Chapter 3
Cytokine Networks and Cancer Stem Cells

Clifford Liongue, Alister C. Ward, Wei Duan and Sarah Shigdar

Abstract Cell-to-cell communication is an integral function of multicellular organisms. Many of these signals are received by a myriad of cell-surface receptors that utilize a range of intracellular signaling pathways to communicate this to the nucleus, rapidly impacting on the transcription of target genes in order to elicit the desired response, such as proliferation, differentiation, activation, and survival. Dysregulation of these important signaling pathways, and networks, often lead to pathological conditions due to inappropriate cell responses with negative consequences. The aberrant signaling pathways have been associated with many diseases, including cancer. Cytokines and chemokines convey a multitude of messages to the target cell, many of which are beneficial for cancers and cancer stem cells, such as proliferation, survival and migration. By hijacking this communication network, cancers and cancer stem cells can become invasive and more pathogenic. Furthermore, by using these communication systems, cancer stem cells are able to evade current therapies. Therefore, novel therapies may be developed to break the communication systems of the cancer stem cells. This chapter explores the role of the cytokines TGF-β, TNF-α, IL-1 and IL-6 and chemokine CXCL8 as well as NF-κB and their role in cancer stem cell survival and maintenance. Emerging therapies are beginning to target the cancer stem cell population, either specifically or synergistically with existing therapeutic options. These novel therapies may hold the key to breaking the communication network of cancer stem cells.

Keywords Cancer • Cancer stem cells • Cytokines • Inflammation • Signaling networks
1 Introduction

During development and throughout life, cells constantly respond to external stimuli and generate an appropriate response, such as proliferation, differentiation, activation and survival. Among these stimuli are cytokines and chemokines, which play an essential role in the development and regulation of a range of cell types, in particular those of the immune and hematopoietic systems. Cytokine signaling plays a major role in the initiation, propagation and resolution of inflammation. Given that cancer is now recognized as a disease of inflammation, it is no surprise that cytokines contribute strongly to the development and propagation of cancer and cancer stem cells. This Chapter aims to explore the contribution of cytokines to the phenotype of cancer stem cell phenotype, as well as identifying possible therapeutic targets and postulating on the effects that personalized medicine may have on the future treatment of cancer patients.

2 Tumor Microenvironment, Inflammation, EMT and Cancer Stem Cells

Inflammation is a natural response to injury (Coussens and Werb 2002). The presence of a tumor triggers an inflammatory response, creating a microenvironment that may enhance the dissemination of cancer cells (Hanahan and Weinberg 2000, 2011). Key features of the tumor microenvironment include endothelial cells, bone marrow derived stromal cells and infiltrating white blood cells, which migrate into the tumor following cytokine and chemokine recruitment via the tumor cells or surrounding stroma (Grivennikov et al. 2010; Hanahan and Weinberg 2011). Within the tumor mass are so-called cancer stem cells (CSCs), which are a small subset of cells with the ability to proliferate and form new tumors (Al-Hajj et al. 2003; Koch et al. 2010). The key functional properties of these cells are self-renewal, multi-potent differentiation and the capacity to generate a heterogeneous lineage of all types of cancer cells comprising a tumor (Shackleton 2010; Clevers 2011). These CSCs also have the potential to lose their epithelial phenotype, becoming motile and invasive, and taking on a mesenchymal phenotype, a process known as the epithelial-to-mesenchymal transformation (EMT), and representing an important step in metastasis (Singh and Settleman 2010; Biddle and Mackenzie 2012; Brabletz 2012).

A number of cytokines and chemokines have been shown to be released during inflammation that play a vital role in the progression of cancer, affecting key CSC phenotypes such as EMT. These include Interleukin (IL)-1, IL-6, IL-8 (CXCL8), tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β) and NF-κB. Communication between cells is not unidirectional, with both immune and tumors cells producing cytokines and chemokines (Lu et al. 2006). Such cues, as along with additional environmental factors, induce the changes that enable cancer cells to metastasize (Hanahan and Weinberg 2011).
Tumor associated macrophages (TAMs) play a major role in the progression of cancer. The secretion of IL-1 and TNF-α by TAMs has been shown to support the steps involved in invasion and metastasis (Biswas et al. 2013). Secretion of TNF-α by TAMs and other immune cells can also up-regulate the secretion of TGF-β by cancer associated fibroblasts (CAFs) and immune cells, which is an inducer of IL-1, IL-6 and IL-8 (Mani et al. 2008). IL-6 is also secreted by CAFs, TAMs, other immune cells, as well as by the cancer cells themselves, forming a positive feedback loop (Shigdar et al. 2014). The cancer cells also secrete IL-8, MCP-1 and RANTES, which promote proliferation of stromal cells, including endothelial cells (Levina et al. 2008), with RANTES and MCP-1 also able to attract tumor-infiltrating lymphocytes (Ji and Zhang 2010). Both IL-6 and IL-8 have been shown to mediate chronic inflammation, suggesting that cancer cells are directly involved in stimulating the inflammatory process (Korkaya et al. 2011, 2012).

