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INSIGHTS

All eyes on a phosphatase in glioma stem cells

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In this issue of *JEM*, Zhang et al. (2021. *J. Exp. Med.* <https://doi.org/10.1084/jem.20202669>) identify a dependency of glioma stem cells on tyrosine phosphatase activity of EYA2 and a new role for this phosphatase at the centrosome, offering a new therapeutic approach to target mitotic activity.

Glioblastoma is an aggressive disease in desperate need of new therapies, as it has the shortest survival and highest mortality of all adult brain tumors (Lapointe et al., 2018; Ostrom et al., 2020). This deadly cancer is thought to be propagated by a population of glioma stem cells (GSCs) with the capacities to self-renew and to resist many conventional therapies (Lathia et al., 2011; Suvà and Tirosh, 2020; Bhaduri et al., 2020). In this issue of *JEM*, Zhang et al. (2021) discover that GSCs overexpress and rely on a tyrosine phosphatase known as Eyes Absent 2 (EYA2), which may represent a new drug target.

The authors begin by comparing gene expression between cultured GSCs, neural stem cells (NSCs), and differentiated glioma cells. They found that EYA2 was the most overexpressed gene in GSCs compared with NSCs and differentiated glioma cells, and they confirmed via The Cancer Genome Atlas that EYA2 was also highly expressed in glioblastomas and conferred a particularly poor prognosis in the most differentiated proneural subtype (Phillips et al., 2006; Cancer Genome Atlas Research Network, 2008). Through shRNA-mediated knockdown, the authors showed that loss of EYA2 impaired proliferation, self-renewal, and survival of GSCs in vitro, and that knockdown of EYA2 inhibited growth of xenografted tumors. The authors used a mutant isoform of EYA2 to confirm that its tyrosine phosphatase activity was required for propagation of glioma cells in vitro and in

xenografts. They also tested chemical inhibitors in GSCs, including the general EYA phosphatase inhibitor benzbromarone; an allosteric inhibitor of EYA2's tyrosine phosphatase activity (Krueger et al., 2014); and a third, novel EYA2 tyrosine phosphatase inhibitor. All three drugs inhibited proliferation of GSCs in vitro. Benzbromarone was tested in xenograft models and prolonged survival. The above experiments revealed that GSCs depended on EYA2 for some essential function.

Returning to the initial approach of comparing differentially expressed genes, the authors found that EYA2 knockdown in GSCs strongly altered expression of genes involved in mitotic spindle formation. Through immunostaining, they showed that EYA2 was localized to the nucleus as classically described. In both cultured GSCs and tissue sections of surgically resected glioblastoma, there was also punctate perinuclear EYA2 immunostaining that colocalized with pericentrin, consistent with centrosomal localization. The authors stained for α -tubulin and noted that the structure of the mitotic spindle was abnormal when EYA2 tyrosine phosphatase activity was inhibited or EYA2 was knocked down. The authors used total internal reflection fluorescence microscopy to visualize tubulin polymerization in vitro. Polymerization of microtubules was not altered by inhibition of EYA2, suggesting that inhibition of EYA2 must alter spindle formation through some other mechanism.



Insights from Robert A. Lindquist and William A. Weiss.

Finally, the authors explored mechanisms of resistance to EYA2 inhibition. In vitro, they found that cells that survived pharmacologic inhibition of EYA2 tyrosine phosphatase showed lower expression of stem cell markers and increased phosphorylation of the MAPK target ERK. They also found increased phosphorylation of ERK upon shRNA knockdown of EYA2 and upon expression of a tyrosine-phosphatase-dead mutant EYA2. These results suggest that MAPK signaling might promote cell survival to compensate for loss of EYA2 function. Dual inhibition with benzbromarone and a MAPK/ERK kinase (MEK) inhibitor further augmented survival in xenografted tumors, suggesting therapeutic synergy and a way to delay emergence of a resistant population.

