

## REVIEW

# Interleukins in glioblastoma pathophysiology: implications for therapy

YT Yeung<sup>1</sup>, KL McDonald<sup>2</sup>, T Grewal<sup>1</sup> and L Munoz<sup>3</sup>

<sup>1</sup>Faculty of Pharmacy, University of Sydney, Sydney, NSW, Australia, <sup>2</sup>Cure For Life Neuro-Oncology Group, Prince of Wales Clinical School, Lowy Cancer Research Centre, University of New South Wales, Sydney, NSW, Australia, and <sup>3</sup>School of Medical Sciences, Discipline of Pharmacology, University of Sydney, Sydney, NSW, Australia

**Correspondence**

Dr Lenka Munoz, School of Medical Sciences/Pharmacology, University of Sydney, Sydney, NSW 2006 Australia. E-mail: lenka.munoz@sydney.edu.au

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Despite considerable amount of research, the poor prognosis of patients diagnosed with glioblastoma multiforme (GBM) critically needs new drug development to improve clinical outcomes. The development of an inflammatory microenvironment has long been considered important in the initiation and progression of glioblastoma; however, the success of developing therapeutic approaches to target inflammation for GBM therapy has yet been limited. Here, we summarize the accumulating evidence supporting a role for inflammation in the pathogenesis of glioblastoma, discuss anti-inflammatory targets that could be relevant for GBM treatment and provide a perspective on the challenges faced in the development of drugs that target GBM inflammation. In particular, we will review the function of IL-1 $\beta$ , IL-6 and IL-8 as well as the potential of kinase inhibitors targeting key players in inflammatory cell signalling cascades such as JAK, JNK and p38 MAPK.

**Abbreviations**

AP, activating protein; BBB, blood–brain barrier; cEBP, CCAAT-enhancer binding protein; GSC, glioblastoma stem cell; GBM, glioblastoma multiforme; HIF, hypoxia inducible factor; HuR, human antigen R; ID, inhibitor of differentiation; IDH, isocitrate dehydrogenase; IR, irradiation; LIF, leukaemia inducible factor; MGMT, O<sup>6</sup>-methylguanine-DNA methyltransferase; MEK, MAPK kinase; MKK, MAPK kinase; Mnk, MAPK-interacting kinase; PTEN, phosphatase and tensin homologue; SASP, senescence associated secretory phenotype; TMZ, temozolomide; TF, tissue factor; TTP, tristetraprolin

**Introduction**

Glioblastoma multiforme (GBM) is the most common and lethal primary malignant cancer of the CNS. The GBM tumour is characterized by extensive heterogeneity, as implicated in its full name (*multiforme: having or occurring in many forms*). From the cellular point of view, GBM harbour multiple cell types, some with increased tumorigenicity and stem-like capacity, which are thought to be responsible for tumour relapse. However, the tumour-associated parenchymal cells – such as vascular cells, microglia, peripheral immune cells and neural precursor cells – also play a vital role in controlling the course of pathology. From a molecular standpoint, GBM heterogeneity is evidenced by the presence of various site- or

tumour-specific mutations. For example, O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) promoter hypermethylation, which is indicative of GBM response to the chemotherapy with the DNA-alkylating agent temozolomide (TMZ), is detected in some but not all GBM tumours, or only in distinct areas of hypermethylation-positive cells surrounded by cells lacking any sign of increased MGMT activity within the same tumour (Von Deimling *et al.*, 2011). Other oncogenic markers not uniformly distributed in GBMs include overexpression of the EGFR mutant EGFRvIII (also known as  $\Delta$ EGFR) or loss of phosphatase and tensin homolog (PTEN), resulting in differential activation/silencing of multiple signalling pathways (The Cancer Genome Atlas Research Network, 2008). The complex network of these cellular and

**Table 1**

Expression and function of interleukins in glioblastoma

Cytokine	Expression	Function
IL-1 $\beta$	GBM clinical samples (Sharma <i>et al.</i> , 2011b; Yeung <i>et al.</i> , 2012) GBM cell lines (Yamanaka <i>et al.</i> , 1994)	$\uparrow$ ERK activity $\uparrow$ proliferation (Meini <i>et al.</i> , 2008) $\uparrow$ p38 MAPK/JNK activity $\uparrow$ VEGF (Yoshino <i>et al.</i> , 2006) $\uparrow$ JNK and sphingosine kinase-1 activity $\uparrow$ proliferation and invasiveness (Paugh <i>et al.</i> , 2009) $\uparrow$ p38 MAPK activity $\uparrow$ IL-6 (Tanabe <i>et al.</i> , 2011; Yeung <i>et al.</i> , 2012) $\uparrow$ stemness factor genes $\uparrow$ invasiveness $\uparrow$ drug resistance (Wang <i>et al.</i> , 2012)
IL-6	GBM clinical samples (Tchirkov <i>et al.</i> , 2001; 2007; Kudo <i>et al.</i> , 2009; Inda <i>et al.</i> , 2010; Yeung <i>et al.</i> , 2012) GBM cell lines (Yamanaka <i>et al.</i> , 1994) Plasma of GBM patients (Reynes <i>et al.</i> , 2011)	IL-6-deficient mice failed to develop GBM (Weissenberger <i>et al.</i> , 2004) $\uparrow$ JAK2-STAT3 activation $\uparrow$ migration and invasiveness (Liu <i>et al.</i> , 2010; Senft <i>et al.</i> , 2011; Zhang <i>et al.</i> , 2012) $\uparrow$ JAK2-STAT3 activation $\uparrow$ proliferation $\downarrow$ apoptosis (Rahaman <i>et al.</i> , 2002) $\uparrow$ JAK2-STAT3 and Jagged-Notch activation $\uparrow$ stemness $\uparrow$ tumour heterogeneity and formation (Jin <i>et al.</i> , 2012) $\uparrow$ tumour heterogeneity (Inda <i>et al.</i> , 2010)
IL-8	GBM clinical samples (de la Iglesia <i>et al.</i> , 2008b; Hong <i>et al.</i> , 2009; Bonavia <i>et al.</i> , 2011) GBM cell lines (Yamanaka <i>et al.</i> , 1994)	$\uparrow$ proliferation and invasiveness (de la Iglesia <i>et al.</i> , 2008a) $\uparrow$ angiogenesis and tumour growth (Bonavia <i>et al.</i> , 2011) $\uparrow$ tumour growth in an autocrine manner (Sun <i>et al.</i> , 2011) $\uparrow$ invasiveness (Wakabayashi <i>et al.</i> , 2004)

molecular differences is believed to confer GBM with unpredictable and often very poor response to therapeutic interventions. The median survival of GBM patients is 15 months, and the overall 5 year survival remains extremely low at approximately 5% (Stupp *et al.*, 2005). Little improvement in overall or progression-free survival has been achieved in the past five decades, reflecting an unmet medical need in the treatment of this cancer.

The deadly nature of GBMs resides in their capacity to diffusely infiltrate throughout the brain tissue, their extreme growth characteristics and their intrinsic resistance to current therapies. The ability of GBM cells to invade healthy brain tissue is critical for the tumour recurrence that accounts for the fatal outcome of the disease. Invading cells, besides seeding micro-tumours, are refractory to most lines of treatment, probably because they transiently arrest from mitosis (Lefranc *et al.*, 2005; Kislin *et al.*, 2009). The identification of glioblastoma stem cells (GSCs) within the tumour (Galli *et al.*, 2004; Singh *et al.*, 2004) had further implications for understanding its resistance to treatment. GSCs are highly invasive and proliferative cells (Cheng *et al.*, 2011), and the standard therapy targets predominantly non-stem cells, while sparing the small population of GSCs (Huang *et al.*, 2010; Beier *et al.*, 2011). Recently, a quiescent subset of endogenous glioblastoma cells has been identified. These cells have properties similar for cancer stem cells and sustain long-term tumour growth through the production of highly proliferative cells (Chen *et al.*, 2012). Finally, the inflammatory microenvironment, which is known to be the driving force for the progression of incipient neoplasias into highly malignant tumours (Mantovani *et al.*, 2008; Solinas *et al.*, 2010), is also found at the GBM lesions. Consistent with its role in other malignancies, inflammatory cytokines greatly enhance the proliferation, invasiveness and/or stemness of GBM cells and thus actively contribute to the global phenotype of GBM.

