

Role of interleukin-6 in cancer progression and therapeutic resistance

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Abstract In the last several decades, the number of people dying from cancer-related deaths has not reduced significantly despite phenomenal advances in the technologies related to diagnosis and therapeutic modalities. The principal cause behind limitations in the curability of this disease is the reducing sensitivity of the cancer cells towards conventional anticancer therapeutic modalities, particularly in advance stages of the disease. Amongst several reasons, certain secretory factors released by the tumour cells into the microenvironment have been found to confer resistance towards chemo- and radiotherapy, besides promoting growth. Interleukin-6 (IL-6), one of the major cytokines in the tumour microenvironment, is an important factor which is found at high concentrations and known to be deregulated in cancer. Its overexpression has been reported in almost all types of tumours. The strong association between inflammation and cancer is reflected by the high IL-6 levels in the tumour microenvironment, where it promotes tumorigenesis by regulating all hallmarks of cancer and multiple signalling pathways, including apoptosis, survival, proliferation, angiogenesis, invasiveness and metastasis, and, most importantly, the metabolism. Moreover, IL-6 protects the cancer cells from therapy-induced DNA damage, oxidative stress and apoptosis by facilitating the repair and induction of countersignalling (antioxidant and anti-

apoptotic/pro-survival) pathways. Therefore, blocking IL-6 or inhibiting its associated signalling independently or in combination with conventional anticancer therapies could be a potential therapeutic strategy for the treatment of cancers with IL-6-dominated signalling.

Keywords IL-6 · Cancer · Therapeutic resistance · STAT-3 · Chemotherapy · Radio-resistance

Introduction

Inflammation has a very strong link with various types of cancer. Malignant cells are highly proliferative in nature, which is facilitated by the inflammatory molecules that are continuously being secreted by other cells and/or tumour cells themselves in a microenvironment [1, 2]. Interleukin-6 (IL-6) is one such inflammatory molecule, which is produced and secreted by various types of cells including the tumour cells. It is involved in the proliferation and differentiation of malignant cells and found to be high in serum and tumour tissues of a majority of cancers, viz. colorectal cancer [3], breast cancer [4], prostate cancer [5], ovarian carcinoma [6], pancreatic cancer [7], lung cancer [8], renal cell carcinoma [9], cervical cancer [10] and multiple myeloma [11]. Elevated levels of IL-6 are associated with aggressive tumour growth and response to therapies in many types of cancer [12, 13]. Patients with high levels of circulating IL-6 are generally associated with poor prognosis and shorter survival, whilst a lower level of IL-6 is associated with better response to therapy [14, 15].

Anticancer drugs and ionizing radiation used during cancer therapy induce inflammatory signalling, mainly in the form of the nuclear factor-kappa B (NF- κ B) pathway [16, 17]. NF- κ B regulates the expression of different pro-inflammatory

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cytokines, chemokines and anti-apoptotic genes and acts as a key molecular link between inflammation and initiation as well as progression of oncogenesis [18]. Chemotherapeutic drugs and radiation also induce IL-6 expression in tumour and stromal cells [12, 13] through the activation of NF- κ B signalling, leading to therapeutic resistance [19, 20]. These evidences suggest that blocking IL-6 or inhibiting the IL-6 downstream signalling pathways may provide therapeutic gain in those cancers which are associated with a higher level of IL-6. This review provides an insight into the current understanding of the role of IL-6 in the regulation of various hallmarks and associated signalling in cancer as well as its contribution to therapeutic resistance. It also provides an insight into how the anti-IL-6 antibody or the inhibitors of pathways downstream to IL-6 signalling can improve the effectiveness of cancer radio- and/or chemotherapy.

Interleukin-6

IL-6 is a glycosylated polypeptide chain having a molecular weight of nearly 25 kDa, depending on the glycosylation and the species. It has a characteristic structure made up of four long α -helices arranged in an up–up–down–down topology [21]. It was first discovered as a B cell differentiation factor (BSF-2) which induces the maturation of B cells into antibody-producing cells [22]. Besides its role in immune regulation, it plays an important role in the maintenance of hepatocytes, haematopoietic progenitor cells, the skeleton, the placenta, the cardiovascular system and the endocrine as well as nervous systems. In the murine haematopoietic system, IL-6 induces the expansion of progenitor cells by stimulating cells from the resting stage to enter the G1 phase [23]. IL-6 also supports various physiological functions by acting as a hepatocyte stimulatory factor and by inducing the acute-phase protein synthesis. It is also known to stimulate osteoclast formation, induce bone resorption and is responsible for neural differentiation [24]. IL-6 supports the survival of cholinergic neurons, induces adrenocorticotrophic hormone synthesis, and, in placenta, causes the secretion of chorionic gonadotropin from trophoblasts [23]. IL-6 also plays a very important role in metabolism. For example, in the absence of IL-6, mice develop glucose intolerance and insulin resistance, whilst IL-6^{-/-} mice exhibit signs of liver inflammation [25]. The secretion and availability of IL-6 is ubiquitous, and it can bind to various types of cells in different tissues. However, its binding on different cell types may differ, resulting in two different types of IL-6-dependent cell signalling (Fig. 1).

IL-6 binds to the IL-6 receptor (IL-6R) on the plasma membrane, and the resultant IL-6/IL-6R complex associates with gp130 and causes gp130 homodimerization to form an activated IL-6 receptor complex, which is a hexameric structure consisting of two molecules each of IL-6, IL-6R and gp130

[26, 27]. The binding of IL-6 to IL-6R occurs at three distinct receptor-binding sites of IL-6R and gp130. However, the Ig-like domain of the human IL-6R is not involved in the direct binding of IL-6 [28]. Upon binding to the receptor and gp130, IL-6 induces various functions by activating cell signalling events [24]. IL-6 triggers signal transduction via two forms of IL-6R: one a transmembrane 80-kDa receptor with a short cytoplasmic domain (mbIL-6R, also known as IL-6R α , gp80 or CD126) and the other a small, extracellular, secretory soluble receptor (sIL-6R) [29]. Classical IL-6 signalling, which is the predominant form of IL-6 signalling, requires membrane-bound IL-6R (mbIL-6R) and is restricted to hepatocytes, some epithelial cells and certain leukocytes (Fig. 1) [26]. IL-6R contains a very short cytosolic domain that lacks the major potential motifs for transduction of intracellular cell signalling. However, gp130 (also known as IL-6R β or CD130) in the same hexameric complex is rich in all these potential motifs required for intracellular signalling, such as SHP-2 domain and YXXQ motif for JAK/STAT signalling. Upon binding with IL-6/IL-6R, the dimerization of gp130 leads to the activation of associated cytoplasmic tyrosine kinases, resulting in the phosphorylation of various transcription factors [24]. gp130 is expressed in almost all organs, including the brain, heart, lung, liver, kidney, spleen and placenta, where it plays an indispensable role in their development, cell survival, growth and tissue homeostasis [30]. gp130 is a common signal transducing receptor and is also used by other members of the IL-6 family cytokines, such as IL-11, IL-12, IL-27, leukaemia inhibitory factor, oncostatin M, etc. [31]. Although the expression of transmembrane IL-6R is limited to the hepatocytes and subsets of leukocytes, gp130 is expressed ubiquitously. Therefore, the IL-6/sIL-6R complex can transduce the IL-6 signal in various cells, which do not express transmembrane IL-6R but express gp130, through a trans-signalling mechanism. sIL-6R is generated by alternative splicing of IL-6R mRNA or by limited proteolysis of mbIL-6R by Zn-dependent metalloproteinase (ADAM10 and ADAM17, a disintegrin and metalloproteinases 10 and 17; Fig. 1) [29–32]. sIL-6R is devoid of the cytoplasmic and transmembrane domains and binds to IL-6 with comparable affinity as the membrane-bound form, thereby mediating gp130 activation in an autocrine or paracrine manner [21]. Consequently, by binding to sIL-6R, IL-6 increases its reach to a wide variety of cells. There is enough evidence to suggest that neural cell, neural stem cells, haematopoietic stem cells, liver progenitor cells and embryonic stem cells depend on sIL-6R in their response to IL-6 [33–36]. The level of sIL-6R present in the human sera increases during inflammation [32, 37]. Knockdown of the IL-6R gene in hepatocytes reduces the levels of sIL-6R by 32 % in the serum, whilst ablation of the IL-6R gene in haematopoietic cells reduces the sIL-6R serum levels by 60 % [38]. These observations suggest that hepatocytes and haematopoietic cells are the main sources of sIL-6R

