Cancer

Cancer is one of the leading cause of mortality worldwide with continuous increase in the number of cancer related deaths every year. According to World Health Organization (WHO), cancer accounted for 8.2 million deaths in 2012 and 60% world’s total new cases initiates in Africa, Central and South America, and Asia. It is expected to reach 22 million annual cases in next two decades. According to Kidwai memorial institute of oncology, India, it is estimated that 0.7 million new cases are reported annually and mortality rate is 0.35 million every year with male dominancy in India. Cancer rate in Karnataka accounts for presence of 0.15 million patients at any given time and about 35,000 new additions every year. More than 100 types of cancer are recognized affecting different organs in the major population including liver cancer, colon cancer, breast cancer, lung cancer, stomach cancer, skin cancer, cervical cancer, head & neck cancer and lymphoma are being major cause for cancer related deaths (Stewart, et al., 2003). The perception of underlying mechanism of cancer progression in the previous decade has enormously increased life expectancy of cancer patients.

Cancer is characterized by unregulated proliferation of cells in a growth factor and density independent fashion in multicellular organisms (Hausman and Cooper, 2004). The ravaging disease is broadly classified into malignant and benign cancer based on their migratory capability where the former display high degree of invasive strength compared to its counterpart. Studies have shown that repetitive mutations in the normal mammalian cell leads to the variation in expression of genes involved in cell cycle regulation, survival and apoptosis to become transformed cell (Hanahan and Weinberg, 2000). This enables the imbalance between cell survival and cell death shifting the equilibrium towards prolonged resistance to apoptosis. The uncontrolled
proliferation of transformed cells leads to the formation of a primary tumor. Small population of tumor cells dislodge from primary tumor and enters circulation to initiate metastasis (Kindt, et al., 2007).


Table 1: Most common type of cancers, estimated numbers of new cases and deaths

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Estimated New Cases</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>224,210</td>
<td>159,260</td>
</tr>
<tr>
<td>Colon and Rectal</td>
<td>136,830</td>
<td>50,310</td>
</tr>
<tr>
<td>Breast (Female-Male)</td>
<td>232,670 – 2,360</td>
<td>40,000 – 430</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>46,420</td>
<td>39,590</td>
</tr>
<tr>
<td>Prostate</td>
<td>233,000</td>
<td>29,480</td>
</tr>
<tr>
<td>Leukemia</td>
<td>52,380</td>
<td>24,090</td>
</tr>
<tr>
<td>Liver Cancer</td>
<td>33,190</td>
<td>23,000</td>
</tr>
<tr>
<td>Non-Hodgkin Lymphoma</td>
<td>70,800</td>
<td>18,990</td>
</tr>
<tr>
<td>Bladder</td>
<td>74,690</td>
<td>15,580</td>
</tr>
<tr>
<td>Kidney</td>
<td>63,920</td>
<td>13,860</td>
</tr>
<tr>
<td>Melanoma</td>
<td>76,100</td>
<td>9,710</td>
</tr>
<tr>
<td>Endometrial</td>
<td>52,630</td>
<td>8,590</td>
</tr>
<tr>
<td>Thyroid</td>
<td>62,980</td>
<td>1,890</td>
</tr>
</tbody>
</table>

Source: http://www.cancer.gov/cancertopics/types/commoncancers
Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) or hepatoma is a most common form of lethal liver malignancy with 4 times more often in men than women and almost equalizes with men after menopause and this was also observed in murine HCC model (Naugler, et al., 2007). HCC is affecting 600,000 people worldwide annually and kills over million contributing to third cause of death in terms of global cancer related mortality (Rajendran, et al., 2011). The disease is characterized by loss of appetite, abdominal tenderness due to fluid accumulation, yellow coloration of skin (hepatic jaundice), fatigue and blood in fecal matter (Koeberle, et al., 2010).

Epidemiology

HCC is mainly associated with old age and it is prevalent in Africa, South-eastern Asia with a high incidence of 40-60 and 18.3-35.5 per 100,000. Western world and central America being with the least incidence of 2.1 per 100,000 and blacks are more susceptible than whites (Whang-Peng, et al., 2010). The major risk factors associated with origin and advancement of HCC are chronic hepatitis B or hepatitis C viral infections, alcoholism, alcoholic hepatitis, liver cirrhosis, Wilson’s disease, non-alcoholic steatohepatitis, obesity, hemochromatosis, diabetes mellitus, alpha-1-antitrypsin deficiency and consumption of carcinogenic aflatoxin B1 (usually produced by Aspergillus species such as A. parasiticus, A. flavus in ground nuts) and anabolic steroids. Alcohol consumption during hepatitis C infection has been shown to act as an additional contributing factor (Nakagawa and Maeda, 2012; Sun and Karin, 2012; Wörns and Galle, 2010).

