

Ritanserin, a novel agent targeting the mesenchymal subtype of glioblastomas

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Patients with glioblastoma (GBM) are uniformly treated with chemoradiation, but recurrence is inevitable in most cases. Advances in the molecular classification of GBMs have shown that 3 major subtypes, termed proneural (PN), classical (CL), and mesenchymal (MES), are consistently seen.¹ The PN and MES subtypes show not only contrasting molecular signatures, but also differential biological and clinical characteristics. For example, in single-cell sequencing studies, survival rates in patients with tumors that had a higher proportion of cells with a mixed signature (PN/MES/CL) were poorer than those with tumors that consisted of predominantly PN cells.² Recent clonal studies using primary GBM cells implicate a MES signature with multitherapy resistance.³ Notably, a switch to the MES subtype upon recurrence is associated with worse outcome in GBM.⁴ Therefore, therapies directed against or preventing the transition to a MES subtype could be beneficial to GBM patients. In a study published in this issue of *Neuro-Oncology*, Purow and colleagues identify a novel therapeutic target for the MES subtype of GBM.⁵ They show that the inhibition of diacylglycerol kinase alpha (DGK α) with a drug, ritanserin, a serotonin receptor inhibitor, or RNA interference specifically kills the MES subtype of glioma stemlike cells (GSCs) in vitro and in vivo. Moreover, they uncover a previously unknown molecular axis (DGK α –GGTase I–NF- κ B) to be operational in MES GSCs.

DGK α is one of the 10 DGK family members that convert diacylglycerol (DAG) to phosphatidic acid (PA), and both DAG and PA are critical lipid second messengers in the plasma membrane.⁶ DGK α has been shown to play essential functions in the regulation of T-cell immunological response.⁷ In cancer, a host of molecular pathways are altered by DGK α , including hypoxia-inducible factor 1 α , mammalian target of rapamycin, c-Met, and vascular endothelial growth factor.⁸ Additional known functions of DGK α include membrane ruffling, cell migration, proliferation, and angiogenesis.⁸ Previous studies by Purow and colleagues have shown that DGK α is a key oncogenic node in GBM and melanoma and is under regulation by

miR-297.^{9,10} Furthermore, ritanserin was found to be a specific inhibitor of DGK α . In this study, the authors examined the expression of DGK α transcript in the database of The Cancer Genome Atlas and found higher expression of this gene in the MES subtype compared with all other subtypes. Next, they examined the effect of ritanserin on the proliferation and self-renewal of GSCs in vitro and found that this drug induces partial death via apoptosis only in MES GSCs. The inhibition was specific as confirmed by apoptosis induced using 2 independent small interfering RNAs against DGK α only in MES GSCs. Furthermore, another serotonin receptor inhibitor that lacks DGK α inhibition (ketanserin) was ineffective in inducing apoptosis in MES GSCs, and a serotonin agonist did not rescue the cytotoxicity induced by ritanserin. Importantly in an orthotopic model, oral ritanserin administration increased survival of mice bearing MES GSCs, but no effects were seen in a non-MES GSC line, pointing to the specific effects of ritanserin against the MES subtype of GBM. Given that MES cells are known to be radioresistant and that nuclear factor-kappaB (NF- κ B) causes activation of PN to MES transition (PMT),¹¹ the authors examined the effects of ritanserin on GSCs undergoing radiation-induced PMT as well as carcinoma cells undergoing an epithelial to MES transition and found strong reduction in viability with this agent.

To delineate the mechanisms underlying the specificity of ritanserin to the MES subtype, the authors hypothesized that DGK α affects NF- κ B via RhoA, given a prior regulatory role of DGK α on RhoA, and NF- κ B in the MES phenotype. Not only did silencing DGK α cause reduction of RhoA activation via geranylgeranylation of Rap1A, an upstream regulator of RhoA, the effects were dependent on geranylgeranyltransferase type 1 (GGTase I) enzyme. In a series of elegant experiments, it was demonstrated that DGK α regulation of GGTase I was directly upstream and that GGTase I inhibition affects not only geranylgeranylation of Rap1a, but also NF- κ B transcriptional function.

