SUMMARY

Cancer, a group of diseases caused by unregulated cell growth, affects multiple body organs. Specific cancer originates from a definite organ but is not restricted to that particular region as the cells rapidly proliferate and metastasize to other distant organs by invasion or migration through blood vessels, lymphatic channels and bones. De-regulation of crucial signaling pathways in cells is thought to be the major reason for cancer initiation and progression. Among the various types of cancer, we studied the regulation of signaling of Stat3 in prostate cancer (PCa, sixth global cause of cancer related death in men) and glioblastoma multiforme (GBM) (most aggressive and malignant form of brain tumor in human with very short patient survival). Amongst the many causal reasons of PCa and GBM, alterations and perturbations in the molecular signaling pathways play a major role. Several signaling pathways have been implicated in their progression including phosphoinositide-3-kinase (PI3K), Wnt-β-catenin signaling, Ras-MAPK and tyrosine kinase signaling pathways viz. EGFR and VEGFR.

The work described in this thesis is divided into three main sections, the first and second parts deal with the cross-regulation of Stat3 with other signaling pathways and the third explores the possible effect of this regulation of Stat3 on tumorigenesis. In the first part of the work, the cross-regulation between Stat3 activation and canonical Wnt/β-catenin signaling is investigated. We found that EGFR mediated activation of Stat3 resulted in over expression and nuclear accumulation of β-catenin. On the other hand, LiCl mediated stabilization of β-catenin resulted in upregulation of Stat3 in PCa. This feedback regulation between two important signaling pathways results in enhanced tumorigenesis.

In the second part, regulation of Stat3 Ser-727 phosphorylation by CK2 was probed. In our study of human glioma patient samples and a rat orthotopic tumor model, Stat3 Ser-727 phosphorylation was found to occur less frequently when compared to casein kinase 2 (CK2), a well known kinase involved in cancer progression. In glioma, increased Stat3 Ser-727 phosphorylation was observed upon CK2 inhibition. Overexpression of CK2 (α, α’ or β subunits) by transient transfection resulted in decreased Stat3 Ser-727 phosphorylation. Stat3 Tyr-705 residue was conversely phosphorylated in similar situations. As CK2 hyperactivates Akt by phosphorylation at
Ser-129, the role of phospho-Akt Ser-129 in Stat3 Ser-727 phosphorylation was further investigated and determined that Akt mediated regulation of Stat3 Ser-727 was independent of CK2 involvement. Interestingly, we found PP2A, a protein phosphatase, to be a mediator in the negative regulation of Stat3 Ser-727 phosphorylation by CK2. In vitro assays prove that Ser-727 phosphorylation of Stat3 affects the transcriptional activity of its downstream targets like SOCS3, bcl-xl and Cyclin D1. Stable cell lines constitutively expressing Stat3 S727A mutant showed increased proliferation and invasion which are characteristics of a cancer cell. Thus, in gliomas, CK2 may negatively regulate Stat3 Ser-727 phosphorylation thereby enhancing tumorigenicity.

In the last part, we establish the effect of reduced Stat3 Ser-727 phosphorylation in vitro and in vivo tumor models. In the in vitro system, stable cell lines constitutively expressing Stat3 S727A mutant (Stat3 mut) showed increased cell survival, increased rate of proliferation and invasion, when compared to either Stat3 WT or empty vector containing cells. Rat tumor models injected with the Stat3 mut cells formed more aggressive tumors when compared to the Stat3 WT or empty vector cells. Thus, the inhibitory effect of Ser-727 phosphorylation on Stat3 activation is reduced in Stat3 mut cells thereby leading to more aggressive tumor formation. Targeting Stat3 activity has been the method of choice for therapies against cancer and our study provides novel axes for exploration in this direction.