Diagnosis

The diagnosis of patients at advanced stages of HCC always results in death of the victim. Early detection and treatment of HCC is critical in enhancing the
prognosis (Rajendran, et al., 2011). Alpha-fetoprotein is a tumor associated antigen that is elevated in the serum of 60-70% of patients with HCC. The concentration of alpha-fetoprotein remains below 10 ng/ml in the serum of normal person. In contrast, the serum levels of alpha-fetoproteins are elevated above 400 ng/ml which makes it a convenient diagnostic marker (Kemmer, et al., 2006; Saffroy, et al., 2007). It is highly recommended to determine the levels of alpha-fetoproteins in patients with cirrhosis, hemochromatosis and hepatitis viral infections at the regular intervals. Transient overproduction of alpha-fetoproteins up to 300 ng/ml is also observed in chronic hepatitis, liver resection and toxic liver injury which may result in misinterpretation as HCC (Kim, 2005). In addition, modalities and imaging techniques such as ultrasound scan, computed tomography (CT) scan, magnetic resonance imaging (MRI) and angiography are the other important diagnostic tools to study the cytological or histological changes in HCC (Arguedas, et al., 2003; Gambarin-Gelwan, et al., 2000). The foresaid imaging techniques may not warrant the accurate detection if HCC is at the initial stages with small tumors (<2 cm). In an event of any misinterpretation, liver biopsy is recommended to ensure the structural organization of tumor cells.

**Treatment**

HCC therapy largely rely on various factors including stage of the cancer, functioning of the liver, volume of the tumor, neovasculature, age of the patient and degree of metastatic lesions. The choice of treatment for HCC is generally of surgical therapy or non-surgical therapy. Based on the stage of HCC, choice of treatment is recommended by specialists. The treatment modalities includes liver resection, percutaneous ethanol injection (PEI), percutaneous radiofrequency ablation (RFA), transarterial chemoembolization (TACE), cryosurgery and percutaneous cisplatin gel infusion (Meguro, et al., 2011; Simonetti, et al., 1997).
Liver is a largest gland of abdominal cavity which is reloaded with the capability to regenerate to its normal size and weight even after the removal of $\frac{3}{4}$th of its size. Liver resection refers to the surgical removal of portion of liver bearing tumor. This remains a first choice of treatment if HCC is diagnosed at early stages and surgical resection ensures long term survival (Llovet, et al., 2004). Unfortunately, surgical sectioning of liver in the patients with cirrhosis and patients with metastasized HCC cannot be treated using this strategy.

Percutaneous ethanol injection, percutaneous radiofrequency ablation and percutaneous cisplatin gel infusion are similar treatment strategies used to collapse the small sized tumors (<5 cm) which are directed by ultrasound techniques. In percutaneous ethanol injection method, small tumors are located using ultrasound scanning and ethanol is gradually injected into the tumor and this method is not very effective for huge tumors (Lencioni and Crocetti, 2005). On the other hand, percutaneous radiofrequency ablation involves positioning of needle into the tumor and generation of frictional heat using electrodes up to 90°C and 15 minutes to have deleterious effect on tumor cells (Lencioni, et al., 1998). Percutaneous cisplatin gel infusion is a newer technique which is in phase-II clinical trials and this technique uses the ultrasound guidance for delivering the cisplatin gel directly into tumor mass.

Transarterial chemoembolization is an effective technique of blocking the blood supply to tumor without obstructing the administration of chemotherapeutic agent. TACE is advised to patients with recurrence of local tumor after surgical resectioning, multiple nodular tumors lacking invasive vasculature and those who are not fit for surgical therapies (Cammà, et al., 2002). Most often TACE treatment involves the infusion of radioopaque lipiodol with cisplatin or doxorubicin through right or left hepatic arteries accompanied by introducing embolic agent (Gelfoam).
(Poon, et al., 2000). Optimally TACE can be implemented to the patients with tumors less than >9 cm. Orthotopic liver transplantation from cadavers or live donors (brain dead patients) has significant benefit and stands unique in the HCC treatment. Liver transplantation can be performed to the patients with tumor burden increasing the Milan criteria. Hepatectomy can be carried out to the patients with single tumor (< 5 cm) or three tumor (< 3 cm) with advanced cirrhosis and orthotopic liver transplantation reduces the likelihood of tumor recurrence (Busuttil and Farmer, 1996). Nevertheless, the elevated recurrence rate is a major consideration irrespective of treatment type. Early detection of HCC and treatment at the initial stages increase the prognosis and survival rate. Unfortunately, HCC is detected at the delayed stages where existing treatment strategies are not much advantageous to the prognosis and survival of patients.