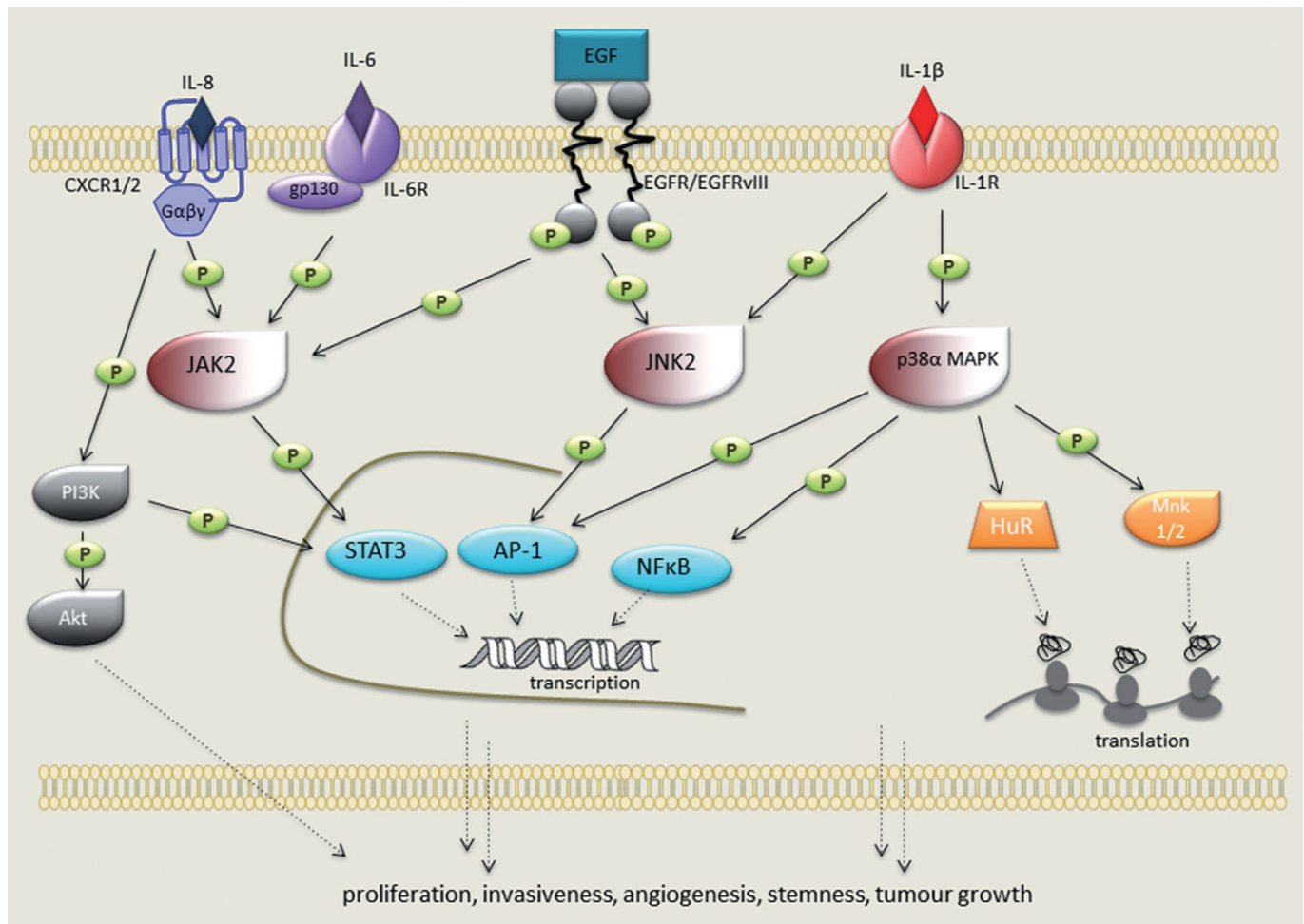
Glioblastomas are surrounded by a pool of pro-inflammatory cytokines, chemokines and growth factors.

Here, we particularly review the tumourigenic effects of inflammatory IL-1 $\beta$ , IL-6 and IL-8 in GBM pathophysiology. Significant up-regulation of these cytokines is found in GBM cell lines as well as in patient samples (Table 1); and some of these interleukins (ILs) have prognostic potential. For example, IL-6 gene amplification in GBM tissue correlates with GBM aggressiveness and decreased patient survival (Rolhion *et al.*, 2001; Tchirkov *et al.*, 2007). A number of cell culture and xenograft studies support the hypothesis that targeting these inflammatory mediators could be beneficial in controlling the insidious behaviour of GBM. Thus, anti-inflammatory agents in conjunction with cytotoxic agents could offer a new and possibly improved approach to GBM therapy. We will therefore also discuss molecular therapeutics that could be used to target the production and activity of these ILs, focusing on inhibitors of signalling cascades activated by IL-1 $\beta$ , IL-6 and IL-8, namely the JAK, p38 MAPK and JNK pathways.

## Role of ILs in GBM pathophysiology

### IL-1 $\beta$

IL-1 $\beta$  is a master pro-inflammatory cytokine that triggers a number of malignant processes by activating various cells to up-regulate key molecules that drive oncogenic events. Elevated levels of IL-1 $\beta$  were observed in a panel of GBM cell lines, including CCF3 and U87MG (Lu *et al.*, 2007), and in human GBM tumour specimens (Sharma *et al.*, 2011b; Yeung *et al.*, 2012). IL-1 $\beta$  receptor (IL-1R) is found in GBM cells and tissues (Sasaki *et al.*, 1998), and we observed increased IL-1R levels in U87MG cells stably overexpressing the EGFRvIII variant (unpubl. data). IL-1 $\beta$  binding to the IL-1R resulted in the activation of NF- $\kappa$ B and MAPK signalling pathways, which cooperatively induced expression of various target genes (Griffin and Moynagh, 2006; McCulloch *et al.*, 2006). IL-1 $\beta$ -induced ERK activation had mitogenic effects on



**Figure 1**

Simplified overview of inflammation-orchestrated signal transduction that induces pro-tumourigenic responses in glioblastoma. GBM tissue and microenvironment contains high levels of IL-1 $\beta$ , IL-6 and IL-8. Binding of IL-6 and IL-8 to IL-6/gp130 complex and GPCRs (CXCR1/2), respectively, leads to the activation (phosphorylation) of JAK2 that stimulates rapid, transient activation of STAT3 transcription factor. PI3K–Akt pathway is also a downstream target of CXCR1/2. IL-1 $\beta$  via its receptor IL-1R is a well-known p38 MAPK and JNK activating cytokine. Upon activation, p38 MAPK and JNK induce the activation of key oncogenic transcription factors NF $\kappa$ B and AP-1 or post-transcriptional gene regulation. This includes p38 MAPK-dependent HuR-shuttling and Mnk1/2 activation, leading to the control of mRNA stability and protein translation. Via these mechanisms, inflammation-activated JAK2, p38 MAPK and JNK signalling pathways are linked to proliferation, invasiveness, angiogenesis, stemness and tumour growth in GBM pathophysiology. Furthermore, JAK2 and JNK2 are also activated by EGFR and constitutively active EGFRvIII. Persistent activation JAK2 and JNK2 pathways via these growth factor receptors possibly contributes to the inflammation-driven GBM progression.

