AIMS OF THE STUDY
Glioblastoma multiforme (GBM) – the most malignant of brain tumors, remains one of the most challenging solid cancers to treat due to its highly proliferative, angiogenic and invasive nature. Hypoxia inducible factor (HIF-1α) which controls the expression of several genes implicated in angiogenesis, metabolism and cell survival [207] is overexpressed in GBM and HIF-1α level correlates with the highest grade of malignancy [208]. Signaling pathways emanating from the insulin-like growth factor-I receptor (IGF-IR) - a transmembrane receptor tyrosine kinase activated by IGF, are associated with the growth and proliferation of several tumor types including glioblastoma [209-211]. Although HIF-1α is a key regulator of cellular response to hypoxia [212] it can also be activated under normoxia in response to IGF [213, 214]. HIF-1α influences tumor growth [215] and serves as a critical link between inflammation and oncogenesis. We have reported that pro-inflammatory cytokine IL-1β regulates HIF-1α in glioma through the induction of IL-1β-HIF-1α autocrine loop [216]. IGF-1 is known to induce HIF-1α accumulation in Kaposi Sarcoma [217] and neuroblastoma [218]. Though HIF-1α-regulated genes are induced by IGF-1 in glioblastoma [219], nothing is known about IGF-1 regulated HIF-1α driven inflammatory responses in GBM. We therefore investigated whether IGF-1 regulates HIF-1α expression to modulate inflammatory responses in GBM under normoxia.

Activated Ras elevates IGF-I mediated induction of the HIF-1α target VEGF [220]. Oncogenic Ras activation occurs in GBMs [221] and inhibition of Ras down-regulates HIF-1α activity in GBM [222]. We have demonstrated IL-1β induced Ras/Akt/ERK mediated HIF-1α activation in glioma cells [216]. CaMKII which translates intracellular changes in calcium is associated with glioma migration [223], and regulates Ras mediated ERK activation [224]. Besides, CaMKII regulates HIF-1α transcriptional activity under intermittent hypoxia [225] as well as under inflammatory conditions [226]. Akt activates mammalian target of rapamycin (mTOR), which is deregulated in glioblastoma [227]. mTOR phosphorylates p70 ribosomal S6 kinase (p70S6 kinase) that regulates translation of proteins involved in cellular proliferation and formation. Moreover, blocking mTOR signaling reduces
glioma cell proliferation [228]. Given the importance of Akt/mTOR signaling in glioma cell survival, significant efforts are being invested in identifying inhibitors that target this pathway [228-230]. Given the importance of Ras/Akt pathway in GBM, we investigated the role of IGF-1 in regulating these signaling cascades in glioma cells.

HIF-1α is involved in host immune response during bacterial infection [231], and LPS is a potent inducer of HIF-1α [232]. Also, HIF-1α accumulation and target gene expression are impaired upon induction of endotoxin tolerance [233]. Activation of toll like receptors (TLRs) which recognize pathogen-associated molecular patterns triggers signaling events that initiate innate immunity and inflammatory response [234]. Interestingly, HIF-1α regulates hypoxia induced TLR4 expression in macrophages [235]. While TLR9 increases metastatic potential of cancer cells through CXCR4 expression [236], the later modulates TLR9 mediated signaling [237]. Importantly, TLR9 is expressed in GBM [238]. Also, TLR activation is modulated by negative regulators such as suppressor of cytokine signaling (SOCS) that feed back upon and inhibit TLR activation [239]. Moreover, SOCS3 which is constitutively expressed in GBM is not only involved in inducing radioresistance [131], but it also regulates CXCR4 function [240]. Interestingly, heightened STAT3 activation plays a critical role in glioblastoma progression and STAT3 inhibitors have shown promise as therapeutics for GBM [241-243]. STAT3 and STAT1 negatively regulate each other through the induction of SOCS [244]. We therefore investigated the role of IGF-1 in regulating cross talk between HIF-1α, SOCS, TLR9 and CXCR4 in glioma cells.

Despite recent advances in understanding molecular mechanisms involved in GBM progression, the prognosis of the most malignant brain tumor continues to be dismal. Ras activation occurs in GBMs [221] and this high level of active Ras has been a target for glioma therapy. RasGRP3 – is an exchange factor that catalyzes the formation of the active GTP-bound form of Ras-like small GTPases [245]. Importantly, Ras activation stimulates its downstream effector Akt that plays a major role in glioblastoma development as ~ 80% of GBM cases express high Akt levels [246].
Iripallidal [(−)(6R,10S,11S,18R,22S)-26-Hydroxy-22-α-methylcycloirid-16-enalNSC 631939] - a bicyclic triterpenoid isolated from Iris pallida belongs to the terpenoid family as Paclitaxel. Paclitaxel is an effective chemotherapy for several types of neoplasms [247]. Iripallidal inhibited cell growth in a NCI 60 cell line screen [248] and induced cytotoxicity in human tumor cell lines [249]. Besides the fact that Iridals are ligands for phorbol ester receptors with modest selectivity for RasGRP3 [248], not much is known regarding their mechanism of action. In addition to RasGRP3 Iripallidal also binds to PKC [248] which is known to induce cells ectopically expressing hyperactive Ras to undergo apoptosis [250]. Not only is STAT3 essential for Ras transformation [251] but constitutively activated STAT3 is negatively regulated by PKC-activated tyrosine phosphatase(s) [252]. As Iridals interacts with PKCα and RasGRP3-molecules that regulate Akt and STAT3 signaling, and since inhibition of Akt/mTOR and STAT3 signaling are being targeted for GBM treatment we evaluated the effect of Iripallidal on glioma cell proliferation and these signaling pathways. Given the central role of HIF-1α in cancer biology, there is considerable interest in identifying compounds that inhibit HIF-1α activity and testing their ability to inhibit tumor growth [253]. Since HIF-1α plays a crucial role in linking inflammatory and oncogenic pathways [216], we determined the ability of Iripallidal to regulate IGF-1-mediated HIF-1α activation and inflammation in glioma.

The overall goal of this study was to evaluate (i) the role of IGF-1 in regulating HIF-1α and inflammatory response in glioma and (ii) to identify small molecule inhibitor that targets aberrant signaling pathways involved in regulating genes crucial for survival responses in GBM.