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The role of IL-6 and STAT3 in inflammation and cancer

David R. Hodge [a], Elaine M. Hurt [b], William L. Farrar [a,*]

[a] *Laboratory of Molecular Immunoregulation, Cytokine Molecular Mechanisms Section, Center for Cancer Research, The National Cancer Institute at Frederick, P.O. Box B, Bldg. 560, Rm. 31-76, Frederick, MD 21702, United States*

[b] *Basic Research Program, SAIC Frederick, The National Cancer Institute at Frederick, Frederick, MD 21702, United States*

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Abstract

The defense of the host from foreign pathogens is the commonly accepted function of the vertebrate immune system. A complex system consisting of many differing cells and structures communicating by both soluble and cell bound ligands, serves to protect the host from infection, and plays a role in preventing the development of certain types of tumours. Numerous signalling pathways are involved in the coordination of the immune system, serving both to activate and attenuate its responses to attack. The ability of the immune system, specifically those cells involved in acute inflammatory responses, to mediate the directed (and sometimes indirect) killing of cells and pathogens, make it a potential threat to host survival. Furthermore, the production and release of various survival factors such as the pleiotropic cytokine IL-6, a major mediator of inflammation and activator of signal transducer and activator of transcription 3, serves to block apoptosis in cells during the inflammatory process, keeping them alive in very toxic environments. Unfortunately, these same pathways serve also to maintain cells progressing towards neoplastic growth, protecting them from cellular apoptotic deletion and chemotherapeutic drugs. Here, we discuss the relationships between cancer and inflammation, and some of the molecular mechanisms involved in mediating the unintended consequences of host defense and tumour survival.

Keywords: Inflammation; STATs; IL-6; Cancer; JAK; Kinase inhibitor; Resistance to chemotherapy

1. Introduction

Cornelius Celsus, a physician in first century Rome, first described the four primary signs of inflammation as heat (*calor*) pain, (*dolor*), redness (*rubor*), and swelling (*tumour*). The use of the Latin derivation "tumour" to describe swelling seems prescient given recent discoveries and developments in oncology. Inflammation follows infection, and is most commonly observed as a part of the defense mechanisms triggered to protect the host from pathogens. However, recent evidence suggests that the immune system more closely resembles a "double edged sword," rather than a benign protector [1]. Tissue damage can also be followed by inflammation, a necessary step to remove necrotic debris. In order to destroy the incoming pathogens, a myriad of cellular and molecular factors are unleashed against the invader, producing an attack that occurs on multiple levels [2–4]. The recruitment of macrophages into the area of the infection represents a major phase of the inflammatory response [5]. The production of reactive oxygen species (ROS) by macrophages, along with highly efficient proteases, degrade the pathogens down to mere fragments of peptides, that are then presented to lymphocytes involved in the second phase of the response [6].

This entire process is controlled by the synthesis and release of various peptide mediators of the immune response, consisting primarily of cytokines and chemokines [7,8]. Typically, cytokines, also known as interleukins, function through the binding and subsequent stimulation of cellular receptors that are associated with

* Corresponding author. Fax: +1 301 846 7042.
E-mail address: farrar@mail.ncifcrf.gov (W.L. Farrar).

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a family of tyrosine kinases known as Janus kinases (JAK) [9]. Chemokines on the other hand, function by binding to heptahelical receptors, triggering the activation of heterotrimeric GTP-binding proteins [10]. Also involved in this process are prostaglandins and leukotrienes [11], lipid second messengers that reinforce the inflammatory response [12]. The overlapping functions of these pathways are numerous, and redundant, for without such a system death would be inevitable in our microbe infested world. However, despite the lethality of the immune system, the host survives due to the protective effects of various inflammatory mediators [13,14]. Indeed, cells of the immune system, and those they are protecting, would certainly perish in the very toxic environment of the inflammatory response were it not for the protective effects of cytokines and chemokines. For example, it has been reported that one of the most common inflammatory cytokines, Interleukin-6 (IL-6), is known to mediate many unwanted, detrimental effects such as resistance to chemotherapeutic drugs [15,16]. It now seems apparent that in addition to driving inflammatory mechanisms, cytokines such as IL-6 have the ability to protect cells from the "byproducts" of inflammation (such as ROS and free-radical damage), and that this protective effect extends also to cells that might have strayed somewhat from normal cell cycle regulatory pathways [17]. A possible scenario depicting the series of events leading to neoplastic growth is shown in Fig. 1.

2. IL-6: a ruthless cytokine

Interleukin (IL)-6 is a potent, pleiotropic, inflammatory cytokine that mediates a plethora of physiological functions, including the developmental differentiation of lymphocytes, cell proliferation, and cell survival and amelioration of apoptotic signals [18–20]. Additionally, IL-6 exerts effects on bone formation, general system metabolism, endocrine functions, and can affect many cells of other tissues and organ systems [21,22]. Depending upon the cell type, IL-6 is able to act through several classic protein kinase cascades such as mitogen activated protein kinase (MAPK), and phosphatidylinositol-triphosphate kinase (PI-3 kinase) (Fig. 2) [23]. The ability of IL-6 to directly activate the signal transducers and activators of transcription (STAT) factors STAT1 and STAT3, via the JAK produces serious unintended

