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Review

The role of Stat3 in glioblastoma multiforme

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common brain tumor and has the worst prognosis. Several signaling molecules have been clearly implicated in the development, progression, and aggressiveness of GBM. Here we review the role of signal transducer and activator of transcription-3 (Stat3) in GBM. We particularly focus on its expression in clinical GBM samples, its role in brain tumorigenicity in cell lines and animal models, and discuss possible therapeutic strategies targeting Stat3. This review also summarizes the current knowledge regarding the role of Stat3 regulation by upstream activators and repressors in promoting GBM progression in both translational and clinical studies.

1. Introduction

Glioblastoma multiforme (GBM) is the most devastating and aggressive tumor of the central nervous system accounting for approximately 50% of all primary brain tumors.[1–3] Surgery, followed by irradiation and concomitant or adjuvant temozolomide, is now considered the standard of care for GBM patients.[4–6] However, despite temozolomide treatment and other innovative therapies, the overall prognosis remains very poor for GBM patients with a median survival of only 12–15 months.[5,7–12]

Successfully identifying the pivotal molecular mediators of GBM progression and tumor resistance is therefore critical to improve overall survival of GBM patients. This review will focus specifically on the role of signal transducer and activator of transcription 3 (Stat3) in promoting GBM progression and summarize the current literature. We will discuss Stat3 signaling in both clinical and translational reports and examine potential therapeutics to inhibit Stat3 in the GBM setting.

2. Stat3 signaling

Stat3 is a member of the Stat family of cytoplasmic transcription factors that are activated by many cytokine and growth factor receptors and downstream substrates (Fig. 1).[13,14] Activation of Stat3 leads to transient Stat3 phosphorylation in normal cells, however, up to 70% of human tumors display persistent Stat3 phosphorylation.[15] Interestingly, genetic alterations leading to Stat3 over-expression or direct mutations within the Stat3 gene have not been reported, implying that upstream alterations or mutations to molecules that regulate Stat3 induce persistent Stat3 activity. Phosphorylated Stat3 initiates transcription of multiple cancer associated genes promoting cell cycle progression, anti-apoptosis, cell survival, angiogenesis, migration, and invasion (Fig. 1).[16–19] Not surprisingly, Stat3 activation has been detected at high frequency in many types of tumors, including GBM, and its activity is required for tumor growth.[17,18]

3. Stat3 activation in primary GBM tissue

The most commonly used method for detecting activated Stat3 levels in GBM patient samples is by immunohistochemistry where phospho-Stat3-specific antibodies are used on frozen or paraffin embedded tumor sections. Several retrospective studies have reported Stat3 phosphorylation in GBM,[20–25] however, detection of positive Stat3 phosphorylation in these studies range from 9% to 83% (Table 1).

Despite using relatively low numbers of primary brain tumor tissues, Lo et al. demonstrated that the amount of Stat3 phosphorylation directly correlated with brain tumor grade,[22] especially when comparing lower grade (Grade I and II) to higher grade (Grade III and IV) tumors. The GBM patient cohort showed constitutively active Stat3 in 66% of samples, compared to only 27%, 29%, and 57% of Grade I, II, and III primary brain tumors, respectively. Another study evaluating a larger patient sample revealed that activation of Stat3 was comparable between anaplastic astrocytomas (AA) (55.6