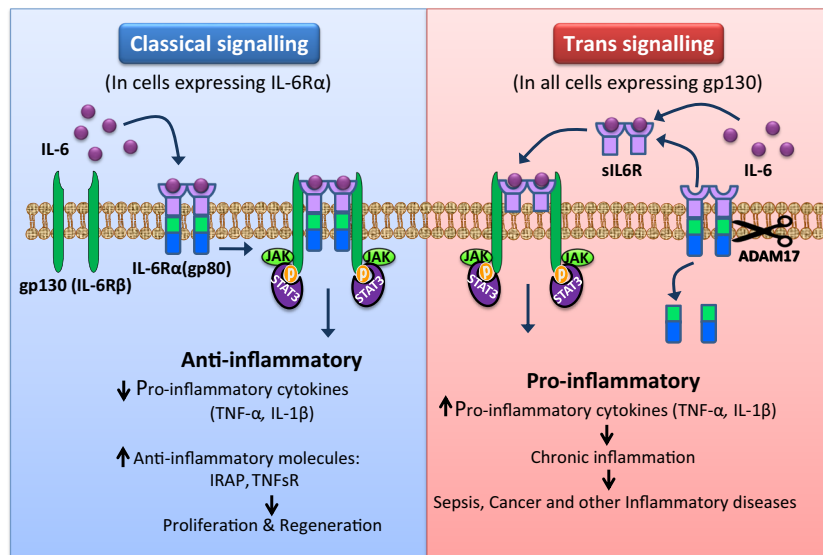


Fig. 1 Classical and trans-signalling of IL-6: In classical signalling, which occurs mainly in leukocytes and liver cells, IL-6 binds to the membrane-bound receptor mbIL-6R α , which then forms a complex with the ubiquitously present cell receptor gp130 (*IL-6R β*). Trans-signalling can occur in any cell expressing gp130. In trans-signalling, IL-6 forms a complex with sIL-6R, which is a small part of mbIL-6R α produced by either metalloproteinase or by alternative splicing. Furthermore, the IL-6–sIL-6R complex binds with gp130 on cells

which do not express mbIL-6R. The inflammatory reactions induce the production of sIL-6R, which elicits response to IL-6 in cells that do not express IL-6 receptor (mbIL-6R α) and/or remain inert to IL-6 signalling in normal physiological conditions. Classical signalling activates the anti-inflammatory pathways and promotes the regeneration of tissues, whereas trans-signalling activates pro-inflammatory pathways and is known to play a significant role in many diseases such as sepsis and cancer

found in the circulation [38]. Like sIL-6R, a soluble form of the signal transducer protein gp130 (sgp130) is also present in the circulation at relatively high concentrations during inflammation and cancer [37, 39]. sgp130 is mainly produced by alternative splicing rather than limited proteolysis, as in the case of sIL-6R generation. Since sgp130 binds to the IL-6/sIL-6R complex in the circulation, it acts as a specific inhibitor of IL-6-mediated trans-signalling [40]. Classic signalling via the mbIL-6R is not affected by sgp130. Its inhibitory action depends on the IL-6/sIL-6R ratio, with trans-signalling inhibition at low concentrations [37].

IL6 expression and secretion

The common characteristic of many of the stimuli that activate IL-6 is that they are associated with tissue damage or stress (e.g. ionizing radiation, UV, reactive oxygen species, viruses, microbial products and other pro-inflammatory cytokines) [12, 41, 42]. IL-6 production is predominantly regulated by changes in the gene expression of various transcription factors such as NF- κ B, CCAAT/enhancer-binding protein a and activator protein 1, the major transcriptional regulator, although posttranscriptional modifications have also been identified [41, 43]. Though the activation of these transcription factors leads to the overexpression of this cytokine during inflammation, its expression is also known to be regulated epigenetically in breast cancer, hepatocellular carcinoma, colon cancer,

prostate cancer and lung cancer through miRNAs (Lin28 and Let-7) [44].

The normal blood circulating level of IL-6 is nearly 1 pg/ml [45, 46], but an increase in its level is found under several conditions such as acute hyperglycemia [47], high-fat meal [48], normal menstrual cycle [49], physical activity [50] and during/after surgery [51]. Inconsistent levels of IL-6 have also been observed during pregnancy, with median values around 128 pg/ml registered at delivery, which drop by more than two fold (~58 pg/ml) immediately afterward [52]. Furthermore, serum IL-6 levels have been found to increase drastically during sepsis [53].

Many physiological factors such as diet, exercise and stress are known to regulate the secretion of IL-6 [47–53]. Exercise is an important stimulus for increased gene expression and production of IL-6 in skeletal muscle, and the majority of circulating IL-6 during exercise originates from contracting muscle, resulting in a 100-fold increase over the normal physiological level [50, 54]. IL-6 produced in the working muscle during physical activity acts as an energy sensor that activates AMP-activated kinase and enhances glucose uptake, metabolism, lipolysis and fat oxidation [50]. IL-6 is also known to sensitize myotubes to insulin and enhances glucose uptake in muscles for high glycogen synthesis. Moreover, the reduced level of muscle glycogen also augments IL-6 production and secretion from muscle cells [55]. In addition to exercise, the expression of IL-6 increases in skeletal muscles under other

conditions as well, such as denervation of muscles and muscular dystrophy, also resulting in the upregulated expression of muscle IL-6 [54]. The adipose tissue produces nearly 30 % of circulating systemic IL-6, where it is closely associated with obesity, impaired glucose tolerance and insulin resistance [56]. Plasma IL-6 concentrations are a predictor of the development of type 2 diabetes, and peripheral administration of IL-6 results in insulin resistance in rodents and humans by causing hyperlipidaemia and hyperglycaemia [56]. Besides muscle cells, e.g. macrophages, mast cells, dendritic cells, B cells and CD4 effector T helper cells in the immune system are amongst the major sources of IL-6 production [22, 57–59]. In addition, IL-6 is also secreted by a variety of non-haematopoietic cells such as fibroblasts, endothelial cells, epithelial cells, astrocytes and malignant cells [2, 33, 42, 60]. Enhanced levels of IL-6 have been found in many cancers, with an inverse relationship between IL-6 levels and response to chemotherapy and hormone therapy [61]. Furthermore, IL-6 expression has also been found higher in recurrent tumours as compared to primary tumours, as well as in recurrent metastatic lesion as compared to primary metastasis [15].

The primary sources of IL-6 in the tumour microenvironment are tumour cells as well as tumour-associated macrophages (TAMs), CD4⁺ T cells, myeloid-derived suppressor cells (MDSCs) and fibroblasts [59–62]. In the tumour microenvironment, IL-6 supports tumorigenesis by directly affecting cancer cells through the modulation of both the intrinsic and extrinsic activities of tumour cells as well as by influencing stromal cells that indirectly support tumorigenesis [63]. For example, in skin and prostate cancer, the autocrine and paracrine secretion of IL-6 induces a complex of cytokine, growth factors and protease network consisting of granulocyte macrophage colony-stimulating factor (GM-CSF), IL-8, MCP-1, vascular endothelial growth factor (VEGF) and MMP-1 and stimulates malignant progression [64]. Basically, tumour cells produce IL-6 for promoting their survival and progression and do not depend on paracrine release of IL-6 by stromal cells [63]. However, both autocrine and paracrine mechanisms of IL-6 are known to influence tumour progression and metastasis through IL-6 trans-signalling [2, 64].

Pleiotropic role of IL-6

Cancer is an inflammatory disease, and the key feature of cancer-related inflammation is the expression of cytokines. Different cytokines play different roles in the onset and resolution of inflammation. However, a ubiquitous and functionally diverse cytokine, IL-6 is a pleiotropic cytokine with pro- and anti-inflammatory properties (Fig. 1). It is an important cytokine regulating the acute-phase response of inflammation [21]. During inflammatory response, tumour necrosis factor alpha (TNF α) induces the expression of IL-6 together with

other inflammatory alarm cytokines, such as IL-1 β , which are involved in the elicitation of acute-phase inflammatory reactions/responses (Fig. 1) [65]. Furthermore, IL-6 controls the level of acute inflammatory responses by downregulating the expression of pro-inflammatory cytokines and upregulating anti-inflammatory molecules, including IL-1 receptor antagonist protein, TNF-soluble receptor and extrahepatic protease inhibitors (Fig. 1) [66]. IL-6 has also been found to counter inflammation by inhibiting TNF α release in experimental endotoxemia [67]. This pleiotropic nature of IL-6 maintains the host–tumour homeostasis.