How then do these cytokines and the inflammatory process contribute towards a CSC phenotype? This ultimately relates to the downstream signaling pathways induced, which act on key genes to modify the phenotype of CSCs. However, this is a multi-step process. Using the analogy of the ‘never-healing wound’, proliferating tumor cells and the surrounding activated stroma secrete cytokines and chemokines to attract immune cells which secrete further factors to induce activation and/or infiltration of other cells. The center of this ‘ball of cells’ becomes hypoxic, leading to the up-regulation of Hypoxia-inducible factor-1 alpha (HIF-1α), which acts in concert with cytokine-induced Snail, Twist1 and Stat3 to induce epithelial-mesenchymal transition (EMT). This then leads to the dissemination of CSCs to distant metastatic sites, where these cells then initiate secondary tumors (Mani et al. 2008), but also leaving behind a population of CSC-like cells (Singh and Settleman 2010; Kong et al. 2011). Indeed, it is thought that the de-differentiation of cancer cells into CSCs, is the first step in the process towards EMT (Fig. 3.1). Moreover, the aberrant activation of EMT enhances cancer cell motility and dissemination but also confers a stem-cell like phenotype, as evidenced by gene expression patterns, and leads to an increase in the CSC population (Chaffer et al. 2011; Hanahan and Weinberg 2011; Brabletz 2012), pointing to the close association between CSCs and EMT. However, cytokines are also important in maintaining CSCs and inducing other phenotypes in these cells in addition to EMT.

### 3 Transforming Growth Factor-Beta

Transforming growth factor-beta (TGF-β) is secreted by immune cells during normal wound healing in order to trigger surrounding epithelial cells to undergo EMT enabling them to migrate to the site of the wound for repair (Biddle and Mackenzie 2012) (Fig. 3.2). However, this process becomes perturbed during cancer development. Immune cells migrate to the area of a tumor in an attempt to heal the ‘wound’ and secrete cytokines, such as TGF-β. The cancer cells, meanwhile, have developed oncogenic mutations that render them more responsive to these
EMT-inducing signals (Biddle and Mackenzie 2012), while also converting TGF-β’s normal growth inhibitory role into a growth promoting role (Padua and Massague 2009; Asiedu et al. 2011). To understand how TGF-β induces EMT, and thus CSCs, an understanding of the signaling pathways involved is required to induce EMT, TGF-β signals via both a Smad-dependent and Smad-independent transcriptional pathway (Fig. 3.3). The Smad-dependent pathway involves phosphorylation of
Smad2 and Smad3, which then form heteromeric complexes with Smad4. These complexes then translocate into the nucleus where they control transcription of EMT target genes (Massague 2000; Derynck and Zhang 2003). The Smad-independent pathway activates Ras/Erk, c-Jun N-terminal kinase (JNK), phosphatidylinositol-3 (PI3) kinase, Par6, and Cdc42 GTPases, which have also been associated with TGF-β-induced EMT (Derynck and Zhang 2003).

Autocrine TGF-β signaling has been linked to EMT and migration, as well as maintenance of a stem cell-like population. Indeed, TGF-β secreted by cancer cells can mediate the differentiation of fibroblasts into myofibroblasts, or CAFs, which then secrete more TGF-β into the microenvironment, thus further promoting EMT (Fig. 3.2). In a recent study, the abrogation of TGF-β autocrine signaling resulted in decreased expression of vimentin and Snail, and enhanced expression of E-cadherin, indicating a reduction in EMT potential. In addition, the cancer stem cell-like markers Integrin β1, Notch 1 and aldehyde dehydrogenase 1/2 were also reduced. Finally, expression of Gli1, a component of the hedgehog signaling pathway involved in stem cell self-renewal was reduced, indicating the importance of this pathway for both EMT and CSCs (Liu et al. 2012).

Another consequence of TGF-β signaling is the decreased expression of the p15INK4b and p21CIP21 tumor suppressor genes, which act by stabilizing cyclin-dependent kinase inhibitors. It is thought that as the balance shifts between
SMAD-dependent and SMAD-independent signaling, the cyclin-dependent kinase inhibitors fail to be induced, thus allowing cell proliferation to continue unchecked. However, at low levels of c-Myc, TGF-β typically induces the expression of p21, a cell cycle inhibitor, which in cancer cells, could contribute towards the CSC quiescent state (Kubiczkova et al. 2012). As well, Notch is activated in the immediate vicinity of active tumor progenitors, and Notch has been linked to p21 activation, thus suggesting that Notch, via p21, drives a quiescent phenotype consistent with CSCs, via some feedback control mechanism (Medema 2013). Once p21 is activated, several other signaling cascades are blocked, including c-Myc (Abbas and Dutta 2009) thus perpetuating the CSC phenotype. However, other pathways are activated which reduce these effects in some of the CSCs, thus leading to the acquisition of an EMT phenotype (Fig. 3.2).