**Role of Pro-inflammatory Transcription Factors in cancer (STAT3 & NF-κB)**

**Signal transducer and activator of transcription 3**

Signal transducer and activator of transcription (STAT) proteins are the latent transcription factors present ubiquitously in cytoplasm of most mammalian cells.. STAT proteins were identified two decades ago in connection with interferon signaling and that are studied extensively. STATs have been identified in diverse group of organisms including Caenorhabditis elegans, zebra fish, drosophila, anopheles and several other organisms (Subramaniam, et al., 2013). STAT family comprises of seven representatives including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6 and each with their unique functions. In an unstimulated cell, STAT proteins reside in inactive form in the cytoplasm and activated by signaling from various growth factors and cytokines. Type-1 interferon, type-2 interferon, leptin, interleukins (IL-4, IL-5, IL-6, IL-9, IL-10, IL-11, IL-12, IL-13,IL-
21, IL-22, IL-23, IL-27), Tumor necrosis factor-α, leukemia inhibitory factor (LIF), stem cell factor, epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), cardiotrophin-1, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), oncostatin M, ciliary neurotrophic factor (CNTF), platelet derived growth factor (PDGF), RANTES/CCL5, macrophage inflammatory protein-1 (MIP-1), monocyte chemotactic protein-1 (MCP-1), transforming growth factor-α (TGF-α) are the important signaling ligands involved in activation of STAT proteins (Siveen, et al., 2014). Signaling events are initiated by binding of specific ligand to the extracellular domain of transmembrane receptor. Intrinsic tyrosine kinase activity of receptor further phosphorylate specific tyrosine residues and activates receptor associated cytoplasmic Janus Kinase (JAK) and Src kinase family proteins. Initially, JAKs were regarded as “Just Another Kinase” and mammalian JAK family protein comprises of 4 classes namely JAK1, JAK2, JAK3 and TYK2 (Tyrosine Kinase 2). On activation, JAK proteins phosphorylate specific tyrosine at Src homology 2 (SH2) domain to initiate the dimerization of STAT (homo/hetero) monomers followed by shuttling of STAT dimer into the nucleus. The dimer interacts with STAT response elements in the promoter of target genes to initiate the events of transcription producing acute phase response (Aggarwal, et al., 2006). Eventually, the role of serine phosphorylation in STATs was demonstrated to modulate the transcription activity (Wen, et al., 1995; Zhang, et al., 1995). Generally, STATs regulate the expression of genes involved in cell survival, proliferation, differentiation, cell migration, inhibition of pathogen assault and immunomodulation.

In addition, Protein Inhibitor of Activates STAT (PIAS) and Suppresser Of Cytokine Signaling (SOCS) are the two major negative regulators of JAK-STAT
signaling pathway. PIAS contains zinc binding segment that can bind to STAT dimer in the cytoplasm and prevents binding to DNA. SOCS is a transcription co-regulator which interact with JAK protein to prevent activation of STATs. Src homology region 2 domain containing phosphatase-1(SHP1 and SHPTP2), phosphatase and tensin homolog (PTEN) and protein tyrosine phosphatase (PTPs) are the other active phosphatases involved in dephosphorylation of STAT and thereby manipulating the relay of extracellular signals to nucleus (Kim and Baumann, 1999; Yamamoto, et al., 2002).

STAT3 was initially identified as DNA binding protein in interleukin-6 induced hepatocytes with a potential to selectively interact with the cis-acting elements of acute phase genes (Akira, et al., 1994). Research in the previous decade revealed that STAT3 is activated by IL-6 family cytokines (Oncostatin M, Leukemia inhibitory factor, Cardiotrophin-1, Ciliary neurotrophic factor, and IL-11) and several other growth inducing cytokines (Bromberg and Darnell, 2000; Ihle and Kerr, 1995). Later, the functions of STAT3 in proliferation of B-lymphocytes, immature myeloid cells, mature granulocytes, differentiation and growth inhibitory action in monocytes, regulation of stem cells was demonstrated in various cellular systems (Levy and Lee, 2002). The entanglement of STAT3 in tumorigenesis was evidenced by ample amount of studies in various cancer cell lines and clinical samples.

Constitutive activation of STAT3 has been observed in many types of solid and liquid tumors including hepatocellular carcinoma, leukemias, lymphomas, prostate cancer, breast cancer, head and neck cancer, multiple myeloma, pancreatic cancer, colon cancer and brain cancer (Rebouissou, et al., 2009; Yu, et al., 2007; Yu, et al., 2009). The critical role of STAT3 in cancer development has been demonstrated in significant number of studies (Calo, et al., 2003). The underlying
mechanism in the constitutive activation of STAT3 is contributed by autocrine function of transformed cells. Hypersecretion of IL-6 in acute myeloid lymphoma and TGF-α in head and neck squamous cell carcinoma are some of the exemplifications for autocrine signaling in constitutive activation of STAT3 (Rubin Grandis, et al., 1996; Schuringa, et al., 2000).