During switch between pro- and anti-inflammatory roles, TNF α and IL-1 β negatively regulate IL-6 signalling at different levels by enhancing the IL-6-induced expression of the suppressor of cytokine signalling (SOCS3, feedback inhibitor) and/or targeting IL-6-induced gene expression via its action on target gene promoters [59, 68–70]. IL-1 β also counteracts IL-6-mediated STAT-3 activation independent of SOCS3 expression [71]. IL-1 β is the major regulator of the pro- and anti-inflammatory nature of IL-6; on the one hand, it reduces the pro-inflammatory activity of IL-6 that results in the inhibition of overshooting immunological reactivity, such as in inflammatory bowel disease or autoimmune arthritis, whilst on the other hand, it delays the anti-inflammatory effects of IL-6 to reinforce the pro-inflammatory processes in the initial phase of inflammation [70]. Similarly, the high concentration of IL-1 β in the tumour microenvironment must maintain the chronic inflammatory environment by suppressing the anti-inflammatory processes of IL-6.

Emerging evidences suggest that IL-6 plays key roles in the acute as well as the transition (resolution) phase of inflammation [68]. Furthermore, IL-6 trans-signalling recruits T cells at the site of inflammation by triggering the expression of T cell-attracting chemokines (CCL4, CCL5, CCL17 and CXCL10) [72]. Moreover, IL-6 also rescues T cells from entering apoptosis by STAT-3-dependent up-regulation of anti-apoptotic regulators (Bcl-2 and Bcl-xL) and modulation of Fas surface expression [73, 74]. IL-6 also regulates the differentiation of recruited T cells towards TH2 by inducing the expression of IL-4. Thus, IL-6 regulates some of the key steps in controlling inflammation and sets the anti-inflammatory environment by promoting TH2 response [57]. Collectively, these evidences suggest that endogenous IL-6 plays a vital anti-inflammatory role in both local and systemic acute inflammatory responses by controlling the level of pro-inflammatory cytokines, mainly. The trans-signalling of IL-6 regulates mainly the pro-inflammatory response; however, IL-6 classical signalling imparts its anti-inflammatory or regenerative activity (Fig. 1) such as regeneration [75], inhibition of epithelial apoptosis and the activation of the acute-phase response [76]. Understanding of the pleiotropic role of IL-6 in cancer

is not very clear; however, the information available from other inflammatory diseases suggest that IL-6 might play both pro- and anti-inflammatory roles in the tumour microenvironment, which is crucial for host–tumour interaction.

Role of IL-6 and associated signalling in cancer

The notion that inflammation drives cancer is now well established. One of the major drivers of this link is NF- κ B, which plays a central role in the secretion and activation of numerous pro-inflammatory cytokines from multiple cell types in the tumour microenvironment, including macrophages, T cells and epithelial cells [43, 57, 58]. Several pro-inflammatory cytokines released by innate and adaptive immune cells regulate cancer cell growth and thereby contribute to tumour promotion and progression. Amongst these, IL-6 is important in the development of human cancer and activates oncogenic pathways, and it is known to be deregulated in cancer [77]. Overexpression of IL-6 in many types of tumours, such as colorectal cancer [3], prostate cancer [5], breast cancer [4], ovarian carcinoma [6], pancreatic cancer [7], lung cancer [8], renal cell carcinoma [9], cervical cancer [10], multiple myeloma [11] and lymphomas [78], suggests a strong link between this cytokine and cancer. The high susceptibility and incidence of liver cancer in males is also found to be associated with high levels of IL-6 [79]. However, in females, oestrogen steroid hormones inhibit IL-6 production and so protect female mice from cancer [79, 80]. Activation of the IL-6/STAT-3 signalling axis is an important event in cancer which promotes tumorigenesis by regulating multiple survival signalling pathways in cancer cells [24]. IL-6 regulates nearly all hallmarks of cancer, such as inhibition of apoptosis [81, 82], promotion of survival [8, 75], proliferation [35, 83], angiogenesis [10], invasiveness and metastasis [62, 84], and is also known to regulate cancer cell metabolism (Fig. 2) [85, 86]. Therefore, there exists a strong link between IL-6 and cancer, similar to the link between cancer and inflammation. Majority of the phenotypes or hallmarks of cancer which are influenced by IL-6 comprise many biological capabilities that are acquired during tumour development. The role of IL-6 in the regulation of hallmarks of cancer will be discussed in detail later.

Evasion of growth suppressors

Cancer cells evade some powerful programmes that negatively regulate cell proliferation. Many of these programmes depend on the action of tumour suppressor genes such as p53 and RB (retinoblastoma gene), which

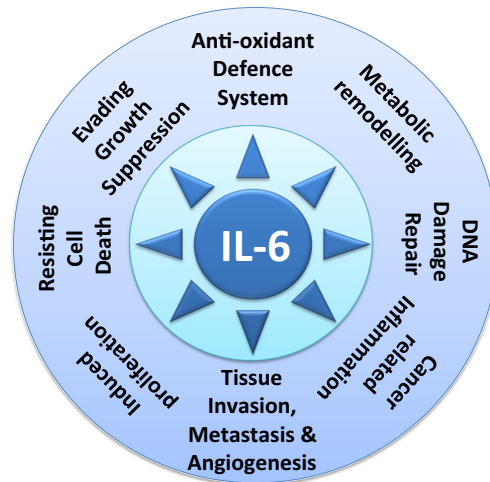


Fig. 2 IL-6 and cancer hallmarks: IL-6 plays an important role in cancer growth and progression by influencing nearly all hallmarks of cancer. The picture illustrates the contribution of IL-6 in some major characteristics of cancer, which are known as cancer hallmarks

undergo either loss or gain of function, respectively, to limit cell growth and proliferation. Rb (retinoblastoma-associated) and TP53 proteins are the essential regulatory nodes within two key complementary cellular regulatory circuits that govern the decisions of cells to proliferate or activate senescence and apoptotic programmes [87]. The hypermethylation of CpG islands in the promoter regions of tumour suppressor genes has been found in many tumours, which allows cancer cells to bypass crucial checkpoints in cell cycle progression and evade apoptotic signals (Fig. 3) [88, 89]. IL-6 is known to increase both the expression of DNA methyltransferase (DNMT-1) [90] and its translocation to the nucleus by DNMT-1 nuclear localizing signal's phosphorylation via PI-3K/AKT signalling [91], thereby increasing the activity of DNMT-1, resulting in CpG island methylation of the promoter region of the p53 gene (Fig. 3) [89]. Contrary to the hypermethylation of tumour suppressor genes, IL-6 is also involved in causing global hypomethylation of retrotransposon long interspersed nuclear element-1 in oral squamous cell carcinoma cell lines, which promotes tumorigenesis in the oral cavity [92]. These epigenetic alterations in tumour cells contribute to the epigenetic silencing of tumour suppressor genes and lead to enhanced tumorigenesis.

Mutations in the RB gene contribute to cellular transformation in various types of malignancies [93]. Normal retinoblastoma protein suppresses the transition from the G1 to the S phase of the cell cycle, which is regulated by the phosphorylation of Rb protein. The active, hypo- or dephosphorylated form of Rb binds with E2F and induces G1 growth arrest. On the contrary, the phosphorylated Rb, which is inactive, cannot bind E2F and activates CDK, thereby facilitating entry of cells into the S phase [94]. In multiple myeloma (MM) cells, IL-6

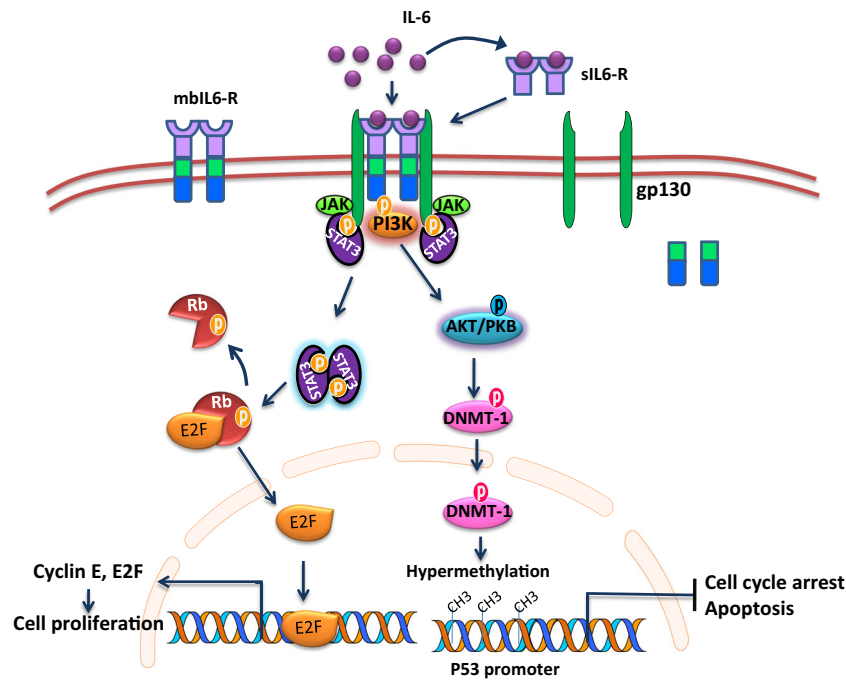


Fig. 3 Role of IL-6 in evading growth suppressors: Normally, E2F remains bound with Rb and localized to the cytosol. IL-6 signalling either induced by mbIL-6R or sIL-6R activates JAK/STAT-3 phosphorylation, which then phosphorylates Rb, resulting in the dissociation of E2F from Rb. The free E2F translocates to the nucleus, where it induces the expression of genes (cyclin E and E2F itself)

