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Cyclospamine-Mediated Hedgehog Pathway Inhibition Depletes Stem-Like Cancer Cells in Glioblastoma

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Brain tumors can arise following deregulation of signaling pathways normally activated during brain development and may derive from neural stem cells. Given the requirement for Hedgehog in non-neoplastic stem cells, we investigated whether Hedgehog blockade could target the stem-like population in glioblastoma multiforme (GBM). We found that Gli1, a key Hedgehog pathway target, was highly expressed in 5 of 19 primary GBM and in 4 of 7 GBM cell lines. Shh ligand was expressed in some primary tumors, and in GBM-derived neurospheres, suggesting a potential mechanism for pathway activation. Hedgehog pathway blockade by cyclospamine caused a 40%–60% reduction in growth of adherent glioma lines highly expressing Gli1 but not in those lacking evidence of pathway activity. When GBM-derived neurospheres were treated with cyclospamine and then dissociated and seeded in media lacking the inhibitor, no new neurospheres formed, suggesting that the clonogenic cancer stem cells had been depleted. Consistent with this hypothesis, the stem-like fraction in gliomas marked by both aldehyde dehydrogenase activity and Hoechst dye excretion (side population) was significantly reduced or eliminated by cyclospamine. In contrast, we found that radiation treatment of our GBM neurospheres increased the percentage of these stem-like cells, suggesting that this standard therapy preferentially targets better-differentiated neoplastic cells. Most importantly, viable GBM cells injected intracranially following Hedgehog blockade were no longer able to form tumors in athymic mice, indicating that a cancer stem cell population critical for ongoing growth had been removed.

Disclosure of potential conflicts of interest is found at the end of this article.