human U373MG cells and significantly increased GBM cell proliferation (Meini *et al.*, 2008). IL-1 $\beta$ -dependent activation of NF- $\kappa$ B, p38 MAPK and JNKs pathways in GBM cells (Figure 1) also led to the up-regulation of VEGF and sphingosine kinase 1, which promoted angiogenesis, migration and invasion respectively (Yoshino *et al.*, 2006; Paugh *et al.*, 2009). Furthermore, IL-1 $\beta$ -mediated up-regulation of hypoxia-inducible factor-1 (Sharma *et al.*, 2011b) mediated molecular responses to hypoxia, which is a crucial component in GBM progression. In addition, GBM cells responded to IL-1 $\beta$  with an exacerbation of the inflammatory environment by secreting high levels of IL-6 and IL-8 (Spooren *et al.*, 2011; Tanabe *et al.*, 2011; Yeung *et al.*, 2012) or up-regulation of COX-2 (Sharma *et al.*, 2011a), an inflammatory enzyme detected in brain tumours and associated with poor prognosis

(Xu and Shu, 2007; Myung *et al.*, 2010). The recent finding that GBM cells without self-renewal capacity can gain this ability after exposure to IL-1 $\beta$  and TGF- $\beta$  further adds to the detrimental role of IL-1 $\beta$  in the tumour microenvironment. In the GBM cell line LN-229, which did not form neurospheres and generated only small tumours *in vivo*, IL-1 $\beta$  in combination with TGF- $\beta$  induced up-regulation of stemness factor genes, increased invasiveness and drug resistance that resulted in amplified tumour growth *in vivo* (Wang *et al.*, 2012). Hence, selectively blocking IL-1 $\beta$  production and/or activity could contribute to control GBM progression.

### IL-6

IL-6 is produced by malignant cells as a response to external stimuli or intrinsic factors, such as oncogenic mutations. For

example, the master pro-inflammatory cytokines IL-1 $\beta$  or TNF- $\alpha$  activate various signalling pathways resulting in stabilization of IL-6 mRNA and increased IL-6 biosynthesis (Tanabe *et al.*, 2010; Spooren *et al.*, 2011; Yeung *et al.*, 2012). Furthermore, as discussed in more detail below, the EGFRvIII variant can induce IL-6 biosynthesis and secretion into the tumour microenvironment (Inda *et al.*, 2010; Jin *et al.*, 2011). The crucial role of IL-6 in glioblastoma development was convincingly demonstrated by Weissenberger *et al.* (2004), who showed that transgenic mice expressing the src oncogene in astrocytes failed to develop GBM on an IL-6-deficient background. Recent studies investigating IL-6 function in GBM progression further improve our understanding on how proliferation, differentiation and invasiveness require IL-6 signalling. Canonical IL-6 signal transduction is initiated by IL-6 binding to heteromeric plasma membrane receptor complexes formed by the IL-6 receptor (IL-6R) and the common signal transducing receptor glycoprotein 130 (gp130). Importantly, IL-6R and gp130 were expressed in GBM tissues and GBM-derived primary cells, including stem cells (Krona *et al.*, 2005; Wang *et al.*, 2009), underscoring that crucial components of this IL-6 circuitry are associated with the tumour cells. Alternatively, IL-6 trans-signalling is triggered by interaction of IL-6 with the soluble form of IL-6R $\alpha$  and subsequent binding of this complex to the membrane-bound gp-130. In both pathways, upon receptor activation, intracellular signalling is propagated by JAK family members (JAK1-3), leading to activation of STAT transcription factors, particularly STAT3 (Figure 1). Indeed, activated STAT3 was found in GBM patients when compared with lower-grade brain tumours (Rahaman *et al.*, 2002; Weissenberger *et al.*, 2004; Abou-Ghazal *et al.*, 2008; Lo *et al.*, 2008; de Groot *et al.*, 2010).

IL-6-induced activation of STAT3 promoted invasion and migration in U251, T98G and U87MG glioblastoma cells (Liu *et al.*, 2010) and correlated with increased expression and secretion of MMP-2, a protease implicated in GBM motility (Li *et al.*, 2010). Furthermore, IL-6 derived from neighbouring cells, in particular microglia, strongly stimulated GBM cell invasion (Zhang *et al.*, 2012). This is in line with data showing that STAT3 inhibition attenuated the invasive and migratory potential of GBM cells (Senft *et al.*, 2011; Michaud-Levesque *et al.*, 2012). Interestingly, IL-6 depleted U87MG cells grafted on the chick chorioallantoic membrane formed a reduced primary tumour (volume <2% compared with negative controls), although a significant number of infiltrated malignant cell clusters was still detected, indicating that sole targeting of IL-6 production might be insufficient to completely block GBM invasiveness. In fact, invasion of GBM cells in this model was reversed upon combination of IL-6 depletion with the administration of VEGF monoclonal antibody bevacizumab (Avastin; Saidi *et al.*, 2009). Despite its potent anti-angiogenic activities, in the model developed by de Groot *et al.* (2010), bevacizumab promoted GBM invasiveness, suggesting that combined inhibition of IL-6 and VEGF might be more efficient in reducing invasiveness and tumour growth.

IL-6-mediated STAT3 activation fostered proliferation and inhibits apoptosis of GBM cells (Rahaman *et al.*, 2002), including GBM stem cells where STAT3 was identified as a downstream mediator of pro-survival IL-6 signalling. When comparing non-stem and stem cells in human GBM tissues,

GSCs expressed higher IL-6R levels, whereas IL-6 ligand levels were higher in non-stem cells. Knock-down of IL-6R or IL-6 significantly reduced growth and neurosphere formation capacity while increasing apoptosis. Targeting IL-6R or its ligand in GSCs thus increased the survival of mice bearing the intracranial human GBM xenografts (Wang *et al.*, 2009). In support of IL-6 promoting stemness of GBM cells, Jin *et al.* (2012) showed that IFN regulatory factor 7-derived IL-6 played a pivotal role in maintaining GBM stem cell properties via JAK/STAT mediated activation of the Jagged–Notch signalling pathway. The same group reported that IL-6–Jagged–Notch cascades conferred GSCs-like features and that IL-6 accelerated formation of GBMs *in vivo*. Furthermore, tumours derived from U87MG cells overexpressing IL-6 were positive for markers of neural stem cells, oligodendrocytes, neurons and astrocytes, as well as endothelial cells, thus showing tumour heterogeneity. In sharp contrast, tumours derived from IL-6-depleted cells were homogenous (Jin *et al.*, 2011). This study confirms and expands the findings from Inda *et al.* (2010) revealing a new tumourigenic function for IL-6. Their study identified IL-6 derived from EGFRvIII-positive GBM cells as a growth factor for GBM cells carrying wild-type EGFR and described IL-6 as a messenger that enhances GBM cellular heterogeneity and tumour growth.

## IL-8

IL-8 is highly expressed and secreted from GBM cell lines, stem cells and human specimens (Table 1). Similar to the regulation of IL-6, IL-8 expression was enhanced by TNF- $\alpha$ , IL-1 $\beta$  or macrophage infiltration (Hong *et al.*, 2009; Yeung *et al.*, 2012). In particular, TNF- $\alpha$  dramatically increased IL-8 synthesis in GBM cells through IL-8 mRNA stabilization (Nabors *et al.*, 2003). In GBM pathophysiology, IL-8 is known as a potent angiogenic factor (Brat *et al.*, 2005). Furthermore, the oncogenic EGFRvIII mutant and loss of tumour suppressor PTEN were linked to significantly higher IL-8 expression levels, which correlated with increased proliferation, neovascularization, invasiveness and *in vivo* tumour growth (de la Iglesia *et al.*, 2008a; Bonavia *et al.*, 2011). IL-8 also acted as an inflammatory chemoattractant for GBM cells promoting their invasiveness (Raychaudhuri and Vogelbaum, 2011; Wakabayashi *et al.*, 2004). Moreover, it is secreted by tumour cells to promote growth in an autocrine manner (Wakabayashi *et al.*, 2004; Sun *et al.*, 2011). It is believed that biological effects of IL-8 are mediated by two highly related G-protein coupled chemokine receptors CXCR1 and CXCR2. However, only CXCR1 has yet been found to be expressed in GBM cell lines (Raychaudhuri and Vogelbaum, 2011). PI3K is one of the main downstream targets of CXCR1/2; with Raf-MAPK/ERK kinase (MEK)–ERK, p38 MAPK and JAK2–STAT3 (Figure 1) also being activated (Waugh and Wilson, 2008).