responsible for the proliferation of cells. Similarly, IL-6 via PI3K/AKT signalling causes the activation of DNMT-1 by its phosphorylation. After phosphorylation, DNMT-1 translocates to the nucleus and hypermethylates the p53 promoter, resulting in silencing of tumour suppressor, pro-apoptotic and other p53 target genes

facilitates the phosphorylation of Rb and, thus, promotes cell growth (Fig. 3). Furthermore, Rb phosphorylation also upregulates IL-6 secretion by MM cells and IL-6-mediated autocrine tumour cell growth [95].

Resistance against cell death

Cancer cells evolve a variety of cytoprotective approaches to limit or circumvent cell death programmes, mainly apoptosis. Besides evading growth suppression signalling, by loss of TP53 function, tumours are also associated with an increase in the expression of anti-apoptotic regulators (Bcl-2, Bcl-xL and Mcl-1) and survival signals (Igf1/2) or downregulated pro-apoptotic factors (Bax, Bim and Puma) [96, 97]. IL-6 regulates the process of apoptosis by activating STAT-3 and NF- κ B signalling (Fig. 4), which transactivates the expression of many anti-apoptotic proteins such as Bcl-2, Bcl-xL, Mcl-1, etc., in cholangiocarcinoma cells [98], cervical cancer [99], gastric cancer cells [81], myeloma cells [82], basal cell carcinoma cells [100] and esophageal carcinoma [101]. In addition, these pro-survival proteins, mainly Bcl-2, promote cell proliferation [102]. As the balance between pro-apoptotic and anti-apoptotic proteins is important for apoptotic decision, the ratio of pro-apoptotic to anti-apoptotic factors is increased with oxidative stress, but the increased levels of IL-6 may alter this ratio in favour of anti-apoptotic signalling, leading to cell

survival, both in IL-6-treated cells and IL-6-expressing transgenic mice [103]. Besides this, IL-6-induced Bcl-2 regulates Bak interactions with mitofusins via inhibition of Bak dissociation from Mfn2 and also inhibits the interaction of Bak with Mfn1. These two mitochondrial events are the major determinants of cell death pathways as they prevent mitochondrial fragmentation during apoptosis [103, 104]. Therefore, Bcl-2 appears to be an essential mediator of IL-6-induced cytoprotection (Fig. 4).

Besides Bcl-2 and Bcl-xL, IL-6 also supports tumour cell survival by inducing the expression of survivin through direct binding of STAT-3 to the survivin promoter [105]. Furthermore, downregulation of survivin at the gene expression level by inhibiting STAT-3 induces apoptosis in tumour cells [105]. IL-6 triggers PI3K/Akt, NF- κ B and MAPK/ERK signalling in prostate cancer cells and results in the upregulation of cyclin A1 expression that promotes tumour cell proliferation in hepatoma, prostate cancer, bladder cancer and in multiple myeloma. IL-6-induced activation of PI3K/Akt signalling further activates I κ B kinase (IKK), which initiates NF- κ B signalling, leading to transactivation of pro-survival and proliferation-inducing proteins (Fig. 4) [83, 106–109]. IL-6/STAT-3 signalling is also required for the survival of intestinal epithelial cells in colitis-associated cancer, where IL-6 produced from lamina propria myeloid cells protects normal and pre-malignant epithelial cells from cell death [75].

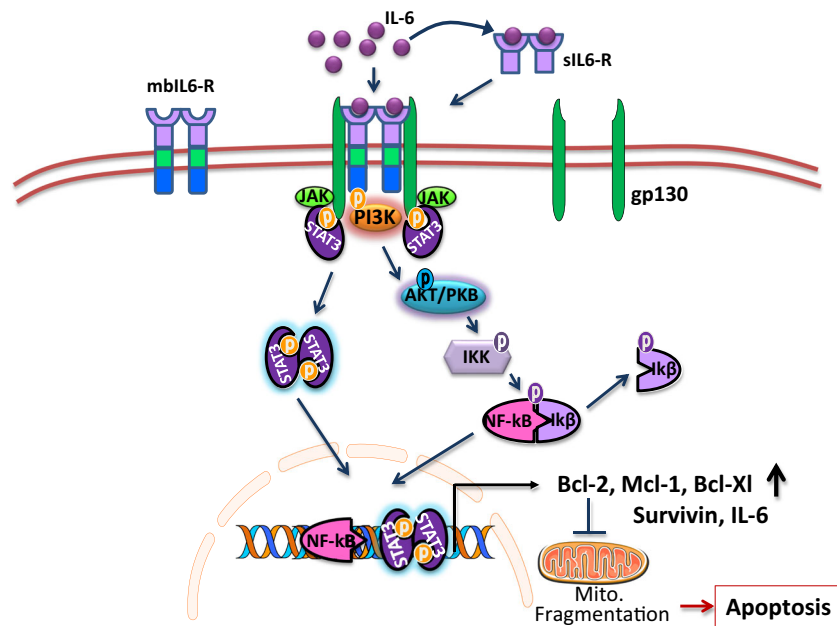


Fig. 4 IL-6 in resisting cell death: IL-6-induced JAK/STAT-3 and NF-κB signalling facilitates the translocation of STAT-3 and NF-κB in the nucleus. Activation of these signalling pathways results in the expression of anti-apoptotic genes (*Bcl-2*, *Bcl-xL*, *Mcl-1*, *survivin*, etc.)

and IL-6 for the constitutive activation of IL-6-dependent signalling in cancer cells. IL-6-induced Bcl-2 expression inhibits stress (endogenous and therapeutic)-induced mitochondrial fragmentation and protects the cells from apoptosis

Collectively, it appears that IL-6 facilitates tumour growth primarily by inhibiting apoptosis and enhancing cell proliferation. Besides deriving the growth potential, cancer cells exploit IL-6 for inducing resistance towards anticancer therapy-induced death pathways. For example, IL-6 confers protection from dexamethasone-induced apoptosis by activating PI3K/AKT signalling and inactivating casapase-9, thereby inhibiting apoptosis in multiple myeloma cells [109]. It is also known to induce resistance in cisplatin-mediated cytotoxicity in prostate cancer cell lines and esophageal squamous cell carcinoma [110, 111]. Furthermore, IL-6-induced Bcl-2 confers protection against hyperoxic damage and oxidant (H_2O_2) injury [103]. Thus, enhanced IL-6 levels appear to confer resistance against chemotherapy in cancer by downregulating cell death pathways.

Induction of proliferation/replicative immortality

The potential growth stimulatory effect of IL-6 in tumour cells is due to the activation of several signalling pathways. IL-6 stimulates tumour cell proliferation and survival by activating the Ras/Raf/MEK/MAPK, PI3K/AKT and JAK/STAT pathways via gp130 tyrosine phosphorylation [83, 101, 108]. In colitis-associated cancer, IL-6 produced by myeloid cells stimulates the proliferation of malignant epithelial cells via NF-κB/IL-6/STAT-3 cascade [75]. These signalling pathways help tumours in the acquisition of unlimited replication potential, which is essentially required to generate large tumours.

Majority of the genes that regulate cell survival and proliferation, such as Bcl-2, Bcl-xL, Mcl-1, Fas, cyclin D1, cyclin E1 and p21, are direct targets of STAT-3. In addition, other transcription factors which promote proliferation, including c-Myc, c-Jun and c-Fos, are also targets of STAT-3 [112]. In tumour cells, STAT-3 activation is mediated through autocrine production and paracrine secretion of IL-6 from stroma and infiltrating inflammatory cells [58–62]. IL-6/STAT-3 signalling also functions as a transcriptional repressor of p53 expression, whilst blocking STAT-3 upregulates the expression of p53, leading to p53-mediated apoptosis [113].