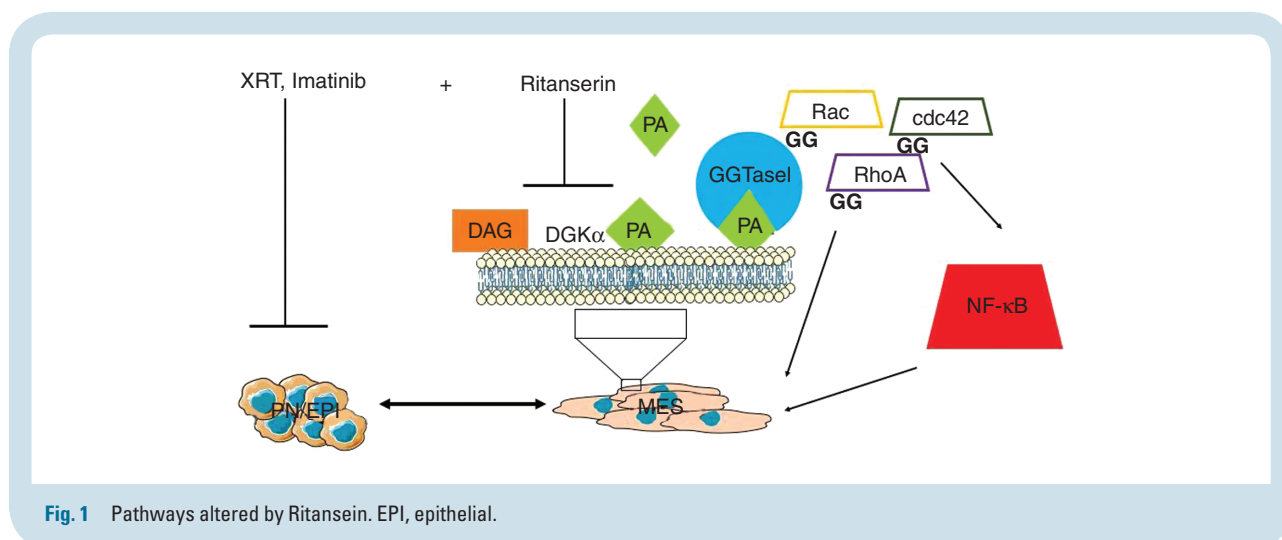


Fig. 1 Pathways altered by Ritanserin. EPI, epithelial.

Given the radioresistant functions of NF- κ B in MES GBMs, combined treatment of ritanserin and radiation caused synergistic growth inhibition in MES GSCs. Mechanistically, DNA damage response pathway activation was attenuated by treatment with ritanserin. Interestingly, the authors found that a 5-day treatment with ritanserin caused not only growth inhibition of GSCs, but a switch from a MES to PN subtype as evidenced by induction of PN markers. The gene product of platelet derived growth factor receptor alpha amplification, a hallmark of PN GBMs, showed marked activation in ritanserin-treated cells, and treatment with a pan receptor tyrosine kinase inhibitor imatinib caused synergistic reduction of cell growth.

Although this is not the first study linking DGK α to oncogenesis, the authors have elucidated the mechanisms by which DGK α promotes survival in the MES subtype of GBMs (summarized in Fig. 1). The authors elegantly demonstrate that PA, the DGK α product, binds to GGTase I and cooperates in the geranylgeranylation of proteins after transcription of the Rho family of GTPases, which in turn activate NF- κ B. Inhibition of DGK α or GGTase I specifically alters the growth and apoptosis in MES GSCs via activation of the NF- κ B cascade. Given that ritanserin is an FDA-approved agent, the regulatory route to GBM clinical trials is less complex when repurposing drugs. However, some biological and clinical issues need to be addressed prior to planning such trials. First, the side effects of ritanserin on serotonin receptor need to be taken into consideration, as these can be long term and include mood disorders. Second, DGK α promotes T-cell maturation, and therefore inhibition of this molecule can affect immune functions. Examination of the effects of ritanserin in genetically engineered mouse models should clarify this. Finally, because GBMs are not clinically classified by transcriptional signatures, choosing the right patient populations (in this case MES) who are likely to benefit in a trial can be challenging. Nonetheless, with the high failure rates of newer drugs in GBM, ritanserin offers hope for GBM patients, and clinical trials with this agent are warranted.

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