While TGF-β can induce CSC phenotypes such as EMT, this effect has been shown to be transitory and required tumor necrosis factor (TNF)-α for more stable phenotypic changes (Mani et al. 2008; Asiedu et al. 2011). Moreover, TNF-α can up-regulate TGF-β expression at the transcriptional level and accelerate TGF-β induced EMT dramatically (Bates and Mercurio 2003).
4 Tumor Necrosis Factor-Alpha

Tumor necrosis factor (TNF) was first identified as a soluble factor released by host cells in response to a bacterial endotoxin that caused necrosis of tumors in both humans and animal models (Balkwill 2009). Amongst the large TNF superfamily, TNF-α has been recognized as a particularly important member (Balkwill 2009). TNF-α has a well-established pro-inflammatory function, shown to be an important mediator of the chronic inflammation of the bowel observed in irritable bowel disease (IBD). Indeed, TNF antagonists have been used quite successfully for the treatment of IBD, Chrohn’s disease and ulcerative colitis (Balkwill 2009; Ben Musa et al. 2014), as well as rheumatoid arthritis, psoriasis, severe chronic asthma, ankylosing spondylitis and sarcoidosis (Balkwill 2009). Moreover, given its pro-inflammatory role, TNF-α has also been shown to act as a tumor initiator by stimulating the production of molecules that lead to DNA damage and mutations such as reactive oxygen and nitrogen species (RONS), and as a tumor promoter by altering cell proliferation and death (Hartnett and Egan 2012). TNF-α mediates its effects through binding to two different receptors with subsequent intracellular signaling occurring through several different pathways (Sethi et al. 2008; Egea et al. 2011). It contributes to malignant transformation through the up-regulation of c-Fos and c-Myc via NF-κB (Wang et al. 2013) (Fig. 3.3).

The role of TNF-α, however, is far from simple since it has also been shown to be cytotoxic and possess anti-tumor effects in several malignant diseases (Soria et al. 2011). The latter are thought to be related to its effects in destroying the tumor vasculature (Balkwill 2009), and may represent an effective therapeutic strategy early in cancer development (Ben Musa et al. 2014). However, it is now known that in advanced stages of cancer, the destruction of the tumor vasculature can lead to enhanced metastasis through EMT (Simon and Keith 2008; Mayol et al. 2009). Interestingly, it appears that low levels of TNF-α act as a tumor promoter and a recent study has shed light on how these low levels are maintained. The microRNA (miRNA) miR-130a, which has been demonstrated to promote cell survival in several cell lines, was shown to directly target the 3’UTR region of TNF-α, repressing its translation. Additionally, TNF-α stimulated enhanced miR-130a levels via NF-κB, providing a negative feedback loop that maintains TNF-α at levels that can promote tumor growth, at least in cervical cancer cells (Zhang et al. 2014). However, as shown quite eloquently in a previous study, ovarian cancer patients with the highest levels of TNF-α experienced the most intense down-regulation following monoclonal antibody therapy against TNF-α, although in mouse studies, a similar blockade of TNF-α resulted in decreased vasculature (Kulbe et al. 2012). Additionally, given that TNF-α therapy has been associated with an increased risk of malignancies, care must be taken when considering this as a therapeutic option (Bongartz et al. 2006).

This data suggests another potential link between TNF-α, EMT and CSCs. If blockade of TNF-α leads to a decreased vasculature, does this lead to a level of hypoxia required to promote CSC formation, and if so is this how miR-130a contributes to tumor promotion? In support of this, hypoxia is known to increase the number of CD133+ CSC within a tumor mass, through the activation of Oct4 and...
Notch signaling (Simon and Keith 2008; Mayol et al. 2009). Additionally, tumor hypoxia increases TGF-β secretion from cancer cells, thus triggering EMT (Jing et al. 2011). TNF-α can also cause the production of reactive oxygen species (ROS) from mitochondria under hypoxic conditions, and both ROS and NF-κB can facilitate EMT in certain cell types (Jing et al. 2011). As well, increased intracellular ROS levels may induce DNA damage within CSCs, resulting in additional mutations that promote disease progression (Tanno and Matsui 2011). Moreover, as described previously, the induction of Notch signaling leads to the activation of p21, resulting in quiescence (Medema 2013). Indeed, it is this link between TNF-α and Notch, whose activation occurs following prolonged exposure to TNF-α, that contributes to the CSC phenotype (Lee et al. 2012).