In various cancers including HCC, STAT3 regulates the gene products associated with tumor proliferation, survival (p53, Bcl-2, Bcl-xL, survivin), cell cycle progression (c-Myc, cyclin D1), enhanced angiogenesis (Vascular endothelial growth factor [VEGF], basic fibroblast growth factor [bFGF], hypoxia inducible factor 1α [HIF-1α]), tissue repair (Tff3, RegIIIβ, RegIIIγ), anti-oxidant (MnSOD, catalase) and metastasis (MMP2, MMP9, RANTES) (Figure 1) (Alvarez and Frank, 2004; Bollrath, et al., 2009; Bowman, et al., 2000; Chan, et al., 2004; Kujawski, et al., 2008; Levy and Darnell, 2002; Niu, et al., 2002; Pickert, et al., 2009; Rebouissou, et al., 2009; Tebbutt, et al., 2002; Waldner, et al., 2010). On the other hand, constituent activation of STAT3 confers immune evasion by downregulating the anti-tumor immunity. In contrast, knock down of STAT3 in mice lead to the embryonic lethality indicating the role of STAT3 in growth and development of an organism (Takeda, et al., 1997). Overactivation of STAT3 confers resistance to apoptosis by transcribing survivin gene in breast cancer cells (Gritsko, et al., 2006). IL-6, IL-22 and leptin are found to be the most vital inducers of JAK-STAT3 pathway in liver carcinoma. Aberrant elevation of serum IL-6 was reported in the patients with hepatocellular carcinoma and chronic liver inflammation (Abiru, et al., 2006). Several reports suggested that blockade of JAK-STAT3 signaling with small molecules and antisense technology interrupts the cell cycle in HepG2 cells in vitro and in vivo (Mohan, et al., 2014). Clinical tissue samples of HCC patients displayed persistent activation of STAT3 and
significant reduction in the activity of SOCS-1. Numerous strategies have been
developed to design the therapeutic agents against JAK-STAT3 pathway in HCC.
Various monoclonal antibodies, novel small molecules which abrogates
proinflammatory cytokine pathways at different stages are under clinical
investigations.
Figure 1: Role of JAK-STAT3 pathway in tumorigenesis
Persistent activation of STAT3 is linked with the activity of cascade of upstream signaling proteins. Overexpression of ligands and receptors, prolonged ligand-receptor interaction and rampant activity of upstream kinases such as JAK and c-Src leads to dysregulation of STAT3 contributing to malignancies (Bowman, et al., 2000; Bromberg, 2002). Studies revealed that upstream events have vital role in constitutive activation of STAT3 in human cancers (Turkson and Jove, 2000). In many tumors IL-6, IL-6R and growth factor receptors necessitates the transmission of extracellular signals to nucleus through over activation of STAT3. Many antagonists, monoclonal antibodies, natural compounds and chemical inhibitors have been designed, evaluated against this pathway by researchers. Prevention of interaction of JAK2, c-Src, Ras, Abl with STAT3, enhancement of dephosphorylation, inhibition of dimerization and nuclear translocation are the potential treatment strategies in targeting JAK-STAT pathway.

Researchers have made a significant contribution in developing inhibitors against JAK-STAT3 pathway in the last decade. A breakthrough in discovery of JAK inhibitors was given by Meydan et al (AG490) in B-cell leukemias. LS-104 is a structural analogue of AG490 with more potency and currently being tested in phase-II clinical trials against acute lymphoblastic leukemia (Wilks, 2008). Recently, ruxolitinib (INCB018424) become the first small molecule JAK1/2 to get FDA approval in 2011 for the treatment of myeloproliferative neoplasms (Seavey and Dobrzanski, 2012). Tofacitinib is a pyrrolopyrimidine derivative with the IC$_{50}$ of 1.3 nM, 1.9 nM, 0.2 nM & 23 nM against JAK1, JAK2, JAK3 & Tyk2 respectively (Menet, et al., 2013; Norman, 2012). Baricitinib and Tofacitinib are structurally related compounds in which former is in Phase-III trials and latter is an approved JAK3 inhibitor for rheumatoid arthritis. In addition, GLPG0634 is an orally-available,
selective JAK1 inhibitor developed against inflammatory diseases. In phase-I clinical trials, it displayed promising results. In 2014, a Biotech company Galapagos announced the beginning of phase-II study with GLPG0634 in Crohn’s disease. Furthermore, cisplatin related compounds CPA-7 and IS3 295 were found to inhibit STAT3 significantly in mammary carcinoma cells (Turkson, et al., 2005; Turkson, et al., 2004). Lestaurtinib (CEP-701), Pacritinib (SB1518), VX-509, XL019, INCB20 and AZD1480 are some of the known JAK inhibitors and few of them have entered clinical trials to treat various cancers. Stattic is a hydroxyanthraquinone based compound with a high selectivity towards the SH2 domain of the STAT3, thereby blocks the activation of STAT3 protein and restrain its translocation into nucleus. Finally, Sorafenib is a FDA approved drug to treat hepatocellular carcinoma which inhibits STAT3 by upregulating the SHP-1 (Tai, et al., 2011).

**Natural compound inhibitors of STAT3 signaling pathway**

Natural compounds induce their effect via interfering with various signaling cascades. In spite of fact that, an ideal therapeutic agent should have single target, natural compounds have been noted for their action in a multifactorial way. However, the compounds which targets a single signaling mechanism often fails to achieve high impact as a therapeutic in complex diseases such as cancer (Hanahan and Papahadjopoulos, 1965). For an instance, natural compounds such as curcumin, betulinic acid, plumbagin, celastrol, butein, diosgenin, guggelsterone, γ-tocotrienol, sanguinarine, capsaicin, thymoquinone, cucurbitacin, uroseolic acid, garcinol, pterostilbene, honokiol, luteolin and caffeic acid have been studied extensively and shown to interfere with JAK-STAT3 signaling in various tumor models (Table 2). Despite of the fact that these compounds abrogate STAT3 signaling, they also manipulate other proinflammatory signaling cascades. Therefore, most natural
compounds induce their action in a multifactorial way and can be a promising therapeutic agents against complex diseases.