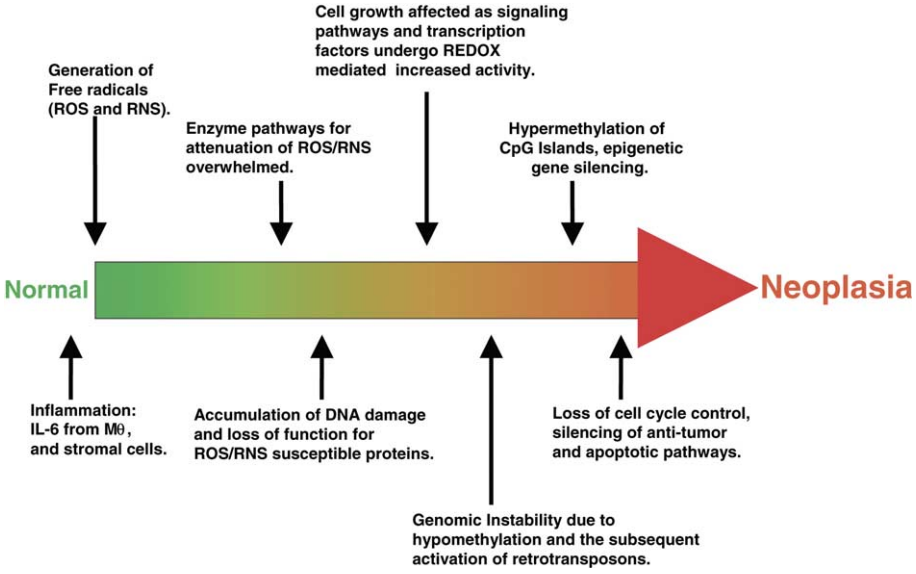

Fig. 1. *Proposed model for progression to neoplastic growth starting from the dysregulation of the inflammatory response.* Following stimulation of the cells (macrophages, neutrophils, stromal, and others) to mediate an inflammatory response, cytokines such as IL-6 are released and activate the requisite signaling pathways involved in host defense. The generation of free radicals, including reactive oxygen and nitrogen species (ROS and RNS, respectively), begins accompanied by changes in REDOX conditions. While having specific purposes, these highly reactive compounds are capable of covalently modifying proteins, nucleic acids, and lipids. Enzyme pathways that function to attenuate the excess free-radicals, *i.e.* superoxide dismutases (MnSOD, Cu/ZnSOD), DNA repair enzyme systems, protein de-nitrosylation, have a limited input capacity and are probably overwhelmed when inflammation is chronic in nature. Simply stated, the repair of DNA damage is really a function of enzyme rates, if the repairs can be affected in the time available between cell cycles point mutations will not be passed on to daughter cells. However, in the event of repeated and constant inflammation, the cytokine signals prevent apoptosis even in the face of DNA damage hence some point mutations are retained. Furthermore, since increases in oxidative free radicals have been shown to drive proliferation by activating various kinases, increased ROS/RNS levels would tend to favor proliferation, decreasing the time available to repair mutations. It is possible that enzymes involved in methylation may be targets of inflammatory dysregulation, as genomic hypomethylation and promoter hypermethylation are commonly observed in many tumour types. These multiple insults eventually lead to unregulated proliferation, and hypoxic conditions that accompany such growth are thought to trigger vascularisation of solid tumours and metastasis.

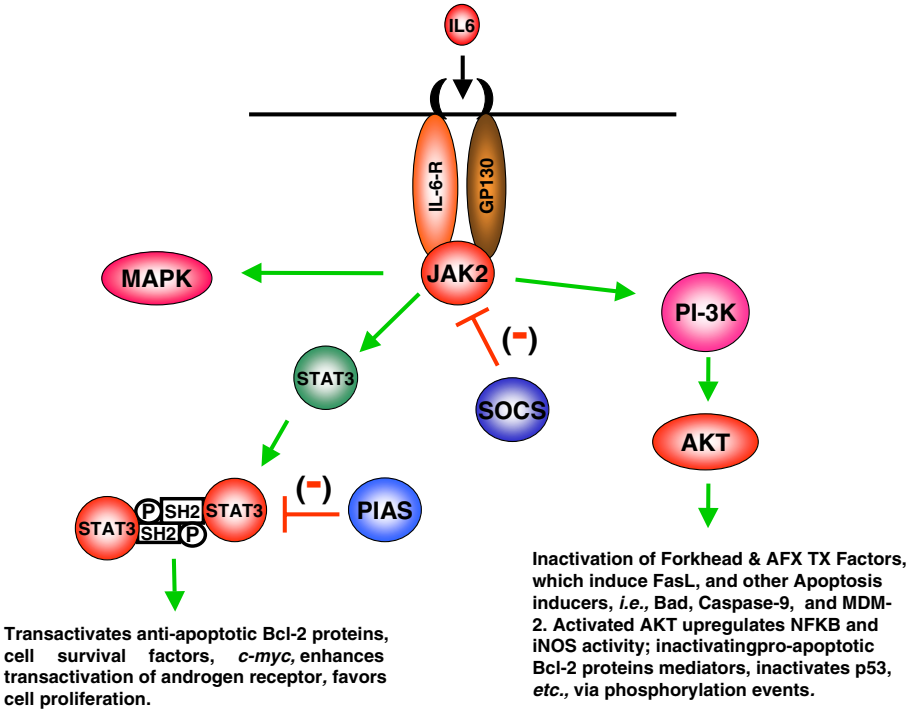

Fig. 2. *IL-6: A pleiotropic cytokine affecting proliferative and anti-apoptotic pathways.* IL-6 is capable of activating three major proliferative pathways as shown. The MAPK pathways and STAT transcription factor activation serves to drive proliferation, as does the PI3K pathways. Activation of AKT inactivates many pro-apoptotic mediators. While this leads to cell survival in an immune related crisis, it can also lead to survival of DNA-damaged cells, and potentially to neoplastic growth. The inhibitors of JAK–STAT (SOCS and PIAS) are shown and described in the text.

consequences when examined in the context of progression to neoplasia [24–28].

The receptor for IL-6 consists of a heterodimeric complex made up of two Ig-like containing proteins, the IL-6 specific chain gp80 (CD126), and gp130 (CD130) [29–31]. Other members of the IL-6 cytokine group (including IL-11, CNTF, cardiotrophin-1 (CT-1), LIF, OSM, and B cell-stimulating factor-3 (BSF3), recognise the gp 130 chain in conjunction with their own specific receptor chains [32].

Numerous cell types respond to IL-6, including astrocytes, lung, ovary, endometrial stromal cells, heart, amnion-derived cells, macrophages, monocytes, microglia, kupffer cells, osteoblasts, multiple myeloma, mast cells, leydig cell precursors (testes), fibroblasts (dental pulp, gingival, nasal turbinate, polyps, synovial), mesangial cells (kidney), human endothelial cells, hepatocytes, and prostatic intraepithelial neoplasia cells [33]. This is significant because of the many different inducers of IL-6 secretion, including IL-1β, tumour necrosis factor-α (TNF-α), prostaglandin E-2 (PGE-2), lipopolysacharide (LPS), and vascular endothelial growth factor (VEGF) [33]. In addition, adjuvants, such as mineral oil (pristane), enterotoxins, growth factors, (gonadotrophins), hypoxia, ionising radiation, and oxidative stress, are able to induce IL-6. The presence of IL-6 in tissues is not an abnormal occurrence, but its unchecked production leading to subsequent chronic inflammation exhibits a strong association with many types of cancer [34–38]. This aspect of IL-6 production illustrates the "ruthless" characteristics of the cytokine, which functions without regard for later consequences.

Chronic inflammatory diseases such as those caused by viral hepatitis, irritable bowel syndrome or colitis, and pancreatitis, all represent a prodromal syndrome that has a strong association with neoplastic disease. One intriguing observation concerning IL-6 comes from the analysis of the Kaposi's Sarcoma-associated herpes virus (KSHV) [39]. It appears that a key mechanism utilised by KSHV to provoke unregulated cell growth and escape from host anti-tumour defenses, is its encoding of a viral version of IL-6, or "virokine" [40]. The KHSV IL-6 virokine acts as normal host cytokines would, bestowing upon the infected cell resistance to immune mediated apoptotic stimuli, thus ensuring the survival and propagation of the viral pathogen.

At the center of these disorders, one usually finds chronic inflammation and IL-6. This phenomenon was examined by several groups in the early to mid 1960s by the use of various inflammation inducers such as pristane (2,6,10,14-tetramethylpentadecane) [41], alum-precipitated tetanus toxoid or pertussis vaccine [42]. Implanting these substances intraperitoneally into BALB/cAn mice lead to the induction of granulomas, a tumour-like localisation of large numbers of macrophages, neutrophils, eosinophils, B and T-lymphocytes,

ultimately leading to the formation of murine plasma cell tumours (PCT) [43]. The indictment of IL-6 as the key mediator of PCT formation came with the production of an IL-6 knockout mouse model. While an IL-6 transgenic mouse (*i.e.* levels of IL-6 above those of wild-type animal) was observed to spontaneously form PCTs, the knockout model was actually resistant to PCT formation [44]. Furthermore, the use of steroidal and non-steroidal anti-inflammatory agents in these animal models acted to ameliorate PCT formation [45].