5 Interleukin-1

The Interleukin-1 (IL-1) cytokine family represent key mediators of inflammation and innate immune response, consisting of 11 cytokines and 10 receptors. Cells of the innate immune system, such as monocytes and macrophages are major sources of the two IL-1 cytokines, IL-1α and IL-1β, which both signal through the same receptor complex consisting of IL-1 receptor 1 (IL-1R1) and IL-1 receptor accessory protein (IL-1Rap). There are two mechanisms for controlling IL-1 signaling, either via the by competitive binding of the IL-1 Receptor antagonist (IL-1Ra) to the IL-1 receptor complex, or recruitment of a decoy receptor IL-1R2 that is unable to induce signal transduction (Dinarello 2011). Signaling pathways activated by IL-1 cytokines include myeloid differentiation primary response gene 88 (MYD88) and interleukin-1 receptor activated protein kinase (IRAK) as well as the NF-κB and PI3K pathways (Weber et al. 2010). The IL-1 family cytokines exist as an “inactive” uncleaved IL-1 which is cleaved to produce an “active” form that is able to signal via its cognate receptor complex (Werman et al. 2004).

Although cancer cells express IL-1α, its role varies depending on its state of cleavage. If IL-1α is uncleaved, it can act as a tumor suppressor, possibly via its ability to form a non-signaling complex with IL-1R1 that may act as an antigen for the immune system. Indeed, uncleaved IL-1α has been shown, at least initially, to reduce tumor load. Conversely, if IL-1α is cleaved it becomes a potent pro-inflammatory cytokine that favors tumor progression by promoting invasiveness and metastasis (Rider et al. 2013). Clearly, dual-function cytokines are unique as they do not require their cognate receptors to be expressed for autocrine signaling, and are able to elicit a function without their canonical signaling cascade. Therefore, dual function cytokines such as IL-1α require careful further study to delineate their exact roles in cancer.

Unlike IL-1α, IL-1β is not a dual function cytokine and requires cleavage of the “inactive” form before it becomes functional (Werman et al. 2004). IL-1β has been implicated in several types of cancer, including glioma, acute myeloid leukemia and colon cancer (Turzanski et al. 2004; Li et al. 2012b; Wang et al. 2012). The glioma
cell line, LN-229 lacks a CSC phenotype, but following addition of IL-1β and TGF-β increased its “stemness” changing it to a more CSC phenotype. This is of clinical relevance, since increased levels of both IL-1β and TGF-β occur in high grade gliomas with poor clinical outcomes for patients (Wang et al. 2012). Similarly, IL-1β can induce colon CSC self-renewal and increase invasiveness in cooperation with zinc finger E-box binding homeobox 1 (Zeb1) (Li et al. 2012b). IL-1β can also act as via antiapoptosis pathways to maintain the blast cells in acute myeloid leukemia (AML) (Turzanski et al. 2004). In addition, IL-1Rap is overexpressed in chronic myeloid leukemia stem cells with an IL-1Rap antibody able to induce antibody-dependent, cell-mediated cytotoxicity to these cells, but not those expressing low levels of IL-1Rap such as normal bone marrow cells (Askmyr et al. 2013).

6 Interleukin-6

The Interleukin-6 receptor (IL-6R) family consists of receptors composed of receptor chains related to the ligand-specific IL-6Rα or the archetypical GP130 (Boulay et al. 2003). The founding member, IL-6, is a pleiotropic cytokine that utilizes a receptor complex consisting of the ligand specific IL-6Rα and shared GP130 receptor subunits which signals via the downstream JAK2 and STAT3. One of the many roles of IL-6 is the maintenance of stem cells (Ernst et al. 1996; Notara et al. 2010). Therefore tight regulation of IL-6 expression and, in particular, its receptor IL-6Rα is required for normal development and homeostasis. The disruption of normal IL-6Rα expression is often pathogenic, and has been reported to have a role in several cancers, such as breast, ovarian and prostate cancers (Knupfer and Preiss 2007).

Serum IL-6 levels have been identified to correlate with poor prognosis for breast cancer patients (Sansone et al. 2007). One key role for IL-6 in breast cancer is to maintain the cancer stem cell population, with disruption of IL-6 promoter methylation able to increase IL-6 levels and thereby increasing cancer stem cell maintenance (D’Anello et al. 2010). Furthermore, IL-6 is also able to induce EMT in breast cancer cells further enhancing their CSC properties (D’Anello et al. 2010). Breast cancers are a heterogeneous population of cells consisting of breast CSC and also differentiated breast cancer cells with the proportion of breast CSC and breast cancer cells varying depending on the microenvironmental conditions. The proportion of breast CSC to breast cancer cells is maintained by IL-6, a function that has also been observed in a prostate cancer cell line (Iliopoulos et al. 2011). Trastuzumab is a monoclonal antibody inhibitor for the v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2) protein (also known as human EGF receptor 2, HER2), with overexpression of ERBB2 associated with aggressively proliferative breast cancer (Schroeder et al. 2014). Moreover, treatment of breast cancer with trastuzumab often leads to an enrichment of the breast CSC population with increased IL-6 expression. Antibody blockade of IL-6Rα is able to reduce the enrichment of the breast CSC population, leading to decreased tumor growth.
Therefore, combination treatment with a compound targeting non-stem cancer cells as well as a CSC targeting drug, such as Metformin, may be useful in avoiding CSC enrichment, with implications for the ability to successfully ablate the cancer (Hirsch et al. 2009; Iliopoulos et al. 2011).