Table 2: Selective small molecule inhibitors of STAT3 signaling pathway

<table>
<thead>
<tr>
<th>Natural/ Small molecule inhibitors</th>
<th>Mechanism of action</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG490</td>
<td>Inhibits JAK2 activity in HCC cell lines</td>
<td><img src="image" alt="AG490" /></td>
<td>(Kusaba, et al., 2007)</td>
</tr>
<tr>
<td>Aloin</td>
<td>Inhibits VEGFR2 mediated STAT3 activation in human colorectal cancer cells and xenograft mice model</td>
<td><img src="image" alt="Aloin" /></td>
<td>(Pan, et al., 2013)</td>
</tr>
<tr>
<td>Apigenin</td>
<td>Inhibits JAK2 activity in breast cancer cells</td>
<td><img src="image" alt="Apigenin" /></td>
<td>(Seo, et al., 2014)</td>
</tr>
<tr>
<td>Atiprimod</td>
<td>Inhibits JAK2/JAK3 in multiple myeloma cells</td>
<td><img src="image" alt="Atiprimod" /></td>
<td>(Amit-Vazina, et al., 2005; Hamasaki, et al., 2005)</td>
</tr>
<tr>
<td>AZD1480</td>
<td>Inhibits all types of JAK proteins in Hodgkin lymphoma cells</td>
<td><img src="image" alt="AZD1480" /></td>
<td>(Derenzini, et al., 2011)</td>
</tr>
<tr>
<td>Compound</td>
<td>Effect</td>
<td>Reference</td>
<td></td>
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<tr>
<td>Butein</td>
<td>Inhibits JAK2, c-Src and upregulates SHP1 in HCC cells</td>
<td>(Rajendra n, et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>Inhibits JAK1 and JAK2 in glioma cells</td>
<td>(Swiatek-Machado, et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Capsaicin</td>
<td>Inhibits JAK1 and c-Src in multiple myeloma cells</td>
<td>(Bhutani, et al., 2007)</td>
<td></td>
</tr>
<tr>
<td>Celestrol</td>
<td>Inhibits JAK1, JAK2 and c-Src in hepatocellular carcinoma cells and xenograft mice model</td>
<td>(Rajendra n, et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Cucurbitacin B</td>
<td>Inhibits STAT3 activation in leukemia cells</td>
<td>(Chan, et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Curcumin</td>
<td>Inhibits JAK1 and JAK2 in murine glioma cell lines and syngeneic mice model</td>
<td>(Weissenberger, et al., 2010)</td>
<td></td>
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<tr>
<td>Diosgenin</td>
<td>Upregulates the expression of SHPTP2 to decrease STAT3 activation in</td>
<td>(Li, et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Molecule</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td>Garcinol</td>
<td>Inhibits JAK1, JAK2 and c-Src in head and neck carcinoma cells and xenograft mice model</td>
<td>(Li, et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>GLPG063</td>
<td>Preferentially inhibits purified JAK1 and in rheumatoid arthritis mice model</td>
<td>(Van Rompaey, et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>Guggelstone</td>
<td>Inhibits JAK2, c-Src and upregulates the expression of SHP1 in multiple myeloma cells</td>
<td>(Ahn, et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Honokiol</td>
<td>Inhibits JAK2 and upregulates the expression of SHP1 in HCC cells</td>
<td>(Rajendra n, et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Lestaurtini b</td>
<td>Inhibits JAK2 in myelofibrosis</td>
<td>(Furqan, et al., 2013; Su, et al., 2014)</td>
<td></td>
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<tr>
<td>Compound</td>
<td>Action</td>
<td>Reference</td>
<td></td>
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<tr>
<td>Pacritinib</td>
<td>Selectively inhibits JAK2 in hematologic disorders</td>
<td>(Hatzimichael, et al., 2014)</td>
<td></td>
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<tr>
<td>Pterostilbene</td>
<td>Inhibits JAK2 in osteosarcoma cells</td>
<td>(Liu, et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>Plumbagin</td>
<td>Inhibits STAT3 in prostate cancer cells and orthotopic xenograft mice model</td>
<td>(Hafeez, et al., 2013)</td>
<td></td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>Inhibits JAK1 and JAK2 (FDA approval in 2011 for the treatment of myeloproliferative neoplasms)</td>
<td>(Seavey and Dobrzanski, 2012)</td>
<td></td>
</tr>
<tr>
<td>Sanguinarine</td>
<td>Inhibits JAK2 and c-Src in prostate cancer cells</td>
<td>(Sun, et al., 2012)</td>
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<tr>
<td>Sorafenib</td>
<td>Inhibits STAT3 by upregulating SHP1 in HCC cells</td>
<td>(Tai, et al., 2011)</td>
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<tr>
<td>Compound</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td>Staurosporin</td>
<td>Selectively inhibits JAK3 in isolated enzyme and in T-cells</td>
<td>(Wilson, et al., 2009)</td>
<td></td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>Inhibits JAK2 and c-Src and upregulates the expression of SHPTP-2 in multiple myeloma cells</td>
<td>(Li, et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>γ-Tocotrienol</td>
<td>Inhibits JAK1, JAK2, c-Src and upregulates the expression of SHP1 in HCC cells</td>
<td>(Rajendra n, et al., 2011)</td>
<td></td>
</tr>
<tr>
<td>Tofacitinib</td>
<td>Inhibits JAK3 and approved for rheumatoid arthritis</td>
<td>(Menet, et al., 2013)</td>
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<tr>
<td>Ursolic acid</td>
<td>Inhibits JAK2 and c-Src in prostate cancer cells and xenograft mice model.</td>
<td>(Shanmug am, et al., 2011)</td>
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<tr>
<td>VX-509</td>
<td>Inhibits JAK3 in rheumatoid arthritis and currently in Phase-II trials</td>
<td>(Kyttaris, 2012)</td>
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</table>
Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

NF-κB is a transcription factor discovered by Baltimore and colleagues in 1986 and pervasively present in almost all the types of mammalian cells (Gilmore, 2006). It was initially identified as DNA binding protein which recognize the specific set of nucleotides in the immunoglobulin kappa light chain joining segment gene in B-cells (Weaver and Baltimore, 1987).