## Underlying molecular mechanism of inflammation in GBM

The pro-tumourigenic activities of inflammatory mediators, in particular the IL-1 $\beta$ , IL-6 and IL-8, are well established, pointing at the need to better understand the signalling mechanisms that underlie their production and activities so that new therapies can be designed.



At present, several models of inflammatory events contributing to the development of cancer exist. First, repetitive injury or infections resulting in a chronic inflammatory response can eventually lead to cancer. Well-known examples for this pathway are *Helicobacter pylori* infection and the development of gastric cancer or hepatitis virus causing hepatocellular carcinoma (Mantovani *et al.*, 2008). Second, cancers that evolve without underlying chronic inflammation can also exhibit tumour-associated inflammation. This inflammatory microenvironment is typically generated by tumour-associated macrophages, which significantly contribute to the tumour mass. Third, malignant cells and oncogenes, such as Ras and Myc, are also potent producers of inflammatory mediators (Sparmann and Bar-Sagi, 2004; Ancrile *et al.*, 2007). Fourth, cell senescence induced by DNA damage enhances the transcription of pro-inflammatory cytokines and the role of senescence-associated secretory phenotype (SASP) in cancer is increasingly being investigated (Rodier *et al.*, 2009). Fifth, chemotherapy or radiotherapy-induced cell death is often necrotic in nature and accompanied by release of inflammatory cytokines. In the healthy individual, the cytokine milieu ensures that primarily humoral immune responses are generated in order to prevent damage due to inflammation (Becker, 2006). However, in cancer patients, these normal humoral responses are skewed and immunosuppressive cytokines (e.g. TGF- $\beta$ ; Barcellos-Hoff *et al.*, 2009) or pro-inflammatory cytokines IL-6 (Pasi *et al.*, 2010) are predominantly expressed. These cytokines then activate oncogenic transcription factors in the remaining cancer cells and stimulate their survival and proliferation.

In GBM pathophysiology, the link between predisposing inflammation and GBM development has not been established; thus, glioblastoma is probably not an inflammation-induced cancer. The inflammation in GBM appears to be driven by oncogenic mutations as well as changes in the microenvironment and is best described as cancer-induced inflammation. Importantly, the evolving inflammation is a response not only to the disease but also to the therapy.

## Oncogene- and tumour suppressor-induced inflammation

This intrinsic pathway of cancer-related inflammation is initiated and orchestrated by the tumour cells themselves without stimulation by prior inflammation. For example, oncogenic H-RasG12V-mediated production of pro-inflammatory cytokines IL-6, IL-8 and growth-regulated alpha protein (GRO1, also known as CXCL1) had emerged as an important contributor to the induction of an inflammatory microenvironment in cancer (Sparmann and Bar-Sagi, 2004; Ancrile *et al.*, 2007). The Ras-dependent up-regulation of IL-6 or IL-8 is predominantly mediated by the activation of signalling pathways that impinge on the transcriptional machinery controlling their expression. Oncogenic Ras is not common in GBM pathophysiology; however, the overexpression of EGFR and/or its mutation EGFRvIII, leading to deregulation of EGFR effector pathways, including Ras and PI3K/Akt, is a hallmark of approximately 88% GBMs (The Cancer Genome Atlas Research Network, 2008). Indeed, the EGFRvIII

mutation in GBM tissues, cell lines and glioblastoma stem cells was associated with strong induction and secretion of IL-6 and related leukaemia inhibitory factor (LIF; Inda *et al.*, 2010). The underlying mechanism appeared to involve EGFR- or EGFRvIII-mediated activation of the Akt-Smad5 pathway, which in turn promoted up-regulation of inhibitor of the differentiation 3 (ID3) protein. EGFRvIII- and EGFR-driven ID3 induction in GBM cells then up-regulated expression of IL-6 and significantly enhanced angiogenesis and neurosphere formation. Pharmacological inhibition utilizing EGFR inhibitors or sh-RNA targeting ID3 confirmed ID3 as the major regulator of EGFR-driven IL-6 expression (Jin *et al.*, 2011).

Several pathways have been identified that facilitate IL-8 up-regulation via EGFRvIII in GBM. For example, EGFRvIII enhanced IL-8 expression through activation of NF- $\kappa$ B, activating protein-1 (AP-1) and CCAAT-enhancer-binding protein (cEBP) transcription factors. Selective pharmacological or genetic targeting of NF- $\kappa$ B or AP-1 pathways blocked IL-8 promoter activity and cytokine secretion (Bonavia *et al.*, 2011). EGFRvIII-dependent GBM cell transformation was also associated with simultaneous overexpression of tissue factor (TF) and its receptors PAR1/2. Consequently, EGFRvIII-positive GBM cells became hypersensitive to TF and subsequent PAR1/2 activation produced abundant amounts of IL-8 (Magnus *et al.*, 2010). Finally, high IL-8 levels correlated with loss of the tumour suppressor PTEN and an inability to activate STAT3. In PTEN-deficient GBM cells, activated STAT3 functioned as a tumour suppressor by occupying endogenous IL-8 promoter and directly repressing IL-8 transcription. Via this mechanism, activated STAT3 inhibited GBM cell proliferation, invasiveness and spreading (de la Iglesia *et al.*, 2008a,b). Together these observations suggest that several pathways activated by oncogenic mutations or loss of tumour suppressors cooperate to increase IL-8 production in GBM cells. Thus, blockade of one pathway will probably not be sufficient to fully inhibit production of IL-8.

## Therapy-induced inflammation

After surgical removal of GBM, conventional treatment consisting of radiation and chemotherapy with the DNA-alkylating agent temozolomide aims to control tumour progression by causing death of remaining cancer cells (Nagasawa *et al.*, 2012). However, almost all GBM tumours recur within 12 months after initial diagnosis. Intriguingly, most recurrent tumours are located at the site of origin and within the irradiated microenvironment.

In the last decade, it has become evident that the long-term consequence of radiation therapy is not only an amalgam of genetic damage and cell loss but also a change in the irradiated microenvironment. Exposure of cells to ionizing radiation (IR) induces the release of various proteins, which can trigger counteractive effects in the irradiated and neighbouring non-irradiated cells (i.e. bystander effect). This function of the radiation-induced microenvironment is exemplified by the IR-induced TGF- $\beta$  activation in normal and cancer cells (reviewed in (Barcellos-Hoff *et al.*, 2009). TGF- $\beta$  released from irradiated T98G glioblastoma cells is a key signalling factor in the radiation-induced bystander effect

by further inducing free radicals and DNA damage in the non-targeted bystander cells (Shao *et al.*, 2008). Just recently, Hardee *et al.* (2012) showed that targeting TGF- $\beta$  in the microenvironment significantly improved response of GBM cells and stem cell to radiation and increased the effectiveness of the therapy. Exposure of GBM cells to radiation also caused upregulation of IL-6 and, with different kinetics, IL-8 (Pasi *et al.*, 2010), suggesting these cytokines are differentially induced by IR. Importantly, IL-8 expression levels were elevated in tumours irradiated *in vivo* (Kim *et al.*, 2010).

GBM tissues often contain non-malignant cells, in particular tumour-associated microglia. Microglia are macrophages of the brain that constitute up to 30% of the tumour mass. Activated microglia are the primary source of inflammatory cytokines in non-malignant neuronal diseases (e.g. Alzheimer's disease) (Bachstetter and Van Eldik, 2010). Interestingly, although the morphological appearance of microglia found in or around GBM tumours suggested microglia activation, these cells did not release the prototypical inflammatory cytokines such as TNF- $\alpha$  or IL-6 (Sliwa *et al.*, 2007; Gabrusiewicz *et al.*, 2011). This is in agreement with *in vitro* data showing that GBM cells down-regulated the production of TNF- $\alpha$  from activated microglia (Kostianovsky *et al.*, 2008). However, irradiation of primary and transformed microglial cells led to an increase of IL-1 $\beta$  and TNF- $\alpha$  mRNA levels (Kyrkanides *et al.*, 1999; Ramanan *et al.*, 2008; Schnegg *et al.*, 2012). This correlates with *in vivo* studies showing that irradiation was associated with microglial activation in rodents (Monje *et al.*, 2003). It is thus conceivable that microglia in recurrent GBM tumours exposed to IR therapy could contribute to local inflammation.