3. Signal transducers and activators of transcription: STATs

Following the discovery of interferons, the signalling pathways responsible for mediating the actions of these cytokines were discovered. The pathways consist of a family of tyrosine kinases, later designated Janus kinases (JAK), bound to a group of receptor proteins that demonstrated specificity for each respective cytokine ligand. Janus, the two faced God who both laughed and cried simultaneously as the guardian of gates and doors, aptly describes these kinases, which act to produce both positive and negative effects on cell growth and proliferation [46]. Cytokines function by specifically recognising their receptors which, as a result of binding to their ligand, undergo conformational changes resulting in the displacement of the Janus kinases [9]. Subsequent to the binding of cytokine, JAKs phosphorylate and activate the signal transducers and activators of transcription (STAT) factors at a single tyrosine residue [47]. This tyrosine residue is strategically located in a domain of the transcription factor that allows it to participate in the formation of dimeric structures. Once phosphorylated, the tyrosine residue is recognised and bound by an SH2 domain on the opposing face of the other STAT protein involved in the dimerisation. This co-recognition of phosphotyrosines by respective SH2 domains forms a stable dimeric complex [48]. The activation and dimerisation of the STAT protein also exposes its nuclear localisation signal, allowing it to translocate to the nucleus where it recognises specific enhancer elements in the promoter and enhancer regions of target genes, serving to initiate transcription [48].

There are seven known STAT proteins, STAT 1, STAT 2, STAT 3, STAT 4 , STAT 5a and 5b, and STAT 6 [49]. Although these factors mediate many important cellular functions, they do not deviate from our current understanding of how transcription factors function. Structurally, STAT factors have DNA binding domains, dimerisation domains, and activation domains. It is known through various experiments, that each STAT transcription factor shows a preference for a particular DNA sequence motif. Generally, the STATs recognise a consensus sequence consisting of 5′-TTMXXX-DMA-3′, where D is A, G, or T and M is A or C, and the number of Xs vary between 3 and 5 bases [50]. Interestingly, STATs can form complexes with other transcription factors and, in some cases, cause these factors to drive their respective phenotype without the presence of the requisite ligand [51]. Furthermore, the actions of STAT factors can be inhibited by the use of drugs that target other transcription factors and nuclear receptors [52]. The role of STAT transcription factors in the development, proliferation, and survival of tumour cells varies with each type of cancer examined, but the strong association of STAT3 with many different types of cancer is well known and summarised in Table 1.

Another member of the STAT family has been found to have an association with cancer, albeit not as frequently observed as STAT3. STAT5 was first identified as a prolactin activated transcription factor in mammary tissue. There are two isoforms of STAT5, *i.e.* STAT5a and STAT5b, showing amino acid homology of almost 95%. STAT5a and STAT5b are also activated by IL-2, IL-3, IL-5, IL-7, IL-9, IL-15, granulocyte macrophage colony-stimulating factor (GMCSF), growth hormone (GH), erythropoietin, and thrombopoietin [53]. Probably as a result of the wide range of activating ligands, STAT5 also appears to be active in some tumour cells [54]. It is likely that the actions of the above cytokines serve to constitutively activate the STAT5 transcription factor in a similar manner to that observed with STAT3. Indeed, we have shown that while both

Table 1
Activated STAT transcription factors in tumour types

Tumour type	Activated STAT factor
Multiple myeloma	STAT3
Acute lymphocytic leukemia (ALL)	STATI, STAT5
Chronic lymphocytic leukemia (CLL)	STATI, STAT3
Acute myelogenous leukemia (AML)	STATI, STAT3, STAT5
Large granular lymphocyte leukemia (LGL)	STAT3
Chronic myelogenous leukemia	STAT5
Lung cancer	STAT3
Breast cancer	STAT3
Renal cancer	STAT3
Prostate cancer	STAT3
Pancreatic carcinoma	STAT3
Melanoma	STAT3
Colon carcinoma	STAT3
Gastric carcinoma	STAT3
Cervical cancer	STAT3
Ovarian cancer	STAT3
Hepatocellular carcinoma	STAT3
Head and neck cancers	STAT3

The STAT3 transcription factor is the most commonly observed member of the STAT family to be present in a constitutively activated state in many tumours. STAT1 and STAT5 are also observed, but at a much lower incidence when compared to STAT3. It should be noted that STAT5 activation is possible following stimulation with other cytokines such as IL-2, for example.

STAT3 and STAT5 can drive cell proliferation in human multiple myeloma cell lines, some upstream functions mediated solely by the ligand activation of signalling pathways, are still needed to fulfill the complete range of anti-apoptotic characteristics and resistance to chemotherapeutic drugs [15]. The repeated association of STAT3 and STAT5 with various cancer types strongly suggests that cytokines play an important role in the development and growth of tumours. Interestingly, although laboratory generated constitutively active mutants have been created for STAT3 and STAT5, naturally occurring counterparts have not been found [55,56].

4. STAT3 in multiple myeloma

Nearly 17 years ago, the role of IL-6 as a growth factor for human multiple myeloma was established. Since that time our understanding of the importance of this cytokine in both the growth and survival of multiple myeloma and normal plasmacytic differentiation has been considerable. Murine plasmacytomas remain the first and most well studied model of inflammation-induced neoplasia. As discussed previously, multiple myeloma, an analogous condition to plasmacytoma in mice, is essentially a quintessential model for the study of inflammation and its effects on tumour growth.

Both the IL-6 knockout mouse and the IL-6 transgenic mouse lead to our understanding of the importance of IL-6 in both the development of normal plasma cells and the development of plasmacytosis, respectively. The mechanism of how IL-6 induces the development of normal plasma cells has been largely elucidated in the past five years, and is now known to involve two key factors, Blimp-1 and xbp-1 [57]. It is through the coordinated expression of these two transcription factors that the majority of the B cell program is extinguished and mature immunoglobulin (Ig) secretion is initiated. In the IL-6 knockout mouse, there is a decrease in the production of high affinity antibodies due to the inability to induce the plasmacytic phenotype [44]. Early evidence indicating the importance of IL-6 in multiple myeloma was elucidated by experiments using transgenic animals. Mice with an IL-6 transgene driven by an Eμ promoter develop polyclonal plasmacytosis [58]. The further crossing of these mice with BALB/c mice, which spontaneously develop plasmacytomas after pristane injection, leads to malignant plasma cells. Furthermore, knocking out the IL-6 gene in Balb/c mice leads to a block in pristane-induced plasmacytosis [44]. In multiple myeloma patients, serum IL-6 levels are elevated and this is correlative with an unfavorable outcome [59,60]. It has also been recently shown that partial reduction of actions or levels of IL-6 can restore sensitivity to chemotherapeutic drugs [61,62]. While the plasma cells of patients with extramedullary disease often produce IL-6 themselves, in the majority of cases IL-6 is produced by the bone marrow stroma and can be further enhanced by the interaction of malignant plasma cells with bone marrow stromal cells through an NFkB-dependent mechanism [63,64].