NF-κB is a cytoplasmic inhabitant present either in its homo- or hetero-dimeric form and united with an inhibitory peptide, Inhibitory κB (IκBα) (Li and Sethi, 2010; Sethi and Tergaonkar, 2009). The ankyrin repeats which are present in the inhibitory protein masks the nuclear localization signal and prevent the translocation of NF-κB into nucleus. Five types of NF-κB subunits have been identified so far and they are RelA (p65), RelB, c-Rel,NF-κB1 (p50) and NF-κB2 (p52) (Gilmore, 2006). All the five transcription factors shares 300 amino acid homology at amino terminal end designated as Rel homology domain (RHD). The RHD essentially possess dimerization and DNA binding domain which facilitates the homo- or hetero-dimerization and binding to specific DNA sequences enabling regulation of respective gene expression (Plaksin, et al., 1993). In addition, RelA, RelB and c-Rel possess carboxy terminal transcriptional activation domains (TADs) which promote the expression of target genes. On contrary, p50 and p52 lacks carboxy terminal transcriptional activation domain which suppress the transcription if not associated with a protein containing TAD (RelA or RelB or c-Rel). p50 and p52
are the processed products of large protein precursors called p105 and p100 respectively. p50-p65 heterodimer is the most common association observed with inhibitory peptide (IκBα) (Nabel and Verma, 1993). Attachment of ubiquitin to IκBα, degradation by proteasome complex and phosphorylation of serine residues translocates the dimer into nucleus to initiate transcription of target genes (Calvisi, et al., 2006).

Multiple inflammatory signals such as stress (hypoxia, heavy metals), free radicals (hydrogen peroxide), radiation (ultraviolet, gamma), oxidized lipids (Low density lipoproteins), bacterial and viral antigens (lipopolysaccharides, EBV latent membrane proteins) cytokines (TNF family, EGF, IL-17, IL-18), tumor promoters (phorbol esters, okadaic acid) and carcinogens (smoke, 7,12 dimethyl-benz(a)anthracene [DMBA]) can activate NF-κB pathway (Aggarwal, 2004; Brasier, 2006; Gilmore, 1999; Perkins, 2007; Tian and Brasier, 2003). NF-κB is a key player in regulation of immune system and small dysregulation may contribute to inflammatory diseases (rheumatoid arthritis, Inflammatory Bowel Disease) neoplastic transformation (cancers) and autoimmune diseases (Monaco, et al., 2004).

NF-κB is reported to regulate the transcription of over 400 genes entailed with inflammation (TNF, Chemokines, IL-1), apoptotic resistance (Bcl-2, Bcl-xL), angiogenesis (VEGF, IL-1, IL-8), immortality (telomerase), cell survival (cIAP, XIAP), cell proliferation (IL-1β, IL-6, c-Myc, cyclin D1, HER2), cellular invasion (IL-8, urokinase type of plasminogen activator [uPA] MMPs), metastasis (VCAM-1, ICAM-1, ELAM-1, inducible nitric oxide synthase [iNOS], CXCR4) and tumor progression (COX2, MMP-9) (Bhat-Nakshatri, et al., 2002; Biswas, et al., 2000; Kawano, et al., 1988; Kim, et al., 2002; Kim, et al., 2000; Li and Sethi, 2010; Novak, et al., 1991; Pahl, 1999; van de Stolpe, et al., 1994).
NF-κB signaling operates in two ways, namely: Classical/canonical pathway and Alternative/noncanonical pathway (Karin, 1999). Both the pathways involve signaling by different ligands and molecular interactions. The ligands for canonical pathway includes TNF-α, IL-1, CpG, LPS acts on cytokine receptor, toll-like receptor (TLR), B-cell receptor, T-cell receptor on the target cell to activate NF-κB pathway (Tergaonkar, 2006). On the other hand, the ligands for noncanonical pathway includes lymphotoxins, CD40L, BAFF, LPS acts on lymphotoxin-β receptor, CD40, B-cell activating factor (BAFF) receptor on the target cells to induce NF-κB pathway, The
noncanonical pathway is required for the generation of B- and T-lymphocytes which operates during the establishment of lymphoid organs (Scheidereit, 2006).

In the classical pathway, binding of anyone of the foresaid ligand to its receptor leads to the assembly of adaptor proteins to the cytoplasmic domain of the receptor. Further adaptor proteins facilitates the activation of IκB kinase (IKK) complex. IKK complex comprises of two molecules of NF-κB essential modulator (NEMO) with IKKα and/or IKKβ. NEMO is also designated as IKKγ. Activated IKK complex essentially phosphorylates and promotes ubiquitylation of IκB, thereby subjecting it to proteasomal degradation. The NF-κB dimers which get activated by classical pathway are RelA, RelB, c-Rel and p50 (Hayden and Ghosh, 2004).