A couple of studies suggest that treatment with temozolomide increases production of inflammatory mediators. For instance, long-term TMZ-treated astroglia cells developed resistance to TMZ, which correlated with up-regulated chemokines CXCL2, CXCL3 and IL-8 (Bruyere *et al.*, 2011). Another study, although limited and not directly addressing chemotherapy-induced inflammation, revealed that TMZ at clinically relevant concentration was not cytotoxic or anti-proliferative for microglia but significantly increased cellular protein synthesis (Vairano *et al.*, 2004). Individual proteins had not been investigated, but given that another alkylating agent cyclophosphamide induced microglial activation and neuroinflammation *in vivo* (Christie *et al.*, 2012), it is tempting to speculate that TMZ could also activate microglia to produce microglia-specific cytokines such as IL-1 $\beta$  and IL-6. This hypothesis is further supported by the discovery that DNA-damaging therapy such as IR can induce cell senescence accompanied with a striking release of pro-inflammatory cytokines (Rodier *et al.*, 2009).

## Senescence-induced inflammation in cancer

Cellular senescence, which is normally associated with ageing, is a process by which cells enter a state of permanent cell cycle arrest. In malignancy, this process constitutes a potent tumour suppressive mechanism. However, cellular senescence can also exert harmful effects in ageing or cancer.

This is related to the acquisition of the SASP phenotype that is manifested by a remarkable secretion of inflammatory cytokines, especially IL-6 and IL-8. As SASP develops after DNA damage with IR, chemotherapy or potent mitogenic stimulation by oncogenic Ras, it is now well documented that cellular senescence is a source of inflammatory factors and thus has tumour-promoting rather than tumour-suppressing properties (Krtolica *et al.*, 2001; Parrinello *et al.*, 2005; Rodier *et al.*, 2009).

Whether cellular senescence contributes to the development of the inflammatory microenvironment in GBM is yet not established. However, literature summarized here suggests that SASP could be one of the sources of inflammatory cytokines found in GBMs. As such, the PTEN status of GBM cells appeared to determine the cell fate after irradiation. PTEN-deficient U87, U251 and U373 cells underwent senescence after IR, whereas PTEN-proficient LN18 and LN428 cells entered apoptosis (Lee *et al.*, 2011). Thus, PTEN-deficient GBM cells might stop to proliferate after IR but are likely to produce inflammatory cytokines that in turn could activate the radioresistant GBM stem cells. Cell surface-bound IL-1 $\alpha$  was identified as an essential regulator of the senescence associated IL-6/IL-8 network (Orjalo *et al.*, 2009), and GBM cell lines as well GBM specimens were found to express IL-1 $\alpha$  mRNA and protein (Tada *et al.*, 1994; Kam *et al.*, 2007; Marcus *et al.*, 2010). Furthermore, glucocorticoids by interfering with IL-1 $\alpha$  signalling suppressed the production of IL-6 and IL-8 from senescent cells (Laberge *et al.*, 2012). Despite their severe side effects, glucocorticoids are used in GBM therapy to control tumour-associated oedema, and their anti-inflammatory efficacy (Piette *et al.*, 2009) could also be attributed to the control of senescence-associated inflammation.

Finally, the contribution of age-associated senescence to the inflammation in GBM pathophysiology should be considered. Age-associated senescent microglia do not function normally, as they fail to respond to certain stimuli and show inflammatory hyper-responsiveness (Lukiw, 2004). As such, ageing microglia are characterized by increased mRNA expression of inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6; Sierra *et al.*, 2007). Given that (a) every third cell in GBM tumour mass is a microglia (Graeber *et al.*, 2002), (b) age is the most significant risk factor for GBM (average disease onset is >60 years) (Behin *et al.*, 2003; Ricard *et al.*, 2012) and (c) that expression of pro-inflammatory genes is increased during aging (Sparkman and Johnson, 2008), one can postulate that not only therapy-induced cell senescence but also age-associated microglial senescence contributes to the development of a permissive inflammatory microenvironment allowing cancer cells to thrive.

## Therapeutic interventions

### Antibodies neutralizing cytokine levels

Cytokine function in an inflammatory disease state can be successfully targeted with antibodies that will neutralize cytokines or their receptors. For example, anti-TNF antibodies infliximab or adalimumab are approved for the treatment of rheumatoid arthritis and investigated for an array of other inflammatory disorders, including cancers (Larkin *et al.*,

2010; Diaz *et al.*, 2011). Similarly, monoclonal antibody against IL-6, siltuximab, has been trialled for ovarian, prostate and renal cell cancer (Rossi *et al.*, 2010; Coward *et al.*, 2011; Fizazi *et al.*, 2012). In GBM, IL-6R antibody tocilizumab has only been tested *in vitro* and exerted anti-proliferative effects in U87MG cells (Kudo *et al.*, 2009). The only antibody against a protein in the tumour microenvironment investigated in GBM clinical trials is bevacizumab (Avastin). Avastin targets VEGF and is the only molecularly targeted drug that is FDA-approved for use in recurrent GBM. Unfortunately, although Avastin has potent anti-angiogenic efficacy in GBM patients, it strongly promotes tumour infiltration (Bai *et al.*, 2011), which does not support its use in GBM therapy.

Large molecules like antibodies have limited brain penetration due to their limited ability to cross the blood–brain barrier (BBB). The anti-angiogenic efficacy of bevacizumab suggests that systematically administered antibodies may be useful as anti-glioblastoma therapies. However, bevacizumab targets VEGF, which has crossed from the brain into the vasculature, and the primary target for signal transduction inhibition appear to be endothelial cells lining the capillary walls and not the tumours cells themselves (Patel *et al.*, 2012). Hence, one must be careful when interpreting the therapeutic potential of monoclonal antibodies, and promising results seen with IL-6 antibody tested against GBM in the absence of brain-specific delivery restrictions (Wang *et al.*, 2009) are difficult to translate into the clinic. Along these lines, limited penetration of the EGFR inhibitor gefitinib into the CNS has been documented in GBM patients (Lassman *et al.*, 2005). Hence, although BBB might be disrupted and leaky at the site of surgical resection and radiotherapy, the delivery of drugs to GBM tumours appears to be facing the challenge of all CNS-targeted drugs. Therefore, clinical development of antibodies focuses on novel drug delivery techniques to bypass the BBB by using viral vectors, liposomal carriers, nanoparticles or regional drug delivery to the brain (Allhenn *et al.*, 2012). Alternatively, small molecules with BBB permeability might offer a more promising way to control inflammation in GBM. These agents could inhibit the interconnected circuits of inflammatory signal transduction pathways such as JAK–STAT, p38 MAPK, JNK and/or NF- $\kappa$ B. In this context, one of the most investigated pathways linking cancer with inflammation is the NF- $\kappa$ B pathway, which has been reviewed in great detail elsewhere (Grivennikov and Karin, 2010; Nogueira *et al.*, 2011). In the following, we will summarize the potential of JAK–STAT3, p38 MAPK and JNK inhibitors for GBM therapy.