IL-6 binding to its receptor induces the homodimerisation of the gp130 IL-6 transducer leading to phosphorylation of JAK1. JAK1 then induces STAT3 phosphorylation and subsequent translocation to the nucleus. In a recent study employing DNA microarrays, Croonquist et al. [65] determined the subset of genes that are regulated in IL-6 treated versus IL-6 starved myeloma cell lines. Of the 138 genes determined to be regulated by IL-6, the majority of them (54%) were found to be involved in cell cycle regulation. The large number of genes induced by IL-6 that are involved in cell cycle regulation underscores again the importance of IL-6 in the growth of multiple myeloma cells, and by extension to other models, foreshadows the importance of this cytokine in many other types of cancer.

There have been several reports that anti-apoptotic genes are also regulated by IL-6 and STAT3, including Bcl-2, Bcl-xL, and Mcl-1 [66,67]. While these genes have been shown to be induced by STAT3, it seems that the most important anti-apoptotic gene is Mcl-1 [68,69]. Antisense inhibition of Bcl-xL was insufficient to inhibit survival, while a knock-down of Mcl-1 inhibited survival in several multiple myeloma cell lines. Overexpression of Mcl-1 was able to promote proliferation of multiple myeloma cells lines even in the absence of IL-6 [69]. It has also been shown that knock-down of Bcl-2 with an antisense oligonucleotide can lead to enhanced sensitivity of the cells to dexamethasone-induced apoptosis [70].

The importance of the JAK/STAT pathway in the survival and proliferation of myeloma is also apparent by the number of ways that this pathway is altered. It has been shown that approximately 48% of multiple myeloma patients have a constitutively active form of STAT3 [71]. It has also been determined that SHP1, a negative regulator of the JAK/STAT pathway, is hypermethylated in 79% (27 of 34) of multiple myeloma patients. The epigenetic silencing of SHP1 appears to be partly responsible for constitutively active STAT3, at least in the U266 multiple myeloma cell line, since treatment of cells with 5-aza-cytidine resulted in demethylation and re-expression of SHP1 and diminished STAT3 activation [72]. Another regulator of the JAK/STAT pathway, suppressor of cytokine signalling 1 (SOCS1), has also been shown to be methylated in approximately 48% of cases [73]. However, the methylation of SOCS1 was found not to have a clinical correlation to outcome [74]. It may be that the correlation of SOCS1 methylation and clinical outcome was not apparent because of the many other ways that the

JAK/STAT pathway can be constitutively activated in multiple myeloma.

Due to the importance of IL6 and the JAK/STAT pathway in multiple myeloma, several therapeutic strategies targeting these pathways have been devised. The production of IL-6 is controlled mainly through the interaction of the bone marrow stromal cells and malignant plasma cells and is an NFkB dependent event [75]. Therefore, the proteasome inhibitor Bortezomib, was tested for its ability to inhibit proliferation and survival of multiple myeloma [76]. While the targets of Bortezomib are now known to involve more than just the NFkB-induced IL-6 production, it has proven to be one of the most useful new therapies in the treatment of multiple myeloma. Bcl-2 antisense therapy has also been shown to have promise when used in combination with other therapies [70]. However, there was minimal reduction in Bcl-2 and response was not correlated with Bcl-2 protein levels [77].

5. Negative regulation and pharmacological interventions of STAT pathways

Blocking the proliferative, anti-apoptotic effects of IL-6 and its downstream target, STAT3 is becoming a very important endeavor in the treatment of cancer. A brief examination of the number of tumours that exhibit activated STAT3 proves the formidable nature of the transcription factor's ability to induce neoplastic growth in the event of pathway dysregulation. Perhaps the reason that no activating mutations of the STAT3 gene have been detected in tumours is due to the ready availability of IL-6. Thus, targeting this pleiomorphic cytokine may serve to reduce, or attenuate the effects of downstream effectors of cell growth and apoptotic resistance. Fig. 3 depicts the potential points in the IL-6-STAT3 pathway in which circumventing the effects of this aspect of inflammation may be possible.

The JAK–STAT system is negatively regulated by several proteins including, the suppressor of cytokine signalling (SOCS family.) The SOCS proteins, also referred to as cytokine-induced SH2 containing proteins (CIS), or Janus kinase binding protein (JAB), function by inhibiting the activity of the JAKs by direct binding to the kinase [78,79]. By virtue of their SH2 domains, these negative regulators of JAK are able to attenuate the actions of the kinase following cytokine stimulation. As mentioned earlier, in some tumour cells these negative effectors (specifically SOCS1) are inactivated by epigenetic silencing of their promoters [73,80]. Next in progression, the protein inhibitors of activated STAT (PIAS) act by interacting with the STAT dimer and blocking transcription [78,81]. In addition to these proteins, there are phosphatases that attenuate signals in the classic sense of removing phopho-signals from activating sites of kinases [82–85].

Targeting the JAK–STAT pathway may be feasible at several points in the cascade. The IL-6 receptor antagonist SANT-7 has shown some promise as an inhibitor of IL-6 action [86]. SANT-7 is able to abrogate the formation of the IL-6 Receptor/GP130/IL-6 complex, thus

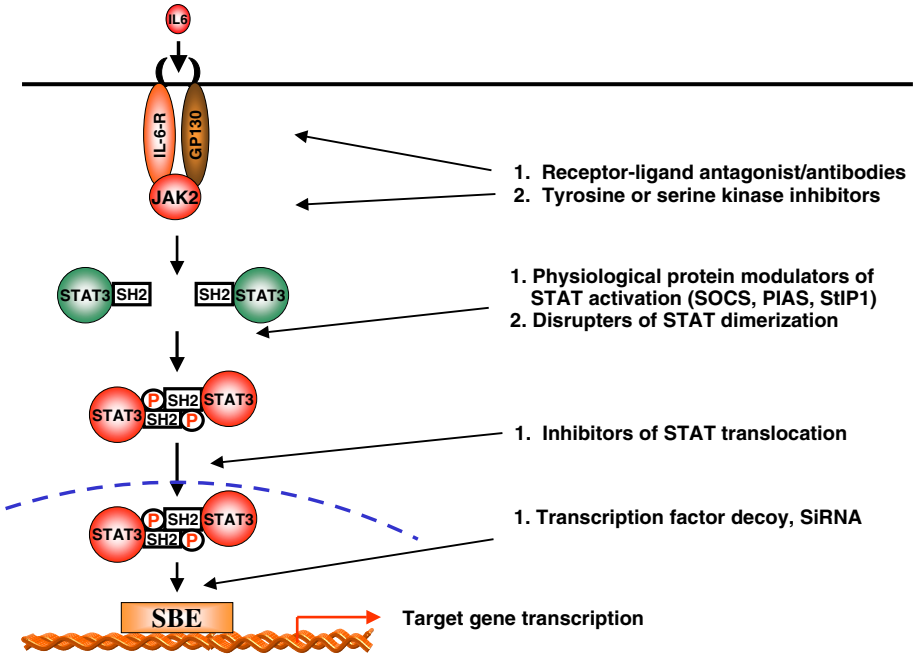

Fig. 3. *Potential therapeutic targets of the IL-6/STAT3 pathway.* Currently available and future drugs or biologicals may serve to block or attenuate the inflammatory signalling and detrimental, constitutive STAT3 activation that favors tumour growth and survival.

blocking the activation of STAT3 among other downstream targets. The effects of blocking an anti-apoptotic cytokine such as IL-6 can be exploited by utilising such inhibitors in parallel with standard chemotherapy drugs, taking advantage of the reduced resistance from the IL-6 inhibition. Furthermore, specific antibodies to the components of the IL-6 receptor complex and the ligand itself may also prove useful in such treatment [87,88]. A "humanised" monoclonal antibody has shown the ability to inhibit IL-6 activity, however, this data was collected in a trial observing the effects of the antibody on rheumatoid arthritis patients, therefore, its efficacy in the treatment of cancer has not yet been established [89].

