PKMYT1 no longer has to play second fiddle to WEE1

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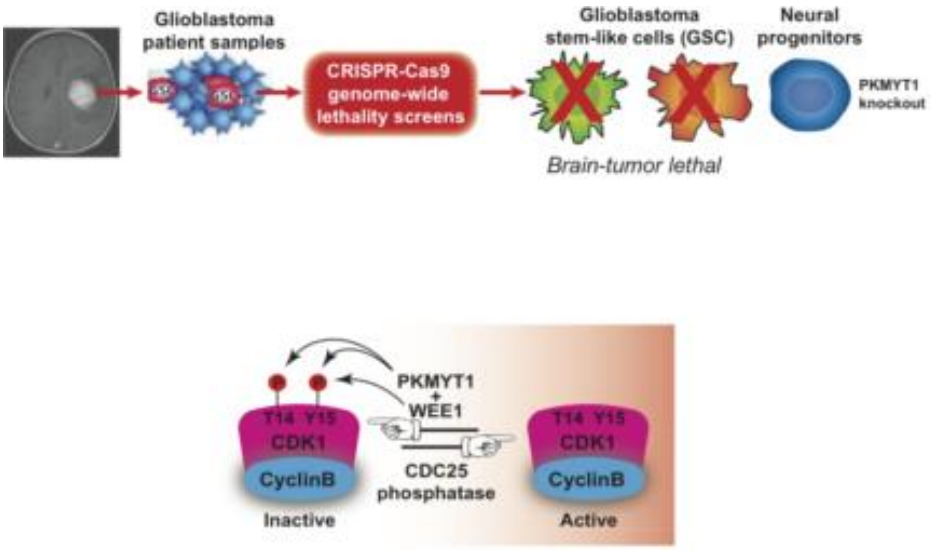

Workflow of CRISPR-Cas9 screen of human glioblastoma stem-like cells (GSCs) and human neural progenitors (top panel). the related kinases PKMYT11 and WEE1 act redundantly to phosphorylate CDK1 on T14 and Y15 to prevent premature mitotic entry (bottom panel).

Image provided by Dr. Patrick Paddison.

A widely held assumption of the burgeoning field of precision oncology is that identification of driver mutations present in tumors allied with computational analysis of other descriptive genomic datasets is sufficient to predict therapeutic targets. However, directly identifying cancer-specific vulnerabilities requires genetic manipulation of genes in patient-derived samples. This has not been a straightforward task until the emergence of a gene editing technology known as CRISPR, which was hailed by Science magazine as the breakthrough of the year of 2015. A Fred Hutch study led by Drs. Chad Toledo and Yu Ding in the Paddison Laboratory (Human Biology and Public Health Sciences Divisions), in collaboration with the Olson (Clinical Research Division) and Clurman (Human Biology and Clinical Research Divisions) Labs, leveraged the CRISPR technology to perform genome-wide screens for genes that when deleted, caused lethality in human glioblastoma stem cells (GSCs), but not in normal neural stem and progenitor cells (NSCs). This study, published in the journal *Cell Reports*, revealed that two kinases, PKMYT1 and WEE1, act redundantly in NSCs but not GSCs and provides a rationale for pursuing PKMYT1 inhibitors.

The investigators first tested the efficacy of a lentiviral-based delivery of a CRISPR-Cas9 system to target a reporter gene (GFP), a non-essential endogenous gene (*TP53*) or an essential endogenous

gene (*MCM2*). All three were efficiently targeted in both GSCs and NSCs. This provided the authors confidence to perform genome-wide screens in two GSC (0131 and 0827) and two NSC isolates (CB660 and U5) using a lentiviral pool containing a CRISPR library containing 64,751 single guide RNA (sgRNAs) targeting 18,080 genes. Deep sequencing of sgRNAs before and after three weeks of growth in self-renewing conditions, followed by empirical analysis of digital gene expression in the biostatistical analysis package R was performed to identify candidate essential genes. The scientists next wondered whether their screen hits would be enriched for genes and pathways commonly altered in glioblastoma yet were surprised to find that only ten GSC-specific lethals out of a total of 946 overlapped with core glioblastoma pathways. Said Dr. Patrick Paddison, "We went into this study thinking we would validate the notion that genomic characterization (e.g., deep sequencing) of patient tumor samples would be predictive of "actionable" therapeutic targets. However, the screen results clearly demonstrate that this is not the case. The genomic alterations (e.g., "driver" mutations) did not predict therapeutic targets, as defined by this screening technique. Instead, we identified 100s of candidate therapeutic targets that are off the radar screen of conventional precision oncology. The results highlight the need to experiment directly in patient samples to identify patient tailored therapeutic approaches."

Both *in vitro* and *in vivo* validation of candidate genes revealed the kinase PKMYT1 as the top candidate, as it was required for the viability of eight out of ten GSC lines tested. PKMYT1 is a kinase that phosphorylates CDK1 (see figure), and at least in *Drosophila*, acts redundantly with the related kinase WEE1 to prevent premature entry into mitosis. With this in mind, the investigators tested the effects of depleting PKMYT1 and WEE1 either alone or in combination by monitoring the mitotic transit times (MTT) of NSCs or GSCs using time-lase microscopy. In NSCs, inhibition of either PKMYT1 or WEE1 alone resulted only in modest increases in MTT (from 37min to 47-51min). However, combined loss of PKMYT1 and WEE1 led to average MTTs of more than 100min. In contrast, inhibition of either PKMYT1 or WEE1 alone led to dramatic increases in MTTs when the same set of experiments was repeated using GSCs, suggesting that PKMYT1 and WEE1 act redundantly in NSCs but not GSCs. Finally, the authors were able to recapitulate PKMYT1 sensitivity in NSCs by concurrently activating the EGFR and AKT, known glioma driver mutation pathways in immortalized NSCs. In conclusion, this study showed both the power and feasibility of CRIPSR-based genome-wide screens using patient-derived samples to identify genes that are essential in transformed cells but not in normal cells. "We are now putting PKMYT1 into our inhibitor development pipeline, which Dr. Jim Olson leads. Our hope is to have a therapeutic strategy in clinical trials within the next 5 years," said Dr. Paddison.

Toledo CM, Ding Y, Hoellerbauer P, Davis RJ, Basom R, Girard EJ, Lee E, Corrin P, Hart T, Bolouri H, Davison J, Zhang Q, Hardcastle J, Aronow BJ, Plaisier CL, Baliga NS, Moffat J, Lin Q, Li XN, Nam DH, Lee J, Pollard SM, Zhu J, Delrow JJ, Clurman BE, Olson JM, Paddison PJ. 2015. Genome-wide CRISPR-Cas9 screens reveal loss of redundancy between PKMYT1 and WEE1 in glioblastoma stem-like cells. *Cell Reports*. 13(11):2425-39.

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