### JAK–STAT inhibitors

The thorough investigation of IL-6 induced JAK–STAT signalling (Figure 1) in GBM pathophysiology and the availability of efficacious JAK–STAT inhibitors resulted in considerable pharmacological investigation for GBM therapy. JAK inhibitor JSI-124 (cucurbitacin I, Table 2) potently inhibits growth of U251 and A172 GBM cells where decreased levels of activated STAT3 led to down-regulation of cyclin B1 and cdc2 expression and induced apoptosis and cell cycle arrest (Su *et al.*, 2008). The same inhibitor proved efficacious in inhibiting STAT3 activation in GBM stem cells and inhibited GSCs proliferation and survival (Wang *et al.*, 2009). *In vivo*, inhibition of JAK–STAT3 activity by curcumin (administered as

curcumin-fortified diet) reduced the proliferation of tumour cells as well as growth and midline crossing of intracranially implanted tumours (Weissenberger *et al.*, 2010). The inhibitor AZD1480 (Table 2) also effectively blocked JAK1, JAK2 and STAT3 phosphorylation in GBM cells, leading to a decrease in cell proliferation (Ioannidis *et al.*, 2011). *In vivo*, AZD1480 inhibits the growth of subcutaneous tumours and increases survival of mice bearing human intracranial GBM tumours (McFarland *et al.*, 2011). WP1066 (Table 2) inhibits the STAT3 pathway by targeting JAK2 (Verstovsek *et al.*, 2008). *In vitro*, this compound induced apoptosis and potentially inhibited viability of U87MG and U373MG cell lines. Systematic *i.p.* administration of WP1066 in mice attenuated the growth of *s.c.* GBM xenografts. Immunohistochemical analysis of excised tumours confirmed that the anti-growth efficacy resulted from inhibiting the STAT3 activity as levels of activated STAT3 (p-STAT3) in the treatment group remained inhibited 3 weeks after initial WP1066 injection, whereas tumours from the control group continuously expressed high levels of p-STAT3 (Iwamaru *et al.*, 2007).

In the context of GBM development, it is important to note that STAT3 is activated not only by JAKs but also by EGFR and EGFRvIII (Figure 1), which are overexpressed in 40–50% of GBMs (see above). STAT3 activation and de-regulated EGFR correlated in 27.2% of investigated tumours. Thus, targeting JAKs alone might not be sufficient to halt growth of tumours with increased EGFR activity. Sorafenib, an oral inhibitor targeting several receptor tyrosine kinases including EGFR, inhibited proliferation in primary and established GBM cell lines. Effects of sorafenib were associated with inhibiting STAT phosphorylation via inhibiting JAK1 and JAK2 (Yang *et al.*, 2010). Combination of EGFR inhibitor gefitinib (Iressa) and JAK2 inhibitor JSI-124 synergistically suppressed STAT3 activation and potently killed GBM cells that expressed EGFR or EGFRvIII. JSI-124 also sensitized GBM cells to TMZ, an alkylating agent used in GBM therapy (Lo *et al.*, 2008). Kohsaka *et al.* (2012) observed increased sensitivity towards TMZ and reported significant positive correlation between expression levels of MGMT and p-STAT3 in 44 GBM specimens. *In vitro*, up-regulation of IL-6, STAT3 and MGMT was accompanied with acquisition of TMZ resistance. Importantly, STAT3 inhibitor WP1066 potentiated TMZ efficacy in TMZ-resistant GBM cell lines by post-transcriptionally suppressing MGMT protein levels. However, the study failed to show *in vivo* efficacy of a WP1066–TMZ combination, suggesting that fast metabolism of STAT3 inhibitor and short half-life of TMZ hindered *in vivo* evaluation.

Together, the encouraging results from these studies indicate that pharmacological inhibition of the JAK2–STAT3 pathway could be considered for the treatment of GBM patients. Availability of ruxolitinib (Table 2), safe and efficacious JAK2 inhibitor recently approved by FDA for the treatment of myelofibrosis (Verstovsek *et al.*, 2010; 2012; Mesa *et al.*, 2012) and currently in clinical trials for the treatment of other malignancies (invasive metastatic breast cancer, multiple myeloma), is a promising starting point for anti-inflammatory GBM therapy.

### p38 MAPK inhibitors

Limited studies have yet addressed the therapeutic potential of p38 MAPK inhibition in GBM, but rather focussed on

**Table 2**

Overview of kinase inhibitors profiled in the review

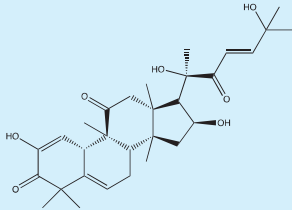
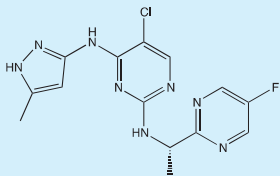
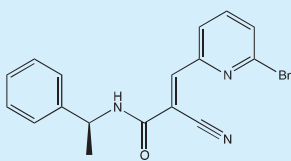
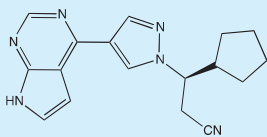
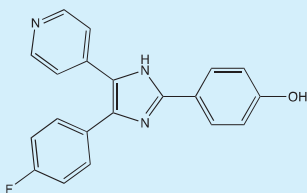
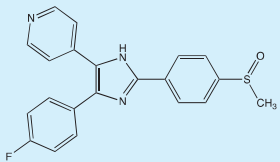
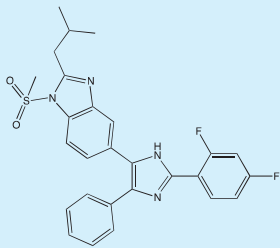
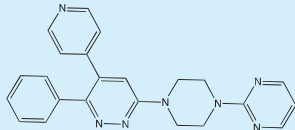
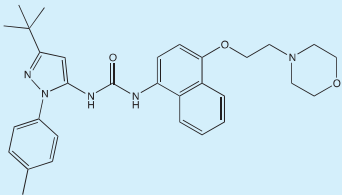
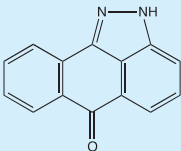
Inhibitor	Structure	Primary Targets	Activity in GBM-related models
JSI-124		<b>JAK2–STAT3</b> pathway (Blaskovich <i>et al.</i> , 2003)	Inhibits growth of U251 and A172 GBM cells (Su <i>et al.</i> , 2008) Inhibits GSCs proliferation and survival (Wang <i>et al.</i> , 2009) Sensitizes GBM cells to gefitinib and temozolomide (Lo <i>et al.</i> , 2008)
AZD1480		<b>JAK2</b> IC <sub>50</sub> = 0.058 µM <b>JAK3</b> IC <sub>50</sub> = 1.36 µM (Ioannidis <i>et al.</i> , 2011)	Inhibits GBM cell proliferation (Ioannidis <i>et al.</i> , 2011) Inhibits growth of subcutaneous GBM tumours (McFarland <i>et al.</i> , 2011)
WP1066		<b>STAT3</b> <i>in vitro</i> IC <sub>50</sub> not available IC <sub>50</sub> (U87MG) = 5.6 µM IC <sub>50</sub> (U373MG) = 3.7 µM (Iwamaru <i>et al.</i> , 2007)	Induces apoptosis, inhibits viability of U87MG and U373MG cells, attenuates the growth of s.c. GBM xenografts (Iwamaru <i>et al.</i> , 2007) Potentiates TMZ efficacy in TMZ-resistant GBM cells (Kohsaka <i>et al.</i> , 2012)
Ruxolitinib		<b>JAK1</b> IC <sub>50</sub> = 3.3 nM <b>JAK2</b> IC <sub>50</sub> = 2.8 nM <b>JAK3</b> IC <sub>50</sub> = 428 nM (Quintás-Cardama <i>et al.</i> , 2010)	FDA approved for treatment of myeloproliferative neoplasms
SB202190		<b>p38α MAPK</b> IC <sub>50</sub> = 50 nM (binding assay) IC <sub>50</sub> = 67 nM (kinase activity assay) (Gallagher <i>et al.</i> , 1997)	Abolishes the GBM-conditioned media-triggered increase in microglial expression of membrane type 1 MMP (MT1-MMP) (Markovic <i>et al.</i> , 2009) Abrogates the GBM conditioned media-induced MT1-MMP activity (Markovic <i>et al.</i> , 2009)
SB203580		<b>p38α MAPK</b> IC <sub>50</sub> = 42 nM (binding assay) IC <sub>50</sub> = 74 nM (kinase activity assay) (Gallagher <i>et al.</i> , 1997)	Sensitizes mismatch repair-proficient tumour cells to temozolomide (Hirose <i>et al.</i> , 2003) Inhibits synthesis of IL-6, IL-8, VEGF in GBM cells (Yoshino <i>et al.</i> , 2006; Yeung <i>et al.</i> , 2012) Inhibits LPA-induced GBM cell migration (Malchinkhuu <i>et al.</i> , 2005)
LY479754		<b>p38α MAPK</b> IC <sub>50</sub> = 4.9 nM <b>p38β MAPK</b> IC <sub>50</sub> = 11.9 nM (de Dios <i>et al.</i> , 2005)	Sensitizes non-migrating GBM cells to cytotoxic therapy with temozolomide (Demuth <i>et al.</i> , 2007) Reduces tumour growth <i>in vivo</i> (Campbell <i>et al.</i> , 2005)
069A		<b>p38α MAPK</b> IC <sub>50</sub> = 0.8 µM (Munoz <i>et al.</i> , 2007)	Inhibits inflammatory response, migration and invasiveness of U251 GBM cells (Yeung <i>et al.</i> , 2012)