Small molecule inhibitors of JAK such as AG-490 (Tyrphostin), have shown some usefulness, but their use is limited because of the cross-specificity of the drug, and the interference with other JAK pathways [90]. Tyrphostin is active against the JAK, but shows some selectivity for JAK2 [90]. In IL-6 signalling, JAK1 is thought to be the primary kinase, with JAK2 and TYK2 playing subroles. Another recently identified tyrosine kinase inhibitor is cucurbitacin I (JSI-124) [91]. JSI-124 showed specificity for the JAK/STAT3 pathway, and also showed a reduction in Akt mediated tumour survival pathways in lung and breast cancer cell lines [91]. A JAK3 specific inhibitor, WHI-P131, was able to block thrombin induced platelet aggregation and degranulation [92]. WHI-P131 was also able to induce apoptosis in anaplastic large cell lymphomas (ALCL) [93]. Due to the specificity of these drugs, they may be useful individually or in combination with other compounds targeting different points in the signalling cascade.

The use of peptide aptamers to block Stat3 signalling has shown some success in reducing the dimerisation, and thus the transactivation and DNA binding activity, of the factor *in vivo* [94,95]. These decoys present small regions of peptides that mimic the activation domains of the actual transcription factor, hence the term peptidomimetic is used to describe them. Such decoy peptidomimetics could conceivably be used to study the various functional domains on the protein. The use of peptidomimetic decoys resembling the tyrosine activation residue on STAT3 has also shown promise by interfering with the JAK activation step. The peptidomimetic Tkip, which binds to the autophosphorylation site of the JAK2 kinase, was shown to inhibit the phosphorylation of STAT1, a potential binding partner of STAT3, in the human prostate cancer cell lines DU145 and LNCaP [96]. Again these types of approaches would be applicable when the inhibition of anti-apoptotic proteins were desired, thus sensitising an otherwise resistant tumour cell to other chemotherapeutic drugs.

STAT transcription factors remain in the cytoplasmic compartment until they are activated by JAK (or other) kinases, which mediate their translocation to the nucleus. Blocking this translocation step would serve to prevent the activation of STAT target genes. In a model consistent with the production of ROS during inflammatory conditions and its subsequent dismutation into hydrogen peroxide by the superoxide dismutases, the nuclear translocation and formation of sequence-specific DNA-bound complexes of STAT3 was enhanced by an increase in hydrogen peroxide levels [97]. Therefore, the generation of specific peptidomimetics targeting the STAT nuclear localisation motifs, or specific drugs that inhibit the transport of the activated transcription factor across the nuclear membrane may prove useful as an adjunct to classic chemotherapy.

A similar strategy to the use of peptidomimetics involves the application of retro-inverso peptides. The somewhat paradoxical retro-inverso (RI) peptides, are created by reversing the sequence of the region of the protein to be targeted (*i.e.* ABCDEF would become FEDCBA) using the *dextro* or D-amino acids instead of the physiological active *levo* or L-form. The fusing of part of the HIV *TAT* protein sequence to the RI peptides has proven useful, as it facilitates transport of the peptide into cells [98]. The use of RI peptides has been shown to reduce the activity of enzymes such as protein kinase C (PKC), and inhibited the metastasis of melanoma cells injected into the lungs of mice by targeting the β1 chain of laminin [99,100]. Therefore, this method may hold promise as a highly specific, yet non-toxic approach in the pursuit of chemo-adjuvants.

Finally, the decoying of transcription factors by the use of stabilised oligonucleotide complexes resembling the binding site of a particular transcription factor has shown some usefulness. The STAT6 transcription factor mediates the transition of TH0 lymphocytes into TH2-lymphocytes by mediating the responsiveness of cells to interleukin-4 (IL-4). Using STAT6 oligonucleotides that mimicked the specific binding domain of the factor, thus blocking the activity of STAT6, the disruption of IL-4 induced cell proliferation of murine TH2 cells and primary human CD4(+) T lymphocytes was observed [101]. In similar experiments, a phosphorothiolate *cis*-element decoy against the estrogen response element (ERE decoy) blocked the binding of the estrogen receptor and abrogated the 17β-estrogen-inducible cell proliferation and induced apoptosis of human breast carcinoma cells by functionally affecting expression of the c-fos gene and reducing AP-1 luciferase gene reporter activity [102]. In both applications of this method, no cross-inhibition was observed with upstream signalling pathways. However, the use of this strategy in humans does present some drawbacks, as the synthesis of sufficient amounts of pharmaceutical grade oligonucleotides for the creation of cis-element decoys will be many-fold higher than those levels required for mice and may prove to be prohibitively expensive.

6. Concluding comments

Only recently have we fully realised the importance of inflammatory cytokines such as IL-6 and their downstream targets *e.g.* STAT3, with respect to their potential roles in the causation of neoplastic disease. The German physician Rudolf Virchow (1821–1902), and others described tumours as wounds that never heal, yet they did not have at their disposal the information regarding the molecular nature of the causative pathways of inflammation in order to pursue their hypothesis. Interestingly, Virchow studied vascular disorders such as inflammation of blood vessels and phlebitis. Remarkably, current evidence is pointing towards a strong connection between inflammation and atherosclerotic plaque formation.

Thousands of years prior to Virchow's observations, the ancient Egyptians used teas brewed from the Willow tree (*Salix babylonica*) for medicinal purposes, and because they believed (correctly of course) it extended life. Images of leaves from the willow and other similar trees appeared in hieroglyphs, signifying the importance of these plants even in the afterlife. Furthermore, as far back as 1650 B.C., hieroglyphs depicting the ancient Egyptian medical literature describe inflammation as heat or warmth. We now know that the salicylic acid (2-hydroxybenzoic acid) in the leaves and bark of this plant inhibit the activity of the cyclooxegenases (COX) enzymes, and that the COX2 form of the enzyme produces inflammatory prostaglandins such a PGE2. PGE2 is a well-characterised and potent inducer of IL-6. The reduction in the incidence of tumour formation has been observed in consumers of tea (*Camellia sinensis*). The medicinal and life extension qualities of this plant were so well established thousands of years ago, that it was used as currency in some cultures. We now know that the polyphenolic compounds, chief among them, epigallocatechin gallate (EGCG), acts to attenuate free-radical species (ROS) and reduce DNA damage [103]. EGCG also inhibits the activity of the human DNA methyltransferase-1 enzyme (DNMT-1), the maintenance enzyme implicated in the epigenetic alterations leading to promoter hypermethylation in tumour cells [104]. In addition, many of these same compounds have shown protection against another serious disorder, atherosclerotic plaque formation, and heart disease. When we consider the current state of modern medical knowledge, perhaps a review of what was known thousands of years ago might be useful in solving some of today's problems.