7 Interleukin-8

Chemokine (C-X-C motif) ligand 8 or interleukin-8 (IL-8) is a proinflammatory chemokine and signals via two cell surface receptors, preferentially CXCR1 but also CXCR2 (Gales et al. 2013). These cell surface 7 transmembrane domain receptors are coupled with α, β and γ G proteins that induce intracellular signaling pathways that mediate cell survival, proliferation, angiogenesis and cell migration (Kobilka 2007), which include PI3K, RAS, JAK/STAT and WNT (Waugh and Wilson 2008). The CXCL8/CXCR1/CXCR2 signaling axis has been implicated in several cancers including melanoma (Singh et al. 2010), kidney (Liang-kuan et al. 2014), breast (Schillace et al. 2014) and pancreatic cancer (Chen et al. 2014).

CXCL8 is expressed by a wide variety of cells, particularly those of the immune system, and atypically by several cancers. Paracrine or autocrine signaling is achieved via CXCR1 (Chen et al. 2014) and CXCR2 (Liu et al. 2011) expressed in breast cancer cells, including CSCs. Indeed, increased CXCL8 serum levels have been shown to increase breast CSC activity, as judged by mammosphere formation (Singh et al. 2013). Similarly, CXCL8 and CXCR1 have been associated with pancreatic CSCs, with expression of CXCR1 correlating with that of pancreatic CSC markers, which has been linked with a lower survival rate due to metastasis of pancreatic cancer cells (Chen et al. 2014). The importance of CXCL8 signaling via CXCR1 has been demonstrated by inhibition of CXCR1, either by repertaxin, a small molecule inhibitor, or by a CXCR1 blocking antibody, which resulted in apoptosis of breast CSC and overall reduction of cancer load (Ginestier et al. 2010). Combined inhibition of CXCL8 and ERBB2, the latter of which is dysregulated in 25% of breast cancers (Slamon et al. 1987), improves treatment in a synergistic manner (Singh et al. 2013).

8 The Central Role of NF-κB Pathway

The NF-κB pathway provides one of the major links between inflammation, CSCs and EMT. Several different stimuli can activate it. These factors include HIF-1α, which is induced in normoxic and cancer cells following stimulation by pro-inflammatory cytokines, such as TNF-α, IL-1β or IL-6 (Lu et al. 2006; Korkaya et al. 2012). Activation of the NF-κB cascade usually results in nuclear translocation and activation of p65 (Fig. 3.3). The p65 protein is a multifunctional transcription factor that elicits its physiologic function by regulating target gene expression upon
NF-κB activation. TNF-α, either from macrophages or the tumor microenvironment up-regulates the canonical NF-κB signaling through activation of IKKβ. Once up-regulated, activation of p65 follows and this protein then translocates to the nucleus where it induces Twist 1 expression thus promoting tumor metastasis via the EMT. This p65-induced EMT is accompanied by an increase in stem cell-like properties (Li et al. 2012a). Following the activation of the NF-κB pathway, several downstream events occur, such as the suppression of apoptosis. As well, the EMT regulators, Snail and Slug, are activated, thus promoting EMT (Korkaya et al. 2012) (Fig. 3.4).

It is interesting to note that NF-κB activation can stimulate different factors, depending on the cell of origin in the tumor. In basal-like breast cancer, NF-κB induces the expression of JAG1, leading to the Notch-dependent expansion of CSCs in a non-cell-autonomous manner (Yamamoto et al. 2013). However, it has also been shown that epigenetic mechanisms are regulated by NF-κB, with IKK-β regulating Lin28B, a RNA binding protein, which sustains the stemness of breast CSCs. This activation of Lin28B decreases Let-7 expression, leading to higher levels of IL-6, thus activating Stat3, and further stimulating NF-κB (Iliopoulos et al. 2011).

During inflammation NF-κB is able to promote the production of ROS, thus damaging DNA in surrounding epithelial cells, though in cancer this effect could cause the relevant mutations required for cancer cell continuation. Once NF-κB is activated

