CHAPTER 6

CONCLUSIONS AND SIGNIFICANCE
Conclusions

- TNF-α stimulated the mTOR pathway by activating both the mTORC1 and mTORC2-mediated pathways in human glioma cell lines - LN18 and LN229.
- The reduced phosphorylation of Rictor at T1135 on TNF-α treatment may indicate that the negative regulation of mTORC2 by S6Kinase might not be effective in LN18 and LN229, when treated with TNF-α.
- Silencing Rictor using siRNA resulted in ~80% decrease in phosphorylation of Akt (PKB) at S473, but this loss of activity failed to affect the viability of LN18 and LN229 cells or alter their response to TNF-α. On these lines it may be inferred that Akt (PKB) activation by PDK2 (Rictor-mTOR complex) may not be important for cell survival.
- Rictor was not involved in proliferation of cells. Upregulation of p21waf1/cip1 on Rictor ablation was higher compared to level induced by TNF-α in control cells. However, the dramatic increase in p21waf1/cip1 levels by silencing Rictor was not effective to result inhibition of proliferation.
- Rictor ablation led to enhanced expression and activity of MMP-9 but did not affect MMP-2 in cell lines as well as primary cells. The enhanced MMP-9 also resulted in increased invasion of cells through matrigel compared to control cells. The presence or absence of Rictor did not affect MMP-9 levels or activity induced by TNF-α suggesting that TNF-α in the tumor microenvironment might overrule the function of Rictor in keeping MMP-9 activity and invasion of glioma cells in check.
- Rictor ablation upregulated PKC-α but had no effect on phosphorylated PKC-α (S657) levels. PKC-α was dispensable for TNF-α-induced MMP-9 expression.
- Rictor knockdown resulted in activation of MEK and ERK. Studies to decipher the molecular mechanism suggested the role of Raf-1 / MEK / ERK-mediated pathway in the secretion of MMP-9 and invasion in Rictor-silenced glioma cell lines and primary cells derived from glioma tumors.
- A cross talk between Raf-1 / MEK / ERK and the PI3K/ Akt (PKB) pathways results in an antagonistic effect by direct phosphorylation of Raf-1 at S259 by Akt (PKB). Downregulation of Rictor resulted decreased level of pAkt (S473)
and thus activated the Raf-1-MEK-ERK resulting in increased MMP-9 and invasion.

- Rictor ablation enhanced the expression and DNA-binding activity of NF-κB in glioma cells. This resulted in upregulation of specific genes that are targets of NF-κB pathway. Amongst the genes, cathepsin B which is important in invasion process and genes involved in inflammation responses such as TNF, IL-1β and pentraxin 3 were significantly upregulated.

- Among the transcriptional regulators that NF-κB interacts with, Rictor silencing resulted in down regulation of HDAC1, 2 and 3 that were differentially regulated in the two cell lines.

- Rictor negatively regulated the expression of IKKβ in glioma cell lines and cells from primary cultures of glioma tumor. Irrespective of the availability of IKKα, it was IKKβ alone that was regulated by Rictor.

- Mechanistic studies revealed that Rictor ablation resulted in activation of Raf-1 / MEK / ERK pathway that was involved in activation of NF-κB pathway. IKKβ expression regulated by Raf-1 / ERK was important for activation of NF-κB and upregulation of its specific targets.

- The present study demonstrates Rictor is the link between the Akt / mTOR and NF-κB pathways mediated by Raf-1 / MEK / ERK signaling in human glioma cells.

**Significance of the study**

The findings from the study identify a novel role for Rictor, a core component of mTORC2 complex, as a negative regulator of MMP-9 activity and tumor invasion mediated via Raf-1 / MEK / ERK pathway. The signaling pathways mediated by Akt, NF-κB and ERK were activated on loss of Rictor expression. These pathways also promoted MMP-9 activity and invasion on stimulation with TNF-α. However, the functions mediated by TNF-α were not altered in the presence or absence of Rictor suggesting that TNF-α overrules the function of Rictor as the negative regulator of MMP-9 and invasion. The study also showed for the first time, the involvement of Rictor in regulating NF-κB pathway. In most tumors, including gliomas, the microenvironment of the tumor comprising of TAMs and abundant inflammatory cytokines play significant role in tumor progression. While most therapeutic strategies focus on eliminating the
tumor cells by focusing on molecular targets, findings from this study underscore the influence of TNF-α (and other cytokines) in effectiveness of anti-cancer agents.