IL-6 has also been found to mediate its multi-lineage haematopoietic effects by shifting stem cells from the G0 to the G1 stage of the cell cycle, thereby inducing the proliferation and making stem cells more responsive to additional haematopoietic growth factors such as IL-3, IL-4, G-CSF, M-CSF or GM-CSF [114]. The autocrine production of IL-6 by non-stem cells activates the JAK1/STAT-3 signal transduction pathway which plays an important role in the conversion of non-stem cells into stem-like cells through the upregulation of Oct-4 (a stem cell marker) [115]. Therefore, IL-6 not only induces the proliferation of cancer cells but also maintains the population of cancer stem cells that induce the reoccurrence of tumours. Since only the cancer stem cells have tumorigenic potential amongst the heterogeneous mass of tumours [116], IL-6 seems to play an important role in the maintenance of equilibrium between non-cancer and cancer stem cells, as observed in breast and prostate cancers

[44]. Thus, IL-6 has been suggested as a potential regulator of normal and tumour stem cell self-renewal.

Cancer-related inflammation

Accumulating evidences suggest that chronic inflammation predisposes cells and tissues to different forms of cancer [117]. Thus, cancer and inflammation have a strong connection, which prompted the use of anti-inflammatory drugs for cancer prevention. Cancer-related inflammation involves the infiltration of TAMs, white blood cells and inflammatory messengers like cytokines, such as TNF, IL-1 and IL-6, and chemokines (CCL2 and CXCL8), which facilitate tissue remodelling and angiogenesis [118]. IL-6 is one of the most highly expressed mediators of inflammation in the tumour microenvironment, and STAT-3-dependent tumorigenesis has been associated with the local secretion of IL-6 and its related trans-signalling within the tumour microenvironment in inflammation-induced colorectal cancer [75]. In addition, production of IL-6 by M2-type macrophages in ulcerative colitis supports the development of colon tumours [119]. These studies have identified a link between IL-6 and tumour-associated inflammation. The primary players in inflammation include transcription factors such as NF- κ B, STAT-3 and primary inflammatory cytokines (IL-1b, IL-6 and TNF α) [1, 120]. NF- κ B is the major regulator of inflammation which is deregulated in many cancers [120]. As a major effector molecule of NF- κ B activation through the STAT-3 pathway, IL-6 appears to be an important component of the NF- κ B/IL-6/STAT-3 cascade involved in tumorigenesis [75]. STAT-3 is required for the maintenance of NF- κ B activation in tumours [121], whilst IL-6 promotes carcinogenesis through inflammation and cell proliferation [35, 44, 106]. Since inflammation enhances the growth and progression of gastrointestinal tumours via the activation of IL-6-mediated STAT-3 signalling, it appears that there is a strong link between IL-6, inflammation and tumour promotion [1, 117, 118].

Metabolic remodelling

Enhanced aerobic glycolysis is one of the prominent phenotypes of a majority of cancer cells which facilitate proliferation and confer protection against death, besides energy production [122, 123]. This induced glycolysis is one of the major factors that contribute to IL-6-induced therapeutic resistance in cancer. IL-6-mediated stimulation of glucose metabolism is dependent on the signal transduction involving the PI-3 kinase and JAK/STAT pathways through the enhanced expression of major glycolytic enzymes hexokinase 2 and PFKFB-3 [124]. It also enhances glucose transport by inducing the expression of glucose transporters GLUT-1 and GLUT-4, which further translocate to the plasma membrane, resulting in a higher

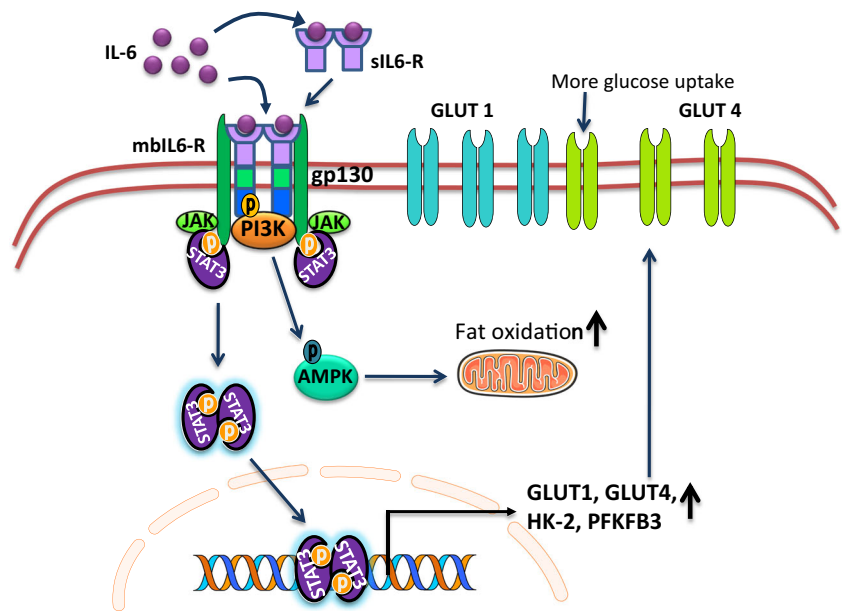
glucose influx in cells (Fig. 5) [124]. In addition, it also increases fatty acid oxidation (Fig. 5) [85]. Furthermore, IL-6-induced p53 regulates glycolytic metabolism through NF- κ B-mediated mechanism, which also increases GLUT-2 and GLUT-4 receptors on the cells to enhance glycolysis [125].

IL-6 also causes 5' AMP-activated protein kinase (AMPK) phosphorylation, which is important for IL-6-mediated glucose uptake and lipid oxidation (Fig. 5) [126] and is known to be involved in obesity-associated cancers [127]. In sickle cell disease, elevated plasma IL-6 levels are correlated with increased rates of glycolysis in red blood cells, as evidenced by the increase in lactic acid and higher 2,3-bisphosphoglycerate levels [128]. More recently, treatment of IL-6^{-/-} mice with diethylnitrosamine (a carcinogenic agent) after a high-fat diet has been shown not to support faster development of hepatocellular carcinoma (HCC) as compared to a low-fat diet, suggesting that IL-6 preferentially may render obese individuals susceptible to HCC [106]. In cachectic tumour-bearing mice, a high level of IL-6 has been found to suppress mTORC1 activity by AMPK activation, thereby rendering the mice irresponsive to glucose administration [86]. p62 is a scaffold protein that binds to the nutrient-sensing component of mTOR, crucial for metabolic reprogramming during cell transformation. Loss of p62 has been found to reduce mTOR activity, resulting in impaired metabolism and higher IL-6 release, causing tumorigenesis [129].

Maintenance of redox potential

Oxidative stress induced either by therapeutic agents or metabolic alterations induces damage to macromolecules, viz. proteins, lipids, membranes and DNA, that play a key role in the development of cancer. NF- κ B is a critical transcription factor that senses redox imbalance and facilitates cytokine gene induction during cellular stress [130]. Endogenous or induced oxidative stress activates transcription factor NF- κ B, which regulates the expression of IL-6 by binding to the promoter region of the IL-6 gene [131, 132]. IL-6, which is the major effector molecule of NF- κ B, itself causes NF- κ B activation in cancer cells, which results in more IL-6 production. Furthermore, this enhanced IL-6 concentration in the tumour microenvironment constitutively activates NF- κ B signalling in the same or neighbouring cells (autocrine/paracrine) [131, 133]. Aberrant NF- κ B regulation has been observed in many cancers, whose sustained activation requires STAT-3. Since NF- κ B and STAT-3 are regulated by IL-6, it appears that the NF- κ B/STAT-3/IL-6 signalling cascade plays an important role in oncogenesis [134]. IL-6 is known to protect cardiac myocytes from oxidative stress-induced apoptosis through STAT-3 signalling and gastric cancer cells by upregulating Mcl-1 expression [81]. In multiple myeloma cells, IL-6 causes radio-resistance by activating NF- κ B in an autocrine manner, which results in the activation of antioxidant defence system

Fig. 5 IL-6 in metabolic remodelling: IL-6-induced JAK/STAT-3 signalling (both classical and trans) induces the expression of major glycolytic enzymes (*HK2* and *PFKB3*) and glucose transporters (*GLUT-1* and *GLUT-4*). The expression of these glycolytic genes ensures aerobic glycolysis in tumour cells. IL-6 also induces fatty acid oxidation in the mitochondria by activating the AMPK pathway



enzymes such as glutathione peroxidase (GPx), superoxide dismutase II (MnSOD) and catalase (Fig. 6) [135].