**Fig. 3.4** The diverse roles of NF-κB (Adapted from (Baud and Karin 2009; Prasad et al. 2010; Li et al. 2012a)). A20 zinc finger protein A20 (also known as TNFAIP3), Bcl-2 B-cell lymphoma protein 2, Bcl-XL also known as Bcl-2 like 1, BFL1 also known as Bcl2A1, CDK2 cyclin-dependent kinase 2, c-IAP-2 cellular inhibitor of apoptosis 2, COX2 cyclooxygenase 2, ELAM1 endothelial adhesion molecule 1, FLIP also known as casp8, HIF-1α hypoxia-inducible factor-1α, ICAM1 intracellular adhesion molecule 1, IEX-1L radiation-inducible immediate early gene (also known as IER3), IL interleukin, iNOS inducible nitric oxide synthase, MCP2 monocyte chemoattractant protein 1 (also known as CCL2), MIP2 macrophage inflammatory protein 2, MMP9 matrix metalloproteinase 9, MnSOD manganese superoxide dismutase, TNF tumor necrosis factor, TRAF1/2 TNF receptor-associated factor, uPA urokinase plasminogen activator, VEGF vascular endothelial growth factor, XIAP X-linked inhibitor of apoptosis protein
in gastric epithelial cells, it stimulates the transcription of IL-1, IL-6, IL-8, TNF-α and cyclooxygenase-2 (COX-2), which can further propagate the NF-κB activation response. How does this then lead to cancer progression, as this response to inflammation would be wound-healing, rather than promoting immortality? One suggestion is that some of its immune and inflammation-related target genes are not activated (Karin et al. 2002). It is also possible that mutations arise in the genes encoding the NF-κB/IκB family members that allows oncogenes to activate NF-κB (Karin et al. 2002; Karin 2009). One recent study investigated the regulation of the NF-κB pathway by miRNAs. This study found that in epithelial ovarian cells, Twist1 negatively regulated NF-κB dependent cytokine production (Yin et al. 2010). This seems counterintuitive as NF-κB increases Twist1 expression. However, this process is mediated by the microRNA, miR-199a which is frequently down-regulated during cancer progression (Fornari et al. 2010; Yin et al. 2010). Yin and colleagues went on to show, through knock-down experiments, that while Twist could influence IKKβ levels, TNF-α was required to induce RANTES production (Yin et al. 2010).

### 9 Therapeutic Prospects

While multiple factors are known to contribute to cancer formation and the cancer stem cell phenotype, cytokines are becoming recognized as one of the most viable targets for cancer therapeutics (Table 3.1). Given the positive feedback loops that drive CSC renewal, agents that can inhibit inflammatory cytokines or block inflammatory signaling pathways could potentially target and eradicate the CSC population. While there have been a number of disappointments, there have been a few successes.

There has been promising pre-clinical data from TGF-β-based therapeutics, the majority of these therapeutics caused harmful off-target effects which have prevented further clinical development (Perrot et al. 2013). TGF-β signaling begins when a ligand binds, and a type II receptor (TβRII) recruits and phosphorylates a type I receptor (TβRI). This TβRI is also known as an activin receptor-like kinase (ALK) and there are seven known type I ALK receptors, though ALK5 is the most specific for TGF-β (Mori et al. 2004). Targeting of the downstream ALK5 induced a range of toxicities, such as heart valve, hemorrhagic, degenerative and inflammatory lesions due to incomplete specificity. Galunisertib (LY2157299), developed by Eli Lilly, demonstrates cardiovascular toxicities (Gueorguieva et al. 2014), but has been used in several studies investigating whether it can sensitize CSCs to chemotherapy (Connolly et al. 2012; Perrot et al. 2013). It is also possible that this drug can be added to current therapeutic strategies to augment and enhance treatment regimens (Bhola et al. 2013) (Table 3.2).

Other options for targeting TGF-β include antisense oligonucleotides and monoclonal antibodies. Trabedersen (AP12009) is an antisense oligonucleotide that targets TGF-β2 mRNA (Schlingensiepen et al. 2011) which showed promise in phase I and phase II trials (Joseph et al. 2013). Efforts are being made to develop it...
as a drug for systemic delivery rather than the prior intra-cranial infusion (NCT00761280). Fresolimumab (GC1008) is a human anti-TGF-β monoclonal antibody that has been investigated for the treatment of advanced malignant melanoma and renal cell carcinoma. In initial phase I trials, no dose-limiting toxicities were observed and preliminary evidence of anti-tumor activity was seen in 25% of patients (Morris et al. 2014). Further studies are now being initiated for the treatment of metastatic breast cancer (NCT01401062).

TNF-α inhibition has fallen far short of expectations due to its high systemic toxicity (Burton and Libutti 2009). Clearly, care must be taken, as seen with the initial trials of TNF-α administration where systemic delivery was associated with severe toxicity and no, or limited, therapeutic effect (Mukaida et al. 2011). However, recent studies using an anti-TNF-α monoclonal antibody, infliximab, have shown disease stabilization in patients with advanced cancers (Harrison et al. 2007, Brown et al. 2008). Etanercept, a soluble TNFR2 fusion protein that binds and neutralizes TNF-α, was also found to stabilize disease in a minority of ovarian cancer patients (Balkwill 2009). There are currently ongoing clinical trials on both of these agents.