In the alternative pathway, interaction of ligand with the corresponding receptor results in the activation of a cytoplasmic protein called NF-κB inducing kinase (NIK). In this pathway, IKK complex does not contain NEMO but comprises of IKKα dimer. Activated NIK phosphorylates the IKK complex, and in turn activated IKK complex phosphorylates p100. Upon phosphorylation, p100 is a large precursor which undergo proteolytic processing to form precise p52. Consequently, p52-RelB heterodimer translocates into nucleus to transcribe target genes (Moynagh, 2005).
Figure 3: Canonical and Noncanonical NF-κB signaling pathways

Constitutive activation of NF-κB have been reported in several cancers due to aberrant signaling by various cytokines (Tak and Firestein, 2001). The role of NF-κB in tumor progression at various stages have been extensively studied (Arkan and Greten, 2011; Karin, 2006). Deletion of NF-κB in hepatocytes and abrogation of TNF-α production by neighboring parenchymal cells lead to induction of apoptosis of hepatocytes and significantly decreased the prevalence of HCC (Pikarsky, et al., 2004). NF-κB induce inflammatory stromal cells to promote the progression of colitis associated colon cancer (Greten, et al., 2004). However, some reports also suggest that, NF-κB can also prevent cancer. For an instance, disruption of NF-κB signaling in
keratinocytes results in squamous cell carcinoma of skin (Seitz, et al., 1998). In addition, the role of NF-κB in oncogenesis is well established in various tumor models. Several chemotherapeutic agents and radiotherapy activate NF-κB pathway in both in vitro and in vivo (Brach, et al., 1991; Prasad, et al., 1994). Activation of NF-κB pathway leads to the expression of target genes which are responsible for radioresistance and chemoresistance and thereby protect the tumor cells from undergoing apoptosis. Therefore, disruption of NF-κB activation prevents the transcription of NF-κB target genes, in turn increases the responsiveness to chemotherapy and radiotherapy to promote apoptosis (Bentires-Alj, et al., 2003; Feinman, et al., 1999; Nakanishi and Toi, 2005; Wang, et al., 1999).

**Synthetic compound inhibitors of NF-κB signaling pathway**

Constitutive activation of NF-κB is entangled with the rampant expression of tumorigenic proteins. Several small molecules have been implemented in targeting persistent activation of NF-κB in various solid and liquid malignancies. Unfortunately many treatment strategies including radiotherapy and chemotherapeutic agents (paclitaxel, cisplatin, vinblastine, 5-fluorouracil, doxorubicin, bortezomib, vincristine, daunomycin and tamoxifen) have been reported to induce cellular stress in turn activates NF-κB proteins in various cancer cells (Baumann, et al., 2008; Bednarski, et al., 2008; Cusack, et al., 1999; Konstantinopoulos, et al., 2008; Kusaba, et al., 2007; Melisi and Chiao, 2007). Therefore, using of chemotherapeutics with stress inducing effect on cancer cells is found to be ineffective. Therefore numerous heterocyclic small molecules have been tested and proved for their inhibitory activity against NF-κB signaling cascade in various inflammatory disease models including cancer, Inflammatory Bowel Disease and rheumatoid arthritis (Gupta, et al., 2010). The potential of sesquiterpene lactones to regulate the activation of NF-κB was
demonstrated by Vincent et al (Duplan, et al., 2014). The effect of SC-514 (thiophene derivative) on RANKL-induced NF-κB pathway in RAW264.7 cells was examined. SC-514 suppressed the RANKL-induced NF-κB signaling and detailed examination proved that SC-514 is a selective inhibitor of IKKβ (Liu, et al., 2013). The new pyrrolopyrazole derivatives displayed a potent inhibition against phosphorylation of IKBα which lead to the elevated levels of p65 in cytoplasm (Zhuang, et al., 2014). Resatorvid (TAK-242) is a cyclohexene derivative which interacts with intracellular domain of Toll-like receptor 4 (TLR4) and suppresses the activation of NF-κB completely. The mechanism of action of resatorvid was demonstrated in LPS induction of transiently TLR4 expressing HEK293 cells (Kawamoto, et al., 2008). Salmeterol (Phenethyl alcohol derivative), Doxazosin (quinazoline derivative), Pefabloc (benzenesulfonyl derivative), BMS-345541 (dimethylimidazole quinoxaline derivative), 1-O-acetylbritannilactone, ben佐xathiole derivatives, Amino-pyrimidine derivatives, and CDDO methyl ester (oleanane triterpenoid) are some of the synthetic small molecules reported to inhibit various proteins of NF-κB signaling cascade and downregulate the progression of inflammatory diseases.

**Natural compound inhibitors of NF-κB signaling pathway**

The therapeutic impact of natural compounds against diverse pathological conditions have been dealt thoroughly by various research groups. As discussed earlier in the previous section, natural compounds induce their effect in a multifactorial way by manipulating multiple signaling cascades in complex diseases. According to our observation based on previous reports, most natural compounds displayed their inhibitory activity against both JAK-STAT3 and NF-κB pathway in common. The natural compounds with dual role in manipulating both the pro-inflammatory pathways are apigenin, curcumin, caffeic acid, ursolic acid, betulinic
acid, garcinol, plumbagin, celastrol, beutein, γ-tocotrienol, diosgenin, guggelsterone, sanguinarine, pterostilbene, capsaicin, thymoquinone, cucurbitacin, honokiol, urosolic acid and luteolin.