When facing a cancer as challenging as glioblastoma, any therapeutic lead is welcome. The standard treatments—including surgery, ionizing radiation, and alkylating

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chemotherapy—can prolong life but are not curative (Lapointe et al., 2018; Ostrom et al., 2020). One major problem is that glioma cells with stem-like properties are less susceptible to radiation and chemotherapy (Bao et al., 2006; Chen et al., 2012). Keys to new treatments may lie in the unique biology of GSCs, and researchers have begun to target GSCs themselves, for instance by differentiating glioma cells into a postmitotic state (Piccirillo et al., 2006; Sabelström et al., 2019). The results of Zhang et al. (2021) are particularly exciting because EYA2 represents not just any novel drug target, but a vulnerability of GSCs. In contrast with differentiation-based strategies, inhibition of EYA2 appears cytotoxic to GSCs. An EYA2 tyrosine phosphatase inhibitor also affected cultured NSC proliferation and survival, but only at doses higher than required to kill GSCs. It should thus be possible to find a dose of EYA2 inhibition that specifically affects glioma cells without significantly affecting normal cells.

The results of Zhang et al. (2021) raise two interesting mechanistic questions: what is EYA2 doing at the centrosome, and why is its tyrosine phosphatase activity required for proper mitotic spindle assembly? While the mitotic spindle forms abnormally upon inhibition of EYA2, Zhang et al. (2021) showed that this was not a direct effect of drugs on microtubule dynamics. They identified genes that were differentially regulated upon EYA2 knockdown, but it remains unclear whether EYA2 acts to regulate those genes through tyrosine phosphorylation at its classical location in the nucleus or at the centrosome. Biochemical approaches may help identify EYA2's binding partners and its direct targets for tyrosine dephosphorylation. It may also be informative to directly observe the process of cell division in light of the common

behaviors that have been found in GSCs and normally occurring neural stem and progenitor cells (Sugiarto et al., 2011; Bhaduri et al., 2020). A recent study demonstrated extensive molecular similarity between glioma cells and normally developing outer-subventricular-zone radial glial stem cells, or “oRG cells,” down to a shared behavior of mitotic somal translocation in which the nucleus migrates a long distance in coordination with cell division (Bhaduri et al., 2020). While Zhang et al. (2021) found that EYA2 itself was differentially expressed between NSCs and GSCs, they also found some perinuclear EYA2 immunostaining in cultured NSCs. It is possible that EYA2 performs a similar function in both cell types (which may account for EYA2 inhibitor toxicity in NSCs); this may be tested by EYA2 overexpression (and inhibition) in NSCs. It thus would be interesting to see whether asymmetric division, mitotic somal translocation, and other common aspects of NSC and glioma cell division are altered by EYA2 gain or loss of function.

A final and far-reaching problem in glioblastoma is intratumoral heterogeneity. After early studies suggested distinct molecular subtypes of glioblastoma (Phillips et al., 2006; Cancer Genome Atlas Research Network, 2008), single-cell analyses showed that instead, every glioblastoma is made of varying proportions of cells of those different subtypes (Patel et al., 2014; Bhaduri et al., 2020). Interestingly, single-cell analysis has also demonstrated extensive molecular heterogeneity among normally developing NSCs (Eze et al., 2021). The striking intratumoral diversity of glioma may allow certain cell populations to evade the selective pressure of a given therapy. Zhang et al. (2021) found that cells that survived EYA2 inhibition had increased MAPK signaling; they found that simultaneously

drugging the MAPK pathway via MEK inhibition was synergistic. It will be worth considering, if and when clinical trials of EYA2 inhibitors in glioma begin, that these drugs be tested in combination with already Food and Drug Administration–approved MEK inhibitors. In the meantime, further analysis of single-cell datasets may reveal whether EYA2 overexpression correlates with, or is independent of, other targetable molecular lesions.

Zhang et al. (2021) identified a novel and specifically targetable dependency of GSCs, with the potential to translate into improved treatments for a deadly cancer. The eyes of the neuro-oncology community will be watching closely.

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