Due to the roles that interleukins play in other health and disease states, including inflammation, caution has also been exercised in the use of agents targeting IL-1, IL-6 and IL-8 in cancer. However, because of their role in other diseases, a wealth of information has been garnered from previous clinical trials. IL-1β has proven a successful therapeutic target in septic shock and rheumatoid arthritis, with the recombinant IL-1 receptor antagonist, Anakinra, having a remarkable safety record (Dinarello 2010). Clinical trials have been conducted with Anakinra in a

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### Table 3.1 Selection of therapeutic strategies for targeting cytokine networks

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<tr>
<th>Name</th>
<th>Target</th>
<th>Type</th>
<th>Clinical trials</th>
<th>References</th>
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<td>Galunisertib</td>
<td>Transforming growth factor receptor</td>
<td>Small molecule drug</td>
<td>III</td>
<td>Gueorguieva et al. (2014)</td>
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<td>Trabedersen</td>
<td>Transforming growth factor-beta 2</td>
<td>Antisense oligonucleotide</td>
<td>III</td>
<td>Joseph et al. (2013)</td>
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<td>Fresolimumab</td>
<td>Transforming growth factor-beta</td>
<td>Monoclonal antibody</td>
<td>II</td>
<td>Morris et al. (2014)</td>
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<td>Infliximab</td>
<td>Tumor necrosis factor-alpha</td>
<td>Monoclonal antibody</td>
<td>III</td>
<td>Harrison et al. (2007), Brown et al. (2008)</td>
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<tr>
<td>Etanercept</td>
<td>Tumor necrosis factor-alpha</td>
<td>Neutralizing protein</td>
<td>III</td>
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<td>Interleukin-1 receptor accessory protein</td>
<td>Monoclonal antibody</td>
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<td>Askmyr et al. (2013)</td>
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<td>Siltuximab</td>
<td>Interleukin-6</td>
<td>Monoclonal antibody</td>
<td>III</td>
<td>Jones et al. (2011)</td>
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<td>CXCR1</td>
<td>Small molecule drug</td>
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<td>Natural product</td>
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<td>DTP3</td>
<td>NF-κB (GADD45β/ MKK7)</td>
<td>Small molecule drug</td>
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Table 3.2 Clinical trials involving LY2157299, an inhibitor of ALK5, lying downstream of TGF-β

<table>
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<th>Study completion date</th>
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<td>NCT01722825 phase I</td>
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<td>A study of LY2157299 in Japanese participants with cancer</td>
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<tr>
<td>NCT01682187 phase I</td>
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<td>NCT01582269 phase II</td>
<td>Recurrent glioblastoma</td>
<td>A study in recurrent glioblastoma</td>
<td>Lomustine</td>
<td>December 2014</td>
</tr>
<tr>
<td>NCT01373164 phase Ib/Ii</td>
<td>Pancreatic cancer</td>
<td>A study in metastatic cancer and advanced or metastatic unresectable pancreatic cancer</td>
<td>Gemcitabine</td>
<td>March 2015</td>
</tr>
<tr>
<td>NCT02154646 phase Ib</td>
<td>Pancreatic cancer</td>
<td>A study of LY2157299 in participants with pancreatic cancer that is advanced or has spread to another part of the body</td>
<td>Gemcitabine</td>
<td>February 2015</td>
</tr>
<tr>
<td>NCT02240433 phase Ib</td>
<td>Hepatocellular cancer</td>
<td>A study of LY2157299 in participants with unresectable hepatocellular cancer</td>
<td>Sorafenib</td>
<td>February 2016</td>
</tr>
<tr>
<td>NCT01220271 phase Ib/Ii</td>
<td>Malignant glioma</td>
<td>A study combining LY2157299 with temozolomide-based radiochemotherapy in patients with newly diagnosed malignant glioma</td>
<td>Temozolomide</td>
<td>January 2016</td>
</tr>
<tr>
<td>NCT02008318 phase I/III</td>
<td>Myelodysplastic syndromes</td>
<td>A study of LY2157299 in participants with myelodysplastic syndromes</td>
<td></td>
<td>April 2016</td>
</tr>
<tr>
<td>NCT01246986 phase II</td>
<td>Hepatocellular carcinoma</td>
<td>A study of LY2157299 in participants with hepatocellular carcinoma</td>
<td>Sorafenib</td>
<td>October 2016</td>
</tr>
<tr>
<td>NCT02178358 phase II</td>
<td>Hepatocellular carcinoma</td>
<td>A study of LY2157299 in participants with advanced hepatocellular carcinoma</td>
<td>Sorafenib</td>
<td>December 2016</td>
</tr>
</tbody>
</table>
number of malignant disorders. One trial combined Anakinra with Dexamethasone to treat patients with smoldering or indolent multiple myeloma (NCT00635154), 1 patient achieved a complete response with Anakinra alone (n = 54), and when combined with desamethasone, 14 patients achieved a response to treatment (n = 29). Additionally, 49 patients out of the 54 were still alive and progression-free at 6 months, with a median duration of response recorded as 41.9 months, indicating some effectiveness. There are currently five other clinical trials currently recruiting to assess the efficacy of Anakinra in combination with other chemotherapeutic agents. However, as IL-1 plays a role in angiogenesis, care must be taken to develop appropriate clinical endpoints.