Conflict of interest statement

None declared. The content of this article does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

References

1. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002, **420**, 860–867.
2. Loetscher P, Seitz M, Clark-Lewis I, *et al*. Activation of NK cells by CC chemokines. Chemotaxis, Ca2+ mobilization, and enzyme release. *J Immunol* 1996, **156**, 322–327.
3. Igonin AA, Armstrong VW, Shipkova M, *et al*. Circulating cytokines as markers of systemic inflammatory response in severe community-acquired pneumonia. *Clin Biochem* 2004, **37**, 204–209.
4. Teplyakov AI, Pryschepova EV, *et al*. Cytokines and soluble cell adhesion molecules. Possible markers of inflammatory response in atherosclerosis. *Ann NY Acad Sci* 2000, **902**, 320–322.
5. Ma J, Chen T, Mandelin J, *et al*. Regulation of macrophage activation. *Cell Mol Life Sci* 2003, **60**, 2334–2346.
6. Maeda H, Akaike T. Nitric oxide and oxygen radicals in infection, inflammation, and cancer. *Biochemistry (Moscow)* 1998, **63**, 854–865.
7. Sehgal PB. Cytokine-induced STAT signalling through the cytoplasmic compartment. *Adv Exp Med Biol* 2001, **495**, 161–168.
8. Schindler C, Strehlow I. Cytokines and STAT signalling. *Adv Pharmacol* 2000, **47**, 113–174.
9. Liu KD, Gaffen SL, Goldsmith MA. JAK/STAT signalling by cytokine receptors. *Curr Opin Immunol* 1998, **10**, 271–278.
10. Soriano SF, Serrano A, Hernanz-Falcon P, *et al*. Chemokines integrate JAK/STAT and G-protein pathways during chemotaxis and calcium flux responses. *Eur J Immunol* 2003, **33**, 1328–1333.
11. Shacter E, Weitzman SA. Chronic inflammation and cancer. *Oncology (Huntingt)* 2002, **16**, 217–226., 229.
12. Liu XH, Kirschenbaum A, Lu M, *et al*. Prostaglandin E (2) stimulates prostatic intraepithelial neoplasia cell growth through activation of the interleukin-6/GP130/STAT-3 signalling pathway. *Biochem Biophys Res Commun* 2002, **290**, 249–255.
13. Shinozaki M, Hirahashi J, Lebedeva T, *et al*. IL-15, a survival factor for kidney epithelial cells, counteracts apoptosis and inflammation during nephritis. *J Clin Invest* 2002, **109**, 951–960.
14. Tourbah A, Linnington C, Bachelin C, *et al*. Inflammation promotes survival and migration of the CG4 oligodendrocyte progenitors transplanted in the spinal cord of both inflammatory and demyelinated EAE rats. *J Neurosci Res* 1997, **50**, 853–861.
15. Hodge DR, Xiao W, Wang LH, *et al*. Activating mutations in STAT3 and STAT5 differentially affect cellular proliferation and apoptotic resistance in multiple myeloma cells. *Cancer Biol Ther* 2004, **3**, 188–194.
16. Frassanito MA, Cusmai A, Iodice G, *et al*. Autocrine interleukin-6 production and highly malignant multiple myeloma: relation with resistance to drug-induced apoptosis. *Blood* 2001, **97**, 483–489.
17. Hideshima T, Nakamura N, Chauhan D, *et al*. Biologic sequelae of interleukin-6 induced PI3-K/Akt signalling in multiple myeloma. *Oncogene* 2001, **20**, 5991–6000.
18. Kamimura D, Ishihara K, Hirano T. IL-6 signal transduction and its physiological roles: the signal orchestration model. *Rev Physiol Biochem Pharmacol* 2003, **149**, 1–38.
19. Heinrich PC, Behrmann I, Muller-Newen G, *et al*. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* 1998, **334**(Pt 2), 297–314.

