclear division	6.8E-23
organelle fission	2.0E-22
cell cycle process	1.2E-21
cell cycle	1.1E-19
microtubule cytoskeleton	1.8E-17
spindle	9.8E-17
cell division	3.8E-15



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