Apoptosis plays a very crucial role in maintaining genomic integrity by selectively removing the population of heavily damaged cells. Reactive oxygen species (ROS) and pro-inflammatory cytokines are generally elevated following exposure to ionizing radiation and during human carcinogenic

processes [136]. Hydrogen peroxide (H_2O_2) is a potent reactive oxygen species that causes mitochondrial dysfunction and cell death. Preconditioning cells with IL-6 decreases H_2O_2 -induced cell death by increasing the expression of prohibitin, which is involved in mitochondrial biogenesis and metabolism, apoptosis and replicative senescence [137]. In addition to signalling through STAT-3, the stress-induced activation

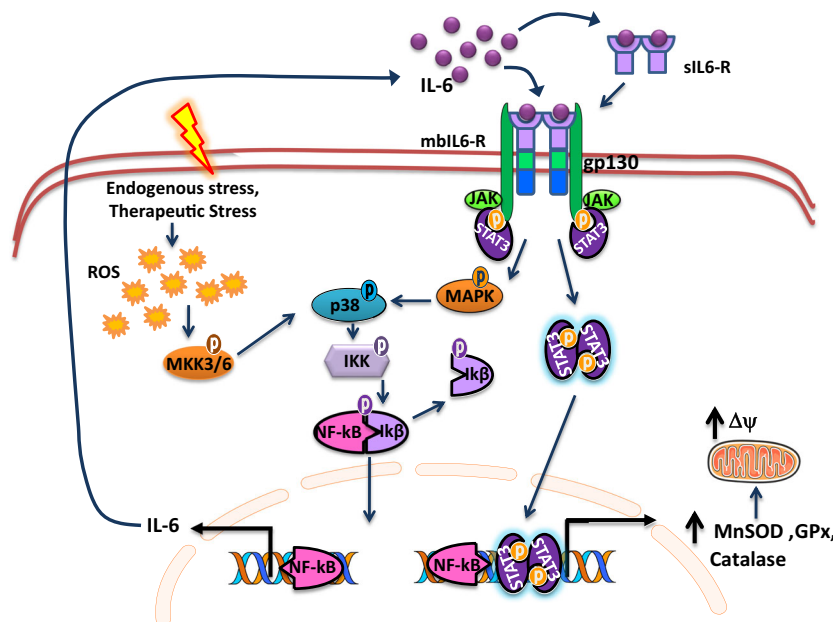


Fig. 6 IL-6 maintains redox balance: p38 senses oxidative stress through ROS-induced phosphorylation of MKK3/6. p38 activation (phosphorylation) induces NF- κ B signalling, leading to the enhanced expression and secretion of IL-6 from affected cells. IL-6 (in classical/trans-signalling fashion) further activates JAK/STAT-3 signalling in both

paracrine and autocrine manner, resulting in the overexpression of the antioxidant enzymes MnSOD, glutathione peroxidase (GPx) and catalase. Altered favourable redox balance also enhances the mitochondrial membrane potential

(phosphorylation) of p38 also induces IL-6 release from cells via the activation of NF- κ B (Fig. 6) [138]. All these studies showed the antioxidant potential of IL-6.

Invasion, metastasis and angiogenesis

During tumour metastasis, cancer cells invade surrounding local tissues to migrate to distant organs by acquiring a mesenchymal phenotype that allows the metastatic cancer cells to migrate from the site of the primary tumour. Upon lodging into the new organ, tumour cells lodge themselves by switching back to an epithelial phenotype and proliferate to form metastatic tumours. The processes by which cells switch between epithelial and mesenchymal phenotypes are widely known as the epithelial-to-mesenchymal transition (EMT) and its counterpart, the mesenchymal-to-epithelial transition [139]. It is well established that inflammation promotes EMT [139, 140]. Elevated levels of IL-6 in the serum have been associated with EMT and invasion along with increased size of tumours, metastasis and decreased survival of colorectal cancer patients [60]. Available evidences suggest that IL-6 facilitates the metastasis of many tumours (e.g. breast, lung, prostate, renal carcinomas, neuroblastoma, melanoma and multiple myeloma) to the bone [141] by increasing CXCR4 expression via STAT-3 and c-Jun [142]. Furthermore, IL-6-activated STAT-3 plays a role in EMT, invasiveness and angiogenesis in bladder cancer by increasing VEGF, MMP9 and DNMT1 expressions [107]. Autocrine production of IL-6 has also been shown to enhance the capacity to invade the extracellular matrix in breast cancer [143].

Supply of nutrients and oxygen as well as the clearance of metabolic by-products required for tumour sustenance are provided by tumour-associated neovasculature. The formation and maintenance of this neovasculature remains always activated as an ‘angiogenic switch’ and helps tumour development [144]. STAT-3 activation by IL-6 facilitates angiogenesis in many cancers by inducing the expressions of VEGF, bFGF and MMP9 in tumour-associated endothelial cells, TAMs and MDSCs [60, 84, 143, 145]. In addition, the Notch ligand, JAG-2, has been reported to be overexpressed in malignant plasma cells from MM patients, which induces the secretion of IL-6 and VEGF [146], whilst in breast cancer, Notch-3-dependent ERK activation via IL-6 appears to activate JAG-1 (Notch ligand) and CA-IX (a hypoxia survival gene). Furthermore, this CA-IX upregulated by IL-6 has also been found to maintain the invasive potential of breast cancer cells and mammospheres [143].

DNA damage and repair

DNA is the primary target of majority of the established anticancer therapies which induce cell death processes in tumour cells. DNA damage has been shown to induce the expression

and secretion of IL-6 from cancer cells, resulting in the activation of the JAK1/STAT-3 signalling pathway. STAT-3 activation not only protects tumour cells against DNA damage but also facilitates the growth of damaged cells by inhibiting induced senescence [147, 148]. Furthermore, inhibition of the IL-6/STAT-3 signalling pathway by the STAT-3 inhibitors, knockdown of gp130, or the neutralization of IL-6 impairs the growth of tumour cells exposed to DNA damage [147]. IL-6 secreted by tumour-associated endothelial cells in the tumour microenvironment has been suggested to protect lymphoma cells from genotoxic chemotherapy [149], whilst persistent DNA damage response has been shown to induce IL-6 secretion in stromal cells such as fibroblasts [148]. The ATM/NEMO/ELKS complex formed by DNA damage-induced phosphorylation of ATM promotes IKK-mediated I κ B α degradation, leading to NF- κ B activation, which further induces the expression of IL-6 (Fig. 7) [150]. In the partial hepatectomy (PH) model of DNA repair, wild-type mice were found to express some of the key DNA repair enzymes such as OGG-1, 8-oxo-GTP, Neil-1 and PARP, whereas these enzymes were absent in IL-6 knockout mice, suggesting a direct role of IL-6 in the repair of DNA damage [151]. Thus, in PH, it appears that IL-6 facilitates the restoration of hepatic mass by activating DNA repair enzymes, followed by accurate replication in proliferating hepatic cells [151]. Loss of Rrm2b function (a key enzyme in de novo deoxyribonucleotide synthesis) is known to cause severe numerical and structural chromosomal abnormalities, which leads to the activation of NF- κ B through ATM phosphorylation and IKK activation, leading to enhanced IL-6 expression and constitutive activation of STAT-3 [152]. Since IL-6 signalling functions by the activation of transcription factors such as STAT-3 and NF- κ B, inhibition of these transactivators or neutralizing the IL-6 may enhance the efficacy of DNA damage-causing chemotherapeutic drugs [153].

IL-6 induces therapeutic resistance in cancer

Accumulating evidences suggest that inflammatory signals from tumour cells and surrounding microenvironment facilitate tumour growth [63, 117]. Furthermore, most anticancer therapies induce inflammation by killing tumour cells and normal tissues [154]. Several inflammatory cytokines are believed to play key roles in therapeutic resistance and lead to tumour regrowth, invasion and angiogenesis. Anticancer therapies induce upregulation of the levels of a variety of inflammatory cytokines, including IL-6, IL-8 and TNF α [8, 12, 13]. Amongst these, IL-6 is known to contribute to poor therapeutic gain, tumour relapse and aggressive tumour growth [2, 8, 12]. IL-6 is also recognised as a key regulator of immunosuppression in patients with advanced cancer [1, 154]. Elevated IL-6 serum levels have been correlated with metastasis and