In addition, several other natural compounds have been investigated against NF-κB signaling pathway (Table 3). Rocaglamide is a flavagline was first synthesized in 1990 by Barry Trost and it is reported to interfere with upstream of IKK signaling at sub-nanomolar concentration in Jurkat T cells (Baumann, et al., 2002). Nimbolide is a triterpene isolated from Neem tree (Azadirachta indica) and was reported to be cytotoxic against colorectal cancer cells and xenograft mice models (Gupta, et al., 2013). Detailed analysis revealed that nimbolide inhibits IKK and thereby sequential suppression of downstream proteins. Epoxyquinol B is a fungal metabolite (pentaketide) was investigated to identify the underlying mechanism of NF-κB inhibition and it was found to inhibit TAK1 complex which resides upstream of IKKβ (Kamiyama, et al., 2008). Numerous natural compounds including betain, herbimycin A, anandamide, geldanamycin have been demonstrated to revoke the pro-inflammatory signaling pathway in various disease models.

Table 3: Selective small molecule inhibitors of NF-κB signaling pathway

<table>
<thead>
<tr>
<th>Natural/Small molecule inhibitors</th>
<th>Mechanism of action</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anandamide</td>
<td>Inhibits IKKβ in human T lymphocytes</td>
<td><img src="image" alt="Structure" /></td>
<td>(Sancho, et al., 2003)</td>
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<tr>
<td>Betain</td>
<td>Inhibits NIK and IKK in human embryonic kidney cells</td>
<td><img src="image" alt="Structure" /></td>
<td>(Go, et al., 2005)</td>
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<tr>
<td>Compound</td>
<td>Action Description</td>
<td>Chemical Structure</td>
<td>Reference</td>
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<tr>
<td>BMS-345541</td>
<td>Selectively inhibits IKK in human acute monocytic leukemia cells</td>
<td><img src="image" alt="BMS-345541" /></td>
<td>(Beaulieu, et al., 2007)</td>
</tr>
<tr>
<td>Doxazosin</td>
<td>Antagonist for alpha1-adrenergic receptor and inhibits NF-κB signaling in breast cancer cells</td>
<td><img src="image" alt="Doxazosin" /></td>
<td>(Hui, et al., 2008)</td>
</tr>
<tr>
<td>Epoxyquinol B</td>
<td>Inhibits TAK1 complex in cervical cancer cells</td>
<td><img src="image" alt="Epoxyquinol B" /></td>
<td>(Kamiyama, et al., 2008)</td>
</tr>
<tr>
<td>Herbimycin A</td>
<td>Preferentially inhibits IKKβ in glioma cells</td>
<td><img src="image" alt="Herbimycin A" /></td>
<td>(Ogino, et al., 2004)</td>
</tr>
<tr>
<td>Isorhapontigenin</td>
<td>Inhibits IKKβ in cardiac myocytes</td>
<td><img src="image" alt="Isorhapontigenin" /></td>
<td>(Li, et al., 2005)</td>
</tr>
<tr>
<td>Nimbolide</td>
<td>Inhibits IKK in colorectal cancer cells and xenograft mice model</td>
<td><img src="image" alt="Nimbolide" /></td>
<td>(Gupta, et al., 2013)</td>
</tr>
<tr>
<td>Compound</td>
<td>Effect</td>
<td>Reference</td>
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<td>----------------------------------</td>
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<tr>
<td>Rocaglamide</td>
<td>Interferes with upstream of IKK signaling in human T lymphocytes</td>
<td>(Baumann, et al., 2002)</td>
<td></td>
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<tr>
<td>Geldanamycin</td>
<td>Inhibits the formation of IKK complex in cervical cancer cells</td>
<td>(Chen, et al., 2002)</td>
<td></td>
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<tr>
<td>Manumycin A</td>
<td>Inhibits IKKβ in HCC cells</td>
<td>(Bernier, et al., 2006)</td>
<td></td>
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<tr>
<td>1-O-acetylbritannilactone</td>
<td>Inhibits IKKβ in vascular muscle cells of Sprague-Dawley rats</td>
<td>(Liu, et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Pefabloc</td>
<td>Interferes with upstream of IKK signaling in rat pancreatic lobules</td>
<td>(Tando, et al., 2002)</td>
<td></td>
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<tr>
<td>Resatorvid</td>
<td>Inhibits TLR4 and suppresses the activation of NF-κB in Human embryonic kidney cells</td>
<td>(Matsunaga, et al., 2011)</td>
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Selective inhibition of IKKβ in synovial fibroblasts (Kishore, et al., 2003)

References:


and the promotion stages of epithelial carcinogenesis. The Journal of clinical investigation 114, 720-728.


of liver metastases with a cooled-tip electrode needle: results of a pilot clinical trial. European radiology 8, 1205-1211.


analog, attenuates cardiac hypertrophy via blocking signaling transduction pathways. Free radical biology & medicine 38, 243-257.


43
cells via the protein tyrosine phosphatase SHP-1. Journal of cellular physiology 227, 2184-2195.


interleukin-6-mediated signaling pathway through STAT3 dephosphorylation. Biochemical and biophysical research communications 297, 811-817.


