Notch Modulates Hedgehog Signaling in Glioblastoma: A Potential Mechanism of Therapeutic Resistance


Abstract

Glioblastoma (GBM) is the most malignant primary central nervous system tumor in adults and is characterized by rapid progression, poor prognosis, and resistance to chemo- and radiotherapy. Research has demonstrated that multiple stem cell maintenance pathways—such as Notch, Hedgehog, and Wnt—are active in malignant brain tumors, and that subgroups of GBM express distinct oncogenes and signaling pathway components. This raises the possibility that GBM might be compensating for targeted-therapy directed against one pathway by activation of others. To assess this, we investigated the long-term effects of Notch inhibition on malignant brain tumor cells and the potential emergence of therapeutic resistance, by treating neurosphere lines with a gamma-secretase inhibitor (GSI). We found that a sub-population of GBM cultures continue to grow in the presence of moderate levels of MRK003. In this population, Notch signaling continued to be suppressed, suggesting that the tumor population had become resistant to Notch inhibition, possibly by upregulating another signaling pathway. To determine the mechanism of escape, we evaluated expression levels of several other developmental pathways. Both Hedgehog and Wnt targets were up-regulated in response to Notch inhibition and Hedgehog seemed to be directly regulated by Notch. We demonstrated that Hes1 binds the Gli1 first intron and inhibits expression but that this can be reversed in the presence of MRK003. We also demonstrated that targeting both Notch and Hedgehog simultaneously with pharmacologic agents decreases cell growth, colony-forming ability, and xenograft formation more dramatically than monotherapy. Moreover, freshly dissected human GBMs are also selectively susceptible to co-inhibition. These findings indicate that targeting multiple stem-cell maintenance pathways is more effective than monotherapy at eliminating GBM cells. Additionally, our findings raise the question of whether potential cross-talk mechanisms should be investigated when developing pathway-targeted therapies.