ABSTRACT

Transcription factors are DNA binding proteins involved in expression of subsequent genes in eukaryotes. Research in past decade explored various class of transcription factors that maintain the homeostatic environment in the cell by regulating proliferation, differentiation, survival and apoptosis. Cytokine are signaling proteins which activates the various cascade of biochemical events including activation of inducible transcription factors in the cytosol. In addition over expression of cytokines have tremendous effect on biological systems. Among the transcription factors, Signal Transducers and Activators of Transcription 3 (STAT3) and Nuclear Factor-κB (NF-κB) have extensive role in cancer biology.

STAT3 is a class of cytosolic protein involved in transducing of extracellular signal to nucleus by IL-6 family cytokines, epidermal growth factors and platelet derived growth factor. Binding of signaling molecule to the extracellular domain of transmembrane receptor stimulates the activation of receptor tyrosine kinase (RTK) with phosphorylation of tyrosine residues in cytoplasmic phase. STAT3 proteins are recruited through Src Homology 2 (SH2) domain and tyrosine 705 (Tyr 705) get phosphorylated by Janus Kinase (JAK). The phosphorylated STAT3 dissociates from receptor and undergo dimerization with another phosphorylated STAT3 monomer through Src Homology 2 domain and translocates into the nucleus. Binding of STAT3 can enhance or repress the transcription of genes and required to regulate cell cycle and prevent apoptosis.

It is reported that, STAT3 is upregulated in many types of cancer including leukemia, lymphoma and carcinoma. Comparison with the other members of STAT family proteins, STAT3 levels are preferentially high in tumor cells but not in surrounding normal tissue, suggesting that STAT3 plays an important role in
enhancing the cancer by overexpression of antiapoptotic genes like Bcl-2, Bcl-xL and survivin. Inhibition of dimerization of STAT3 is identified as potential target for new drug to combat cancer.

NF-κB is a host of cytoplasm resides either as homo-dimeric or hetero-dimeric form along with a inhibitory peptide-kappa B (IκB). RelA, RelB, C-Rel, NF-κB1 & NF-κB2 are the members of NF-κB family which are involved in the regulation of cell survival, growth, differentiation, cell adhesion and inflammation. In NF-κB pathway, interaction of extracellular domain of receptor with signaling molecule initiates chain of events in cytoplasm including assembly of adapter proteins, activation of IKKK, IKK followed by phosphorylation and ubiquitylation of IκB, which leads to degradation by proteasome. Exposure of nuclear localization signal in transcription factor translocates the protein into the nucleus which binds to sequence 5-GGGRNWYYCC-3 where R is A or G; N is any nucleotide; W is A or T; Y is C or T called κB-site and upregulate the expression of genes including Bcl-2, Bcl-xL, cIAP, survivin, cyclin D1, TRAF1, TRAF2. NF-κB transcribe genes involved in cell growth and differentiation, survival and inflammation. Studies have shown that, activation of NF-κB in cells which have undergone transformation are protected from apoptosis results in cancer progression. This transcription factor is very active in multiple myeloma, prostate cancer, breast cancer and leukemias.

Chapter – 1: This chapter deals briefly with the literature survey on role of STAT3 and NF-κB in development of various cancers and existing small molecule targeting pro-inflammatory signal transduction pathways.

Chapter – 2: This chapter deals with targeting of JAK-STAT pathway in hepatocellular carcinoma by synthetic small molecules. In this work, we present the
biological evaluation against Hepatocellular Carcinoma (HCC) cells (IC\textsubscript{50} = 7.3 µM), thereby identifying 2-(1-(4-(2-cyanophenyl)1-benzyl-1H-indol-3-yl)-5-(4-methoxyphenyl)-1-oxa-3-azaspiro(5,5) undecane (CIMO) as a potent inhibitor of the JAK-STAT pathway with selectivity over normal LO2 cells (IC\textsubscript{50} = \textgtr 100 µM). The lead compound, CIMO, suppresses proliferation of HCC cells, and achieves this effect by reducing both constitutive and inducible phosphorylation of JAK1, JAK2 and STAT3. Interestingly, CIMO displayed inhibition of Tyr-705 phosphorylation which is required for nuclear translocation of STAT3, but it has no effect on Ser-727 phosphorylation. CIMO accumulates cancer cells in the sub-G1 phase and decreases STAT3 in the nucleus, and thereby causes downregulation of genes regulated \textit{via} STAT3. Suppression of STAT3 phosphorylation by CIMO and knock down of STAT3 mRNA using siRNA transfection displayed a similar effect on viability of HCC cells. Furthermore, CIMO significantly decreased the tumor development in an orthotopic HCC mouse model through the modulation of phospho-STAT3, Ki-67 and cleaved caspase-3 in tumor tissues. Thus, CIMO represents a chemically novel and biologically active compound, which targets the JAK-STAT pathway in potential cancer treatment.

Chapter – 3: Tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) is a pleotropic cytokine known to involve in the progression of several pro-inflammatory disorders. Many therapeutic agents have been designed to counteract the effect of TNF in rheumatoid arthritis as well as a number of cancers. In the present study we have evaluated the anti-cancer activity of novel biscoumarin \textit{in vitro} and \textit{in vivo}. 3,3’-((2-butyl-5-chloro-1H-imidazol-4-yl)methylene)bis(4-hydroxy-2H-chromen-2-one) (BIHC) was found to be the good cytotoxic agent against the HepG2 cell line while exhibiting less toxicity towards normal hepatocytes. Furthermore, BIHC inhibited the proliferation of various
HCC cells in a dose- and time-dependent manner. Moreover, we have demonstrated the downregulation of p65 phosphorylation and other NF-κB regulated gene products upon BIHC treatment, and on the phenotypic level the compound shows inhibition of CXCL12 induced invasion of HepG2 cells. Also, we demonstrate that BIHC inhibits infiltration of macrophages to the peritoneal cavity, and suppresses the activity of TNF-α in vivo in mice primed with thioglycollate broth and lipopolysaccharide. We comprehensively validated the TNF-α inhibitory efficacy of BIHC in inflammatory bowel disease mice model.