20. Steelman LS, Pohnert SC, Shelton JG, *et al*. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. *Leukemia* 2004, **18**, 189–218.
21. Ershler WB. Interleukin-6: a cytokine for gerontologists. *J Am Geriatr Soc* 1993, **41**, 176–181.
22. Wolvekamp MC, Marquet RL. Interleukin-6: historical background, genetics and biological significance. *Immunol Lett* 1990, **24**, 1–9.
23. Yang L, Wang L, Lin HK, *et al*. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. *Biochem Biophys Res Commun* 2003, **305**, 462–469.
24. Miki S, Iwano M, Miki Y, *et al*. Interleukin-6 (IL-6) functions as an *in vitro* autocrine growth factor in renal cell carcinomas. *FEBS Lett* 1989, **250**, 607–610.
25. Eustace D, Han X, Gooding R, *et al*. Interleukin-6 (IL-6) functions as an autocrine growth factor in cervical carcinomas *in vitro*. *Gynecol Oncol* 1993, **50**, 15–19.
26. Van Wagoner NJ, Oh JW, Repovic P, *et al*. Interleukin-6 (IL-6) production by astrocytes: autocrine regulation by IL-6 and the soluble IL-6 receptor. *J Neurosci* 1999, **19**, 5236–5244.
27. Leu CM, Wong FH, Chang C, *et al*. Interleukin-6 acts as an antiapoptotic factor in human esophageal carcinoma cells through the activation of both STAT3 and mitogen-activated protein kinase pathways. *Oncogene* 2003, **22**, 7809–7818.
28. Okamoto M, Lee C, Oyasu R. Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells *in vitro*. *Cancer Res* 1997, **57**, 141–146.
29. Hammacher A, Ward LD, Weinstock J, *et al*. Structure-function analysis of human IL-6: identification of two distinct regions that are important for receptor binding. *Protein Sci* 1994, **3**, 2280–2293.
30. Lust JA, Jelinek DF, Donovan KA, *et al*. Sequence, expression and function of an mRNA encoding a soluble form of the human interleukin-6 receptor (sIL-6R). *Curr Top Microbiol Immunol* 1995, **194**, 199–206.
31. Taga T, Kishimoto T. Role of a two-chain IL-6 receptor system in immune and hematopoietic cell regulation. *Crit Rev Immunol* 1992, **11**, 265–280.
32. Autissier P, De Vos J, Liautard J, *et al*. Dimerization and activation of the common transducing chain (gp130) of the cytokines of the IL-6 family by mAb. *Int Immunol* 1998, **10**, 1881–1889.
33. Suganuma M, Okabe S, Kurusu M, *et al*. Discrete roles of cytokines, TNF-alpha, IL-1, IL-6 in tumour promotion and cell transformation. *Int J Oncol* 2002, **20**, 131–136.
34. Ayre JE. Cervical cancer: chronic inflammation, stress and adaptation factors. *Acta Unio Int Contra Cancrum* 1956, **12**, 20–27.
35. Dalgleish AG, O'Byrne KJ. Chronic immune activation and inflammation in the pathogenesis of AIDS and cancer. *Adv Cancer Res* 2002, **84**, 231–276.
36. Farrow B, Evers BM. Inflammation and the development of pancreatic cancer. *Surg Oncol* 2002, **10**, 153–169.
37. Nelson WG, De Marzo AM, DeWeese TL, *et al*. The role of inflammation in the pathogenesis of prostate cancer. *J Urol* 2004, **172**, S6–S11.
38. Schwartsburd PM. Chronic inflammation as inductor of procancer microenvironment: pathogenesis of dysregulated feedback control. *Cancer Metastasis Rev* 2003, **22**, 95–102.
39. Samaniego F, Pati S, Karp J, *et al*. Human herpesvirus 8 k1-associated nuclear factor-kappa b-dependent promoter activity: role in kaposi's sarcoma inflammation? *J Natl Cancer Inst Monogr*, 15–23.
40. Osborne J, Moore PS, Chang Y. KSHV-encoded viral IL-6 activates multiple human IL-6 signalling pathways. *Hum Immunol* 1999, **60**, 921–927.
41. Potter M, Sklar MD, Rowe WP. Rapid viral induction of plasmacytomas in pristane-primed BALB-c mice. *Science* 1973, **182**, 592–594.
42. Potter M, Maccardle RC. Histology of developing plasma cell neoplasia induced by mineral oil in balb/c mice. *J Natl Cancer Inst* 1964, **33**, 497–515.
43. Potter M. Plasma cell tumour-formation and antigen-binding myeloma proteins in mice. *Brit Med J* 1971, **2**, 320.
44. Gado K, Silva S, Paloczi K, *et al*. Mouse plasmacytoma: an experimental model of human multiple myeloma. *Haematologica* 2001, **86**, 227–236.
45. Potter M, Wax JS, Anderson AO, *et al*. Inhibition of plasmacytoma development in BALB/c mice by indomethacin. *J Exp Med* 1985, **161**, 996–1012.
46. Nosaka T, Kitamura T. Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) in hematopoietic cells. *Int J Hematol* 2000, **71**, 309–319.
47. Yang J, Chatterjee-Kishore M, Staugaitis SM, *et al*. Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation. *Cancer Res* 2005, **65**, 939–947.
48. Sasse J, Hemmann U, Schwartz C, *et al*. Mutational analysis of acute-phase response factor/Stat3 activation and dimerization. *Mol Cell Biol* 1997, **17**, 4677–4686.
49. Takeda K, Akira S. STAT family of transcription factors in cytokine-mediated biological responses. *Cytokine Growth Factor Rev* 2000, **11**, 199–207.
50. Seidel HM, Milocco LH, Lamb P, *et al*. Spacing of palindromic half sites as a determinant of selective STAT (signal transducers and activators of transcription) DNA binding and transcriptional activity. *Proc Natl Acad Sci USA* 1995, **92**, 3041–3045.
51. Chen T, Wang LH, Farrar WL. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. *Cancer Res* 2000, **60**, 2132–2135.
52. Wang LH, Yang XY, Zhang X, *et al*. Transcriptional inactivation of STAT3 by PPARgamma suppresses IL-6-responsive multiple myeloma cells. *Immunity* 2004, **20**, 205–218.
53. Stewart WC, Baugh Jr JE, Floyd ZE, *et al*. STAT 5 activators can replace the requirement of FBS in the adipogenesis of cells. *Biochem Biophys Res Commun* 2004, **324**, 355–359.
54. Baskiewicz-Masiuk M, Machalinski B. The role of the STAT5 proteins in the proliferation and apoptosis of the CML and AML cells. *Eur J Haematol* 2004, **72**, 420–429.
55. Bromberg JF, Wrzeszczynska MH, Devgan G, *et al*. Stat3 as an oncogene. *Cell* 1999, **98**, 295–303.
56. Ariyoshi K, Nosaka T, Yamada K, *et al*. Constitutive activation of STAT5 by a point mutation in the SH2 domain. *J Biol Chem* 2000, **275**, 24407–24413.
57. Klein B, Tarte K, Jourdan M, *et al*. Survival and proliferation factors of normal and malignant plasma cells. *Int J Hematol* 2003, **78**, 106–113.
58. Kovalchuk AL, Kim JS, Park SS, *et al*. IL-6 transgenic mouse model for extraosseous plasmacytoma. *Proc Natl Acad Sci USA* 2002, **99**, 1509–1514.
59. Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine Growth Factor Rev* 2002, **13**, 357–368.
60. Foti E, Ferrandina G, Martucci R, *et al*. IL-6, M-CSF and IAP cytokines in ovarian cancer: simultaneous assessment of serum levels. *Oncology* 1999, **57**, 211–215.
61. Salem M, Elbaz O, Zahran M, *et al*. Malignancy: identification of predictors of disease status and progression in patients with myeloma (MM). *Haematologica* 2000, **5**, 41–45.
62. Thavasu PW, Ganjoo RK, Maidment SA, *et al*. Multiple myeloma: an immunoclinical study of disease and response to treatment. *Hematol Oncol* 1995, **13**, 69–82.