Table 2

Continued

Inhibitor	Structure	Primary Targets	Activity in GBM-related models
BIRB796		<b>p38<math>\alpha</math> MAPK</b> $K_D = 0.097$ nM (Regan <i>et al.</i> , 2002) <b>JNK2</b> $K_D = 4.6$ nM <b>JNK3</b> $K_D = 62$ nM (Gruenbaum <i>et al.</i> , 2009)	
SP600125		<b>JNK1</b> $IC_{50} = 40$ nM <b>JNK2</b> $IC_{50} = 40$ nM <b>JNK3</b> $IC_{50} = 90$ nM (Bennett <i>et al.</i> , 2001)	Inhibits LPA-induced migration and invasiveness of GBM cells (Malchinkhuu <i>et al.</i> , 2005) Inhibits adenosine receptor stimulated increase of MMP-9 (Gessi <i>et al.</i> , 2010) Amplifies senescence in TMZ-treated U87MG cells (Ohba <i>et al.</i> , 2009) Inhibits IL-1 $\beta$ -induced IL-6, VEGF and sphingosine kinase 1 upregulation (Yoshino <i>et al.</i> , 2006; Paugh <i>et al.</i> , 2009; Tanabe <i>et al.</i> , 2011) Blocks IL-8 promoter activity and expression in EGFRvIII-bearing cells (Bonavia <i>et al.</i> , 2011)

The *in vitro*  $IC_{50}$  values were taken from the primary literature (where available), for selectivity profiles of these inhibitors see, e.g. (Fabian *et al.*, 2005; Bain *et al.*, 2007).

improving the understanding of the role of p38 MAPK isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) in various aspects of GBM pathophysiology.

p38 MAPK activity contributes to the highly invasive capacity of GBM cell lines and regulates the expression of MMPs, which are responsible for the proteolytic degradation of extracellular matrix, which is the major obstacle for cell motility. MMP secretion from GBM cells stimulates the migratory response in a p38 MAPK-dependent manner. Accordingly, inhibition of p38 MAPK blocked MMP secretion and invasion of GBM cells (Park *et al.*, 2002; Malchinkhuu *et al.*, 2005). IR-induced EGFR activation, which triggers p38 MAPK activation, along with Akt and PI3K signalling, also increased MMP2 expression and heightened invasiveness of PTEN deficient GBM cells (Park *et al.*, 2006). Furthermore, GBM-released factors manipulated the tumour-associated microglia via toll-like receptor induced p38 MAPK signalling, which up-regulates membrane type 1-MMP to activate pro-MMP2 and thereby support GBM expansion (Markovic *et al.*, 2009).

In addition, p38 MAPK may affect GBM malignancy via modulating cytokines and growth factors in the tumour microenvironment. p38 MAPK has a key role in the production of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 from activated microglia and also regulates signalling of these cytokines in GBM cells (Figure 1), further exacerbating local inflammation. These cytokines exert their pro-tumourigenic effects in GBM pathology by increased expression of other inflammatory (e.g. COX-2; Xu and Shu, 2007), invasiveness-promoting (e.g. MMPs; Markovic *et al.*, 2009; Sarkar and Yong, 2009) or angiogenic (e.g. IL-8 and VEGF; Yoshino *et al.*, 2006) mediators. GBM cells could potentially up-regulate these mediators via p38 MAPK-dependent phosphorylation of transcription factors such as NF- $\kappa$ B (Figure 1). Post-transcriptional gene

regulation has also been implicated as one mechanism of the tumour response to the inflammatory microenvironment, and in this regard, p38 MAPK-mediated shuttling of the mRNA stabilizing human antigen R (HuR, Figure 1) protein is linked to increased stability of VEGF, TGF- $\beta$ , IL-6, IL-8 and TNF- $\alpha$  mRNA in GBM cells (Nabors *et al.*, 2003). HuR depletion led to transcript destabilization, reduced protein expression, a significant decrease in tumour volume and increased sensitivity to chemotherapeutic drugs (Filippova *et al.*, 2011). Further supporting p38 MAPK activity in regulating mRNA stability, p38 MAPK-mediated hyperphosphorylation deactivated the RNA destabilizer tristetraprolin (TTP), resulting in stabilization of IL-8 and VEGF mRNA, and promoted GBM cell proliferation and viability (Suswam *et al.*, 2008). Moreover, MAPK-interacting kinase 1 and 2 (Mnk1/2) are translation-controlling serine/threonine kinases that are activated by p38 MAPK (Figure 1) and Mnk1 knockdown in U87MG reduced tumourigenic activity in nude mice (Ueda *et al.*, 2010). Finally, activity of p38 MAPK influences the response of GBM cells to DNA-alkylating agents and pharmacological inhibition of p38 MAPK sensitized GBM cells to TMZ-induced toxicity (Hirose *et al.*, 2003; 2004).

Despite increasing evidence for p38 MAPK as an anti-GBM target, currently *in vivo* data are limited in order to validate the effectiveness of p38 MAPK inhibition in GBM. Indirectly supporting the use of p38 MAPK inhibitors for GBM therapy comes from research on minocycline, a semi-synthetic broad-spectrum and lipophilic tetracycline antibiotic approved by the FDA that is able to cross the BBB and inhibit microglial activation and inflammation in CNS disease models. One of the main targets of minocycline is p38 MAPK (Nikodemova *et al.*, 2006), and the anti-inflammatory effect of minocycline correlates with inhibition of microglial

p38 MAPK phosphorylation and decreased GBM invasiveness and expansion *in vitro* and *in vivo* (Liu *et al.*, 2011; Markovic *et al.*, 2011).

Commonly, the prototypical p38 MAPK inhibitors SB203580 (Table 2) and SB202190 (Table 2) have been utilized to examine p38 MAPK in GBM malignancy (Hirose *et al.*, 2003; Malchinkhuu *et al.*, 2005; Markovic *et al.*, 2009; Bonavia *et al.*, 2011). A better clinical candidate, the p38 MAPK inhibitor LY479754 (Table 2), was shown to inhibit GBM invasiveness *in vitro*. In addition, LY479754 also sensitized arrested, non-migrating cells to cytotoxic therapy with TMZ (Demuth *et al.*, 2007). We recently showed that a BBB-permeable p38 MAPK inhibitor, 069A (Table 2, Munoz *et al.*, 2007), can potentially target both malignant and non-malignant microglial cells in GBM tumours and attenuate the development of an inflammatory microenvironment and GBM invasiveness (Yeung *et al.*, 2012). Possibly in combination with other treatments, these promising results mandate further development and testing of suitable p38 MAPK inhibitors against invasive glioblastoma *in vivo*. In clinic, however, BBB permeability might be problematic as p38 MAPK inhibitors have been developed for rheumatoid arthritis therapy and were intentionally made highly polar in order to avoid BBB permeability. This restricts the use of current p38 MAPK inhibitors for CNS-related diseases. Certainly, a p38 MAPK inhibitor with satisfying preclinical safety might be able to control the local inflammation at the tumour site, but a new p38 MAPK inhibitor with improved BBB permeability could have far more potential in GBM therapy.