IL-6 targeting therapeutics have also been trialed in a number of malignant disorders, and have typically utilised monoclonal antibodies. CNTO 328 is a humanized monoclonal antibody (Guo et al. 2012). However, results have been limited. One study in patients with non-hormone responsive metastatic prostate cancer (NCT00433446) was not completed due to significant disease progression. Another clinical trial assessing the efficacy of CNTO 328 in myelodysplastic patients was terminated due to a lack of sufficient efficacy (NCT01513317). There is currently only one trial active, which will assess the efficacy of CNTO 328 in patients with high-risk smoldering multiple myeloma (NCT01484275). However, some efficacy has been observed in patients with renal cell carcinoma and ovarian cancer (Guo et al. 2012), suggesting it may be an effective therapeutic in some cancers, either as single or in combination therapy.

The use of IL-8 targeted therapies has been investigated in inflammatory diseases but to date, there is limited information on the efficacy in cancer. In vitro and in vivo studies have demonstrated inhibitory effects on tumor growth, angiogenesis and tumor dissemination, but these results have not be exploited in clinical trials (Skov et al. 2008). There is currently one pilot study investigating the safety profile in early breast cancer patients (NCT01861054) and an ongoing study to evaluate reparixin with weekly paclitaxel in patients with HER2-negative metastatic breast cancer (NCT02001974). Again, while safety has not proven to be an issue to date, only time will tell whether this therapeutic strategy is efficacious.

The NF-κB pathway is an intriguing transcription factor to target. This is because non-steroidal anti-inflammatory drugs, such as aspirin and salicylates, have been shown to be effective at inhibiting NF-κB activation (Karin et al. 2004). Indeed, cancer rates in aspirin users has been shown to be far reduced compared to the normal population (Cuzick et al. 2009). The most commonly accepted mechanism by which this is thought to occur is via inhibition of COX and thus prostaglandin production, although a prostaglandin inhibitor, indomethacin, failed to elicit an effect on NF-κB. Aspirin and salicylates do, however, inhibit some NF-κBs target genes (Karin et al. 2004). One of the issues with targeting the NF-κB is its role in inflammation, and thus any therapeutic should be transient so as not to cause immunosuppression (Baud and Karin 2009). Additionally, care must be taken so as to not enhance the production of IL-1β and related cytokines during bacterial infections, which has been a surprising side-effect of NF-κB inhibition (Greten et al. 2007; Baud and Karin 2009). While there are problems with targeting this pathway, there are a few drugs that have entered or are about to enter clinical trials.
Curcumin, derived from turmeric, is a natural product that has been shown to block IKK activation (Hussain et al. 2008). It has been well tolerated and there have been no associated toxicities. While there have been concern over the absorption of curcumin, bioavailability has been demonstrated in pancreatic cancer patients, although there remains no clinical trial on the effectiveness of this drug as a cancer therapeutic. Curcumin has been shown to decrease the levels of TNF-α, NF-κB, IL-6, IL-8, IL-10 and COX-2 in colorectal and pancreatic cancer, and multiple myeloma (Gupta et al. 2013) and may be beneficial given alongside other chemotherapeutics. Clinical trials are shortly to start investigating the potential of curcumin to prevent chemotherapy-induced fatigue in breast cancer patients about to receive radiotherapy (NCT01740323).

One approach that has been taken by scientists at Imperial College London was to investigate target genes downstream of NF-κB in an attempt to avoid some of the serious toxic side effects associated with other NF-κB targeted therapies. Tornatore and colleagues identified a protein complex, GADD45β/MKK7, that appeared to play a critical role in allowing cancer cells to survive. Using high-throughput screening, the investigators found two molecules that disrupted this protein complex with no toxicity to normal cells. This new drug, DTP3, will be entering clinical trials late in 2015 for the treatment of multiple myeloma (Tornatore et al. 2014).

10 Conclusion

Multiple factors in the tumor microenvironment contribute to the alteration of tumor cell function and behavior, including the transition to CSCs. CSCs represent a very fluid population of cells within the tumor mass that are reactive to environmental cues including cytokines secreted from both cancer cells and various tumor associated cell populations. Collectively, this microenvironment strongly influences progression of the tumor, with the influx of immune cells and their involvement in both paracrine and autocrine signaling directly contributing to metastatic disease. As a result, a new influx of therapeutics targeting these cytokine networks are being trialed, with some positive results being achieved. However, understanding of intricate details of gene regulation, such as via microRNAs and epigenetic changes, and how these impact remain largely unknown. More studies are required to further delineate these pathways to elucidate how cytokines that play a role in our immune response can be safely targeted to effectively eradicate cancer stem cells.

References


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Li Y, Wang L, Pappan L, Galliher-Beckley A, Shi J (2012b) IL-1b promotes stemness and invasiveness of colon cancer cells through Zeb1 activation. Mol Cancer 11:87