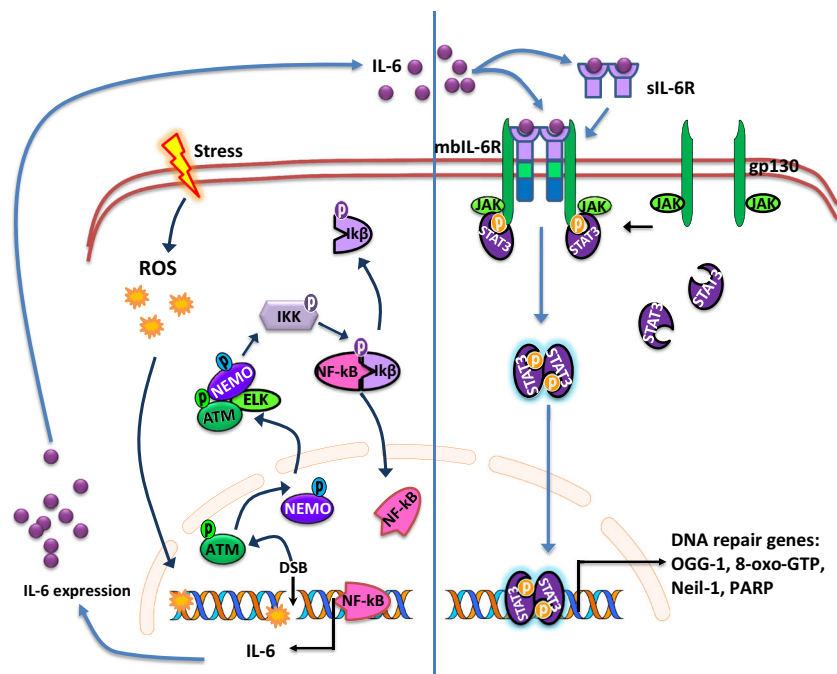


Fig. 7 Role of IL-6 in DNA repair: IL-6 facilitates DNA repair by inducing the expression of DNA repair enzymes in cancer cells. The induction of DNA damage phosphorylates the ATM, leading to the phosphorylation of IKK through the phosphorylation of various

downstream kinases. IKK phosphorylation activates NF- κ B signalling, resulting in sustained IL-6 expression and constitutive activation of STAT-3 signalling, which induces the expression of DNA repair enzymes

morbidity in prostate cancer and therapeutic resistance in ovarian cancer, whilst patients with reduced levels of IL-6 respond better to therapy [14, 15]. It appears that tumour cells produce IL-6 as a protective mechanism against drug-induced death, as in the case of prostate cancer where inhibition of IL-6 secretion increases the sensitivity of prostate cancer cells to anticancer drugs [12, 110]. Clinical studies on combinations of docetaxel and zoledronic acid in prostate cancer patients with bone metastasis have shown interesting data in relation to IL-6. Patients who responded to therapy had a 35 % decrease in overall serum IL-6 levels, whilst patients who did not respond had a 76 % increase in serum IL-6 levels [155], lending support to the notion that IL-6 confers therapeutic resistance in prostate cancer [5, 12]. The autocrine secretion of IL-6 by breast cancer cells is also shown to confer therapeutic resistance; however, it does not affect their growth. Further studies showed that drug-sensitive breast cancer cells do not express IL-6, whereas multidrug-resistant breast cancer cells produced high levels of IL-6 [156]. IL-6-mediated STAT3 activation has been reported to cause therapeutic resistance in tumours by inducing several pro-survival pathways [12, 105, 110]. However, IL-6-induced drug resistance is associated with increased expression of the multidrug resistance gene, *mdr1*, and upregulation of C/EBP β and C/EBP δ (CCAAT enhancer-binding protein family of transcription factors) [156]. Moreover, in colorectal cancer, IL-6 secreted from stromal cells induces CYP2E1 and CYP1B1 expression (CYP450

enzymes have a significant role in xenobiotic activation) through the JAK/STAT and PI3K/AKT pathways, which causes tumour initiation and promotion via the activation of chemical carcinogens [157].

IL-6-induced modifications of the stromal cell function appear to be important for tumour growth and angiogenesis as an anti-IL-6 receptor antibody has been found to inhibit tumour angiogenesis and growth by blocking tumour–stroma interaction [60]. Cancer-associated adipocytes can also promote radio-resistance by secreting IL-6 [158], whilst IL-6 and IL-8 secreted by mesenchymal stem cells activate macrophages in the microenvironment of human colorectal and ovarian cancers, causing chemoresistance [159, 160]. Besides causing resistance to chemo- and radiotherapy, autocrine secretion of IL-6 (induced by therapy) also confers resistance to some targeted therapies; for example, aflibercept (anti-VEGF, inhibitor)-resistant epidermoid carcinoma cells and herceptin (trastuzumab, anti-HER2)-resistant breast cancer cells secrete a large amount of IL-6 and show hyperactivation of STAT-3 signalling, causing resistance to therapy [161, 162].

Besides therapeutic resistance, IL-6 also minimizes clinical outcome by promoting the maintenance of a highly therapeutic-resistant cancer stem cell population which is mainly responsible for tumour reoccurrence [116]. IL-6 secreted from either cancer cells or tumour microenvironment (immune cells and tumour stromal cells) not only facilitates tumour growth but also acts as a major obstacle in obtaining

therapeutic gain and tumour-free survival [163]. Therefore, targeting IL-6 and its associated signalling may hold greater promise in minimizing therapeutic resistance in cancer. IL-6 inhibition could sensitize tumour cells to anticancer drugs and radiation by increasing DNA damage and cell death, and mitigate tumour regrowth by inhibiting subsequent angiogenesis and reducing cancer stem cell population.

Targeting IL-6 for therapeutic gain

IL-6 plays an important role in tumour progression and therapeutic resistance through inhibition of cancer cell apoptosis and stimulation of tumour-promoting factors such as proliferation, angiogenesis, etc. These effects are mediated by several signalling pathways; however, STAT-3 plays a central and major role

[1, 112]. Targeting the IL-6/JAK/STAT-3 pathway has shown promising results in many types of cancer and various inflammation-related diseases. The clinical correlation of increased serum IL-6 concentrations and advanced tumour stages in various malignancies, as discussed earlier in the review, makes a strong case of blocking IL-6 signalling for therapeutic gain. Therefore, inhibiting IL-6 signalling or minimizing the level of IL-6 can be a potential therapeutic strategy for those cancers which are characterized by overproduction of IL-6. IL-6 signalling can be targeted in multiple ways, such as the use of specific monoclonal antibodies against IL-6 or IL-6R [15, 19, 31], by using synthetic/semi-synthetic compounds as specific inhibitors of IL-6 downstream signalling molecules or by kinase inhibitors (like JAK inhibitor) [164]. All these approaches evaluated in experimental models and reached clinical trials are summarized in Table 1.

Table 1 Multiple therapeutic approaches for targeting IL-6, IL-6R and IL-6-associated signalling

| S. no. | Target molecule | Type of cancer | Therapeutic approach | References |
|--------|-----------------|-------------------------------|--|----------------|
| 1 | IL-6 | Prostate cancer | IL-6 siRNA | [12] |
| | | | Siltuximab (CNTO328)—anti-IL-6 antibody | [165] |
| | | Colorectal cancer | Siltuximab (CNTO328) | [3, 166] |
| | | Ovarian cancer | Siltuximab (CNTO328) | [166] |
| | | | Lysophosphatidic acid | [167] |
| | | Renal cancer | Siltuximab (CNTO328) | [166] |
| | | Lung cancer | Siltuximab (CNTO328) | [166, 168] |
| | | Multiple myeloma | Siltuximab (CNTO328) | [166, 169] |
| | | Lymphoma | BE-8 (monoclonal antibody) | [170] |
| | | Prostate cancer | Siltuximab, zoledronic acid | [15, 171] |
| 2 | IL-6R | Breast cancer | Siltuximab, zoledronic acid, PMA (medroxyprogesterone acetate) | [15, 172, 173] |
| | | Colon cancer | MR16-1, tocilizumab (anti-IL-6R antibody) | [3, 60] |
| | | Oral squamous cell carcinoma | Tocilizumab | [174] |
| | | Lung cancer | Tocilizumab | [175] |
| | | Multiple myeloma | Tocilizumab | [176, 177] |
| | | Ovarian cancer | Tocilizumab | [178] |
| | | Breast cancer | Tocilizumab | [179] |
| 3 | JAKs | Lung cancer | TG101209 (JAK inhibitor) | [180] |
| | | Multiple myeloma | TG101209 | [181] |
| | | Ovarian cancer | AG490 | [182] |
| | | Acute myeloid leukaemia | Ruxolitinib (JAK1/JAK2 inhibitor) | [183] |
| | | Colitis-associated cancer | CEP-33779 (JAK inhibitor) | [184] |
| | | Acute myeloid leukaemia | SB1518 (JAK2) | [185] |
| 4 | STAT-3 | Glioblastoma | Sorafenib (multiple kinase inhibitor) | [186] |
| | | Breast cancer | SD-1029 | [187] |
| | | Ovarian cancer | SD-1029 | [187] |
| | | Lymphoma | JSI-124 | [188] |
| | | Glioblastoma | JSI-124 | [189] |
| | | Breast cancer | JSI-124 | [190] |
| | | Hepatocellular carcinoma | Sorafenib (Nexavar) | [191] |
| | | Breast cancer | Sorafenib | [192] |
| | | Advanced renal cell carcinoma | Sorafenib | [193] |
| | | Colorectal cancer | Sorafenib | [194] |

