Insulin-like growth factor (IGF-1) is known to induce hypoxia inducible factor (HIF-1α) regulated genes in glioblastoma multiforme (GBM). As HIF-1α links inflammatory and oncogenic pathways in GBM, we investigated whether IGF-1 affects HIF-1α to regulate inflammatory response in glioma cells under normoxia. IGF-1 induced Ras/Akt/ERK pathway and Calmodulin-dependent kinase II (CaMKII) regulated HIF-1α transcriptional activity in glioma cells. Increase in HIF-1α was concurrent with decreased Toll-like receptor (TLR9) and CXCR4 expression and elevated suppressor of cytokine signaling (SOCS3) levels. Interestingly, while synthetic CpG containing oligodeoxynucleotide TLR9 agonist (CpG DNA) decreased IGF-1 mediated increase in HIF-1α activity, siRNA mediated knockdown of HIF-1α decreased TLR9 levels. This suggested that IGF-1 induced HIF-1α-TLR9 axis is regulated by both positive and negative feedback loops. Importantly, TLR9 agonist reversed the effect of IGF-1 on CXCR4 and SOCS3 expression. While knockdown of HIF-1α abrogated IGF-1 mediated increase in SOCS3 it elevated IGF-1 induced decrease in CXCR4 levels. Thus HIF-1α positively and negatively regulates SOCS3 and CXCR4 expression respectively, in glioma cells. Though TLR9 agonist had no additive effect on IGF-1 mediated increase in pro-inflammatory cytokines IL-1β, IL-6 and IL-8, treatment with TLR9 agonist alone elevated expression of these pro-inflammatory cytokines. Our studies indicate that a complex HIF-1α-TLR9 cross-talk sustains a self-regulating cycle of inflammatory response through intrinsic negative and positive feedback mechanisms.

The highly resistant nature of GBM to chemotherapy prompted us to evaluate the efficacy of bicyclic triterpenoid Iripallidal against GBM in vitro. Iripallidal (i) induced apoptosis, (ii) inhibited Akt/mTOR and STAT3 signaling, (iii) altered molecules associated with cell cycle and DNA damage, and (iv) inhibited telomerase activity and colony forming efficiency of glioma cells. In addition, Iripallidal displayed anti-proliferative activity against non-glioma cancer cell lines of diverse origin. Importantly, Iripallidal down-regulated IGF-1 induced increase in HIF-1α transcriptional activation and increase in pro-inflammatory cytokines. The efficacy of Iripallidal to serve as a dual-inhibitor of Akt/mTOR and STAT3 signaling; alongwith
its ability to abrogate IGF-1 induced increase in HIF-1α transcriptional activation and release of pro-inflammatory cytokines, warrants further investigation into its role as a therapeutic strategy against GBM.