63. Makarov SS, Johnston WN, Olsen JC, *et al*. NF-kappa B as a target for anti-inflammatory gene therapy: suppression of inflammatory responses in monocytic and stromal cells by stable gene transfer of I kappa B alpha cDNA. *Gene Ther* 1997, **4**, 846–852.
64. Chauhan D, Uchiyama H, Akbarali Y, *et al*. Multiple myeloma cell adhesion-induced interleukin-6 expression in bone marrow stromal cells involves activation of NF-kappa B. *Blood* 1996, **87**, 1104–1112.
65. Croonquist PA, Linden MA, Zhao F, *et al*. Gene profiling of a myeloma cell line reveals similarities and unique signatures among IL-6 response, N-ras-activating mutations, and coculture with bone marrow stromal cells. *Blood* 2003, **102**, 2581–2592.
66. Puthier D, Derenne S, Barille S, *et al*. Mcl-1 and Bcl-xL are co-regulated by IL-6 in human myeloma cells. *Brit J Haematol* 1999, **107**, 392–395.
67. Spets H, Stromberg T, Georgii-Hemming P, *et al*. Expression of the bcl-2 family of pro- and anti-apoptotic genes in multiple myeloma and normal plasma cells: regulation during interleukin-6 (IL-6)-induced growth and survival. *Eur J Haematol* 2002, **69**, 76–89.
68. Song L, Li Y, Sun YX, *et al*. IL-6 inhibits apoptosis of human myeloma cell line XG-7 through activation of JAK/STAT pathway and up-regulation of Mcl-1. *Ai Zheng* 2002, **21**, 113–116.
69. Song L, Li Y, Sun Y, *et al*. Mcl-1 mediates cytokine deprivation induced apoptosis of human myeloma cell line XG-7. *Chinese Med J (Engl)* 2002, **115**, 1241–1243.
70. Chanan-Khan AA. Bcl-2 antisense therapy in multiple myeloma. *Oncology (Huntingt)* 2004, **18**, 21–24.
71. Quintanilla-Martinez L, Kremer M, Specht K, *et al*. Analysis of signal transducer and activator of transcription 3 (Stat 3) pathway in multiple myeloma: Stat 3 activation and cyclin D1 dysregulation are mutually exclusive events. *Am J Pathol* 2003, **162**, 1449–1461.
72. Zhang Q, Raghunath PN, Vonderheid E, *et al*. Lack of phosphotyrosine phosphatase SHP-1 expression in malignant T-cell lymphoma cells results from methylation of the SHP-1 promoter. *Am J Pathol* 2000, **157**, 1137–1146.
73. Galm O, Yoshikawa H, Esteller M, *et al*. SOCS-1, a negative regulator of cytokine signalling, is frequently silenced by methylation in multiple myeloma. *Blood* 2003, **101**, 2784–2788.
74. Depil S, Saudemont A, Quesnel B. SOCS-1 gene methylation is frequent but does not appear to have prognostic value in patients with multiple myeloma. *Leukemia* 2003, **17**, 1678–1679.
75. Chauhan D, Uchiyama H, Akbarali Y, *et al*. Multiple myeloma cell adhesion-induced interleukin-6 expression in bone marrow stromal cells involves activation of NF-kappa B. *Blood* 1996, **87**, 1104–1112.
76. Hideshima T, Chauhan D, Hayashi T, *et al*. Proteasome inhibitor PS-341 abrogates IL-6 triggered signalling cascades via caspase-dependent downregulation of gp130 in multiple myeloma. *Oncogene* 2003, **22**, 8386–8393.
77. Chauhan D, Li G, Podar K, *et al*. The bortezomib/proteasome inhibitor PS-341 and triterpenoid CDDO-Im induce synergistic anti-multiple myeloma (MM) activity and overcome bortezomib resistance. *Blood* 2004, **103**, 3158–3166.
78. Starr R, Hilton DJ. Negative regulation of the JAK/STAT pathway. *Bioessays* 1999, **21**, 47–52.
79. Yoshimura A. The CIS family: negative regulators of JAK–STAT signalling. *Cytokine Growth Factor Rev* 1998, **9**, 197–204.
80. Yoshikawa H, Matsubara K, Qian GS, *et al*. SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. *Nat Genet* 2001, **28**, 29–35.
81. Jimenez-Lara AM, Heine MJ, Gronemeyer H. PIAS3 (protein inhibitor of activated STAT-3) modulates the transcriptional activation mediated by the nuclear receptor coactivator TIF2. *FEBS Lett* 2002, **526**, 142–146.
82. Schaper F, Gendo C, Eck M, *et al*. Activation of the protein tyrosine phosphatase SHP2 via the interleukin-6 signal transducing receptor protein gp130 requires tyrosine kinase Jak1 and limits acute-phase protein expression. *Biochem J* 1998, **335**(Pt 3), 557–565.
83. Weidler M, Rether J, Anke T, *et al*. Inhibition of interleukin-6 signalling and Stat3 activation by a new class of bioactive cyclopentenone derivatives. *Biochem Biophys Res Commun* 2000, **276**, 447–453.
84. Mitsuhashi S, Shima H, Tanuma N, *et al*. Protein phosphatase type 2A, PP2A, is involved in degradation of gp130. *Mol Cell Biochem* 2005, **269**, 183–187.
85. Tanuma N, Shima H, Nakamura K, *et al*. Protein tyrosine phosphatase epsilonC selectively inhibits interleukin-6- and interleukin- 10-induced JAK–STAT signalling. *Blood* 2001, **98**, 3030–3034.
86. Honemann D, Chatterjee M, Savino R, *et al*. The IL-6 receptor antagonist SANT-7 overcomes bone marrow stromal cell-mediated drug resistance of multiple myeloma cells. *Int J Cancer* 2001, **93**, 674–680.
87. Imazeki I, Saito H, Hasegawa M, *et al*. IL-7 functions in cynomolgus monkeys blocked by a humanized antibody to human IL-6 receptor. *Int J Immunopharmacol* 1998, **20**, 345–357.
88. Huang YW, Vitetta ES. A monoclonal anti-human IL-6 receptor antibody inhibits the proliferation of human myeloma cells. *Hybridoma* 1993, **12**, 621–630.
89. Yoshizaki K, Nishimoto N, Mihara M, *et al*. Therapy of rheumatoid arthritis by blocking IL-6 signal transduction with a humanized anti-IL-6 receptor antibody. *Springer Semin Immunopathol* 1998, **20**, 247–259.
90. Wang LH, Kirken RA, Erwin RA, *et al*. JAK3, STAT, and MAPK signalling pathways as novel molecular targets for the tyrphostin AG-490 regulation of IL-2-mediated T cell response. *J Immunol* 1999, **162**, 3897–3904.
91. Blaskovich MA, Sun J, Cantor A, *et al*. Discovery of JSI-124 (cucurbitacin I), a selective Janus kinase/signal transducer and activator of transcription 3 signalling pathway inhibitor with potent antitumour activity against human and murine cancer cells in mice. *Cancer Res* 2003, **63**, 1270–1279.
92. Malaviya R, Zhu D, Dibirdik I, *et al*. Targeting Janus kinase 3 in mast cells prevents immediate hypersensitivity reactions and anaphylaxis. *J Biol Chem* 1999, **274**, 27028–27038.
93. Amin HM, Medeiros LJ, Ma Y, *et al*. Inhibition of JAK3 induces apoptosis and decreases anaplastic lymphoma kinase activity in anaplastic large cell lymphoma. *Oncogene* 2003, **22**, 5399–5407.
94. Nagel-Wolfrum K, Buerger C, Wittig I, *et al*. The interaction of specific peptide aptamers with the DNA binding domain and the dimerization domain of the transcription factor Stat3 inhibits transactivation and induces apoptosis in tumour cells. *Mol Cancer Res* 2004, **2**, 170–182.
95. Buerger C, Nagel-Wolfrum K, Kunz C, *et al*. Sequence-specific peptide aptamers, interacting with the intracellular domain of the epidermal growth factor receptor, interfere with Stat3 activation and inhibit the growth of tumour cells. *J Biol Chem* 2003, **278**, 37610–37621.
96. Flowers LO, Subramaniam PS, Johnson HM. A SOCS-1 peptide mimetic inhibits both constitutive and IL-6 induced activation of STAT3 in prostate cancer cells. *Oncogene* 2005, **24**, 2114–2120.
97. Carballo M, Conde M, El Bekay R, *et al*. Oxidative stress triggers STAT3 tyrosine phosphorylation and nuclear translocation in human lymphocytes. *J Biol Chem* 1999, **274**, 17580–17586.
98. Ramanathan S, Pooyan S, Stein S, *et al*. Targeting the sodium-dependent multivitamin transporter (SMVT) for improving the

oral absorption properties of a retro-inverso Tat nonapeptide. *Pharm Res* 2001, **18**, 950–956.
99. Ricouart A, Tartar A, Sergheraert C. Inhibition of protein kinase C by retro-inverso pseudosubstrate analogues. *Biochem Biophys Res Commun* 1989, **165**, 1382–1390.
100. Zhao M, Kleinman HK, Mokotoff M. Synthesis and activity of partial retro-inverso analogs of the antimetastatic laminin-derived peptide, YIGSR-NH2. *J Pept Res* 1997, **49**, 240–253.
101. Wang LH, Yang XY, Kirken RA, *et al*. Targeted disruption of stat6 DNA binding activity by an oligonucleotide decoy blocks IL-4-driven T (H)2 cell response. *Blood* 2000, **95**, 1249–1257.
102. Wang LH, Yang XY, Zhang X, *et al*. The cis decoy against the estrogen response element suppresses breast cancer cells via target disrupting c-fos not mitogen-activated protein kinase activity. *Cancer Res* 2003, **63**, 2046–2051.
103. Yamamoto T, Hsu S, Lewis J, *et al*. Green tea polyphenol causes differential oxidative environments in tumour versus normal epithelial cells. *J Pharmacol Exp Ther* 2003, **307**, 230–236.
104. Fang MZ, Wang Y, Ai N, *et al*. Tea polyphenol (−)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res* 2003, **63**, 7563–7570.