Antibodies targeting IL-6 or IL-6 signalling have been extensively investigated in experimental tumour models and clinical trials for a variety of cancers. Elsilimomab (BE-8), a murine monoclonal antibody, and siltuximab (CNTO 328), a chimeric antibody with strong affinity to IL-6 and licenced for the treatment of Castleman's disease, are used for the treatment of prostate cancer, ovarian cancer, renal cell carcinoma and colorectal cancer in combination with other chemotherapeutics [3, 6, 166]. In prostate cancer, siltuximab has been shown to downregulate the genes downstream of IL-6 signalling along with decreased STAT-3 expression [165]. Tocilizumab, a FDA-approved drug for rheumatoid arthritis and Crohn's disease, is the humanized antibody specific for IL-6R that recognises both soluble and membrane-bound receptors and blocks its signalling [195]. BE-8, a murine anti-IL-6 monoclonal antibody, has also been used in the treatment of lymphoma and multiple myeloma [196]. Sequestering the enhanced IL-6 showed a significant increase in the therapeutic efficacy of paclitaxel in mouse models of epithelial ovarian cancer by reducing tumour angiogenesis [197]. Tocilizumab has also been used in the treatment of oral cell carcinoma, prostate cancer, renal cancer, multiple myeloma and breast cancer [15]. It inhibits the growth of prostate and breast cancer cells by reducing STAT-3-induced VEGF expression. In addition, it reduces bone metastasis of breast cancer cells [142]. Tocilizumab has been shown to improve cachexia developed by IL-6 overexpression in lung cancer patients [175]. The combination of anti-IL-6R with herceptin and aflibercept enhances therapeutic gain, also in targeted therapy-resistant breast cancer and epidermoid carcinoma, respectively [161, 162]. However, blocking IL-6 signalling can be harmful and may result in some adverse effects, such as gastrointestinal, nasopharyngeal and upper respiratory tract infections, gastrointestinal haemorrhage, thrombocytopenia and neutropenia [198]. Therefore, selective blocking of trans-signalling (the major pathway involved in inflammation and related diseases like cancer) using sgp130Fc (a recombinant fusion protein of soluble gp130 and human IgG₁ Fc) may be useful in achieving therapeutic gain in cancer [76].

Besides antibodies, a number of other compounds are currently available that inhibit IL-6 signalling. Zoledronic acid (ZA) is the most potent nitrogen-containing bisphosphonate compound which has been used in adjuvant therapies to inhibit bone metastasis caused by multiple cancers [172]. It inhibits growth, besides inducing apoptosis by reducing IL-6 secretion in prostate cancer cell lines, which is suggestive of its use in the treatment of prostate cancer either alone or in combination with chemotherapy [171]. Radiation activates IL-6/STAT-3 signalling, which stimulates tumour invasion and EMT changes and promotes the survival of tumour cells after therapy, thereby conferring resistance to therapy [12, 84, 156]. However, use of siRNA against IL-6 inhibits tumour regrowth after radiotherapy in prostate cancer and sensitizes

tumour cells to radiation by increasing cell death and DNA damage [12]. Its inhibition also mitigates tumour regrowth by eliminating radiotherapy-triggered MDSC and subsequent angiogenesis after radiation exposure [199]. Therefore, inhibition of IL-6 could be a potential therapeutic strategy for increasing the radiation response of tumours. Similarly, medroxyprogesterone acetate (MPA) is a synthetic compound used as an endocrine therapeutic agent for patients with breast cancer which is known to reduce serum IL-6 levels [173]. Several studies have shown that the use of an inhibitor for downstream targets of IL-6 signalling can also be a good approach for enhancing therapeutic gain. For example, the use of JAK inhibitor (TG101209) enhances the efficacy of radiotherapy in lung cancer [180]. Similarly, WP1066 and CEP3379, other JAK2 inhibitors, suppress the growth of gastric cancer and colorectal tumours by inhibiting IL-6/JAK2/STAT-3 signalling [184, 200]. Sorafenib, which is a multiple kinase inhibitor, causes dephosphorylation of STAT-3 and prevents AKT signalling in glioblastoma cells and prostate cancer, respectively [186]. AG490, a potent JAK inhibitor, targets STAT-3 signalling, reduces the invasion of human pancreatic cancer cells *in vitro* and induces apoptosis in gastric cancer cells [201, 202]. Moreover, AG490 is also effective in murine ovarian cancer, where it induces the expression of anti-tumour cytokines [182]. Another JAK inhibitor, ruxolitinib, is currently in clinical trials for leukaemia [183].

Inhibition of STAT-3 phosphorylation by the use of STAT-3 inhibitors can also be a good approach to block IL-6 signalling. JSI-124 (STAT-3 inhibitor) inhibits STAT-3 phosphorylation at serine 727 and sensitizes B-leukaemia cells to apoptosis [203]. JSI-124 suppresses breast cancer cell growth by downregulating STAT-3 activation in tumour-associated B cells. Stattic and Eriocalyxin B (a diterpenoid) also inhibit STAT-3 phosphorylation and induce apoptosis in MDA-MB-231 and HepG2 cells [204, 205]. Besides inhibiting STAT-3 phosphorylation and transcriptional activation, Decoy oligodeoxynucleotides target the DNA-binding domain of STAT-3 by competing against endogenous DNA *cis* element, which results in reduced cell growth and increased apoptosis [206]. G-quartet oligodeoxynucleotides can also be used as a STAT-3 inhibitor which suppresses the growth of prostate, breast, and head and neck cancers in nude mice [206, 207]. Instead of inhibiting STAT-3 phosphorylation, these oligodeoxynucleotides inhibit the binding of STAT-3 dimer on DNA. Moreover, small-molecule inhibitors can also be used to inhibit STAT-3. A small-molecule non-peptide STAT-3 inhibitor named S3I-201 (a.k.a. NSC74859) selectively inhibits STAT-3 DNA-binding activity *in vitro* and blocks the formation of STAT-3:STAT-3 dimer, which leads to the inhibition of STAT-3-dependent gene transcription and the blockade of the proliferation and survival of human breast carcinoma cells [208]. STA-21 also selectively inhibits STAT-3 DNA-binding activity *in vitro*, disrupts STAT-3:STAT-3

dimerization and suppresses STAT-3-mediated gene transcription along with cell growth inhibition and apoptosis through the caspase pathway in a human breast carcinoma and rhabdomyosarcoma model [209, 210]. Catechol-containing compounds, IS3 295, galiellalactone and peptide aptamers have been found to inhibit the DNA-binding ability of STAT-3 and further signalling [208, 211].

Conclusion

IL-6, which is produced by tumours and many other cells in the tumour microenvironment, facilitates tumour growth and sustenance by influencing and regulating nearly all hallmarks of cancer, besides contributing to therapeutic resistance. Recent studies have enhanced our knowledge regarding the potential role of IL-6 in the cross talk between tumour and its microenvironment. IL-6/JAK/STAT-3 appears to be the primary pathway through which IL-6 regulates majority of the tumour-promoting functions. Therefore, neutralizing IL-6 or the IL-6 receptor to prevent the initiation of signalling or inhibiting the activity of two other members, JAK and STAT-3, to prevent the execution in the end has established therapeutic efficacy in cellular and systemic models of cancer. Blocking IL-6 using monoclonal antibodies against either IL-6 or IL-6R has shown promising results in preclinical studies and clinical trials. Phase I and II clinical trials have established the efficacy of monoclonal antibodies either as a single agent or in combination with other chemotherapeutic drugs, radiation and targeted therapies in various types of cancer. Subsequently, certain synthetic molecules, such as ZA, MPA, polyphenols, etc., have also shown promising results in various cancers by regulating IL-6 levels. The use of small-molecule inhibitors of JAK and STAT-3 alone or in combination with radiation or anticancer agents has also resulted in promising anti-tumour effects in various cancers. However, undesirable effects (such as infections, gastrointestinal haemorrhage, thrombocytopenia and neutropenia) of anti-IL-6 therapy have also been reported in patients with various inflammatory diseases. Therefore, selective inhibition of IL-6 trans-signalling using molecules such as sgp130Fc in the future may result in better therapeutic gain without the side effects associated with anti-IL-6 therapy currently under evaluation.

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