### JNK inhibitors

JNKs are an evolutionarily conserved sub-group of MAPKs activated by MAPK kinases 4 and 7 (MKK4 and MKK7), which integrate a wide array of stimuli to phosphorylate and stimulate JNKs. The major JNK target is the transcription factor AP-1, which is composed of Fos and c-Jun family members. Thereby, the JNK/c-Jun pathway modulates expression of a plethora of AP-1 target genes that control inflammatory response, cell proliferation, apoptosis as well as invasion. Besides regulating proto-oncogenes Jun, Fos and Myc; JNKs have also been linked to p53-dependent senescence and apoptosis. Overall, pro- and anti-apoptotic effects of JNK signalling on tumour development appear to be determined by stimuli and tissue specificity, signal intensity and crosstalk between JNK isoforms (Wagner and Nebreda, 2009).

In GBM, JNKs and c-Jun phosphorylation correlate with the grade of malignancy and patients' age (Antonyak *et al.*, 2002). JNK2 is the major activated JNK isoform in GBM (Tsuiki *et al.*, 2003). This isoform is unique among all MAPKs as it possesses autophosphorylation activity and constitutive substrate kinase activity *in vitro* and *in vivo* (Nitta *et al.*, 2008). JNK2 is also activated by oncogenic EGFRvIII (Figure 1) (Antonyak *et al.*, 1998) and supports tumourigenesis *in vivo* through increased proliferation and tumour formation (Cui *et al.*, 2006). In addition to its role in apoptosis, JNKs have been linked to glial-derived neurotrophic factor (Lu *et al.*, 2010) and lysophosphatidic acid-induced migration and invasiveness of GBM (Malchinkhuu *et al.*, 2005). SP600125-mediated JNK inhibition antagonized adenosine receptor

stimulated increase of MMP-9 levels (Gessi *et al.*, 2010). Furthermore, SP600125 (Table 2) amplified senescence in TMZ-treated U87MG cells, suggesting that SP600125 potentiates TMZ-dependent cell death pathways (Ohba *et al.*, 2009).

Within the tumour microenvironment, JNKs likely potentiate carcinogenesis by promoting local inflammatory responses. For example, IL-1 $\beta$ -induced IL-6 production, which contributes to increased tumour development and invasiveness, is inhibited by SP600125 in an inflammatory model of GBM (Tanabe *et al.*, 2011). Inhibition of JNK signalling also suppressed angiogenesis and invasiveness of GBM cell via inhibiting IL-1 $\beta$ -induced VEGF production and sphingosine kinase 1 upregulation, respectively (Yoshino *et al.*, 2006; Paugh *et al.*, 2009). Together, these studies indicate that therapeutic manipulation of oncogenic IL-1 $\beta$  activities can be managed with JNK inhibitors; however, the JNK isoforms responsible for these effects have yet to be identified. Interference with the JNK pathway also decreased EGFR-dependent tissue factor expression (Rong *et al.*, 2009). TF is the main cellular initiator of coagulation that significantly contributes to two fundamental features of GBM, namely hypoxia and necrosis. EGFR-mediated TF expression depends on AP-1 transcriptional activity and is associated with JNK and JunD activation. These mechanisms are likely to work *in vivo* as elevated/mutated EGFR highly correlated with TF expression in GBM specimen (Rong *et al.*, 2009). Recently, Bonavia *et al.* (2011) showed that the pharmacological targeting of the JNK-NF- $\kappa$ B pathway with SP600125 efficiently blocked IL-8 promoter activity and expression in EGFRvIII-bearing cells. However, interpretation of results derived from the pan-JNK inhibitor SP600125 should have taken into consideration the vast number of off-target kinases that this compound inhibits (Bain *et al.*, 2007).

A significant number of potent and selective JNK inhibitors have recently been developed, some with BBB permeability (He *et al.*, 2011; Plantevin Krenitsky *et al.*, 2012). These inhibitors either target peripheral JNK1 in obesity and diabetes, or the JNK3 isoform expressed in CNS to reduce neurodegeneration. Yet JNK2 appears to be the main JNK isoform involved in GBM pathophysiology and JNK2 selective inhibitors are yet to be developed. Interestingly, BIRB796 (Table 2), a potent type II inhibitor of p38 MAPK that also binds with high affinity to JNK2 (Gruenbaum *et al.*, 2009), could provide an opportunity as dual-kinase inhibitor.

### Conclusion

ILs play a major role in various inflammatory diseases including cancer. For example, IL-13 is a cytokine that modulates inflammation, apoptosis and/or tumour immunosurveillance in many different cancers (Wynn, 2003). However, its beneficial activity in glioblastoma is limited by increased expression of IL-13 receptor  $\alpha$ 2 (IL-13R $\alpha$ 2) on GBM cells. IL-13R $\alpha$ 2 binds and internalizes IL-13 with high affinity, but it does not mediate signal transduction. Accordingly, the decoy receptor IL-13R $\alpha$ 2 is a promising drug target (Hsi *et al.*, 2011; Balyasnikova *et al.*, 2012). As we review here, IL-1 $\beta$ , IL-6 and IL-8 in the close vicinity of malignant cells promote carcinogenesis through activation of proliferative mechanisms and/or activation of signalling cascades involved in angiogenesis, migra-

tion and invasiveness. Furthermore, these ILs are potent activators of signalling pathways, which regulate cell survival and promote resistance to chemotherapy. Hence, targeting these inflammatory cytokines in the GBM microenvironment with small molecule kinase inhibitors in combination with cytotoxic agents could achieve therapeutic benefits for patients with recurrent GBM. As demonstrated recently, resistance of GBM and stem cells to radiation can be attenuated by inhibiting TGF- $\beta$  in the tumour microenvironment (Hardee *et al.*, 2012). The magnitude of radiosensitization seen in this study is comparable with the radiosensitization achieved by adding the cytotoxic agent TMZ to the radiation therapy. Given that concurrent combination of TMZ and radiation is the standard and best available approach to treat GBM, it is reasonable to expect increasing basic and clinical research in targeting the GBM microenvironment. Among the diverse signalling networks involved in inflammation, JAK, p38 MAPK and JNK pathways have emerged as critical convergent points that warrant further pursuit as drug targets. Availability of ruxolitinib, assuming it will reach therapeutic concentrations in the brain, and significant *in vivo* data supporting beneficial JAK inhibition in GBM pathophysiology, could launch trials assessing anti-IL-6 targeted approaches for GBM therapy in the near future. Testing of p38 MAPK or JNK inhibitors in GBM therapy could be next, but *in vivo* data and FDA-approved inhibitors of these kinases are not yet available.

It is important to note that success of anti-inflammatory therapies in GBM does not entirely depend on the availability of suitable drugs. Treatment of glioblastoma is challenged by the extreme heterogeneity of these tumours requiring a highly personalized approach. EGFR, PTEN, MGMT and p53 status, isocitrate dehydrogenase (IDH) mutation and/or deletion of chromosome arms 1p and 19q (Riemenschneider *et al.*, 2010; Ricard *et al.*, 2012) are known to determine the response of GBM cells to radiation and chemotherapy, as well as efficacies of newly developed kinase inhibitors. Hence, the future success of kinase inhibitors, including those targeting inflammatory pathways, lies in the ability to identify vulnerable tumours based on their specific genetic alterations.

Research summarized in this review suggests that the future direction for GBM therapy could benefit from the design of synergistic multi-target drug combinations that would target both oncogenic cellular signalling in cancer cells as well as the inflammatory microenvironment. Although the significance of inflammation for GBM pathology and *in vivo* evaluation of therapies directly targeting inflammation is still missing, accumulating *in vitro* evidence indicates that inflammation-based therapies could provide useful tools in combating GBMs, most likely in the combination with standard therapeutic regimens. The extreme heterogeneity of GBMs and the lack of kinase inhibitors with sufficient BBB permeability remain challenging aspects for future research. In addition, the plethora of possible drug combinations might exhibit unknown and unacceptable toxicity profiles. Importantly, however, future research in the inflammatory microenvironment will not only improve our understanding of GBM development, progression and therapy resistance but also provide new opportunities for therapeutic strategies.

## Conflict of interest

All authors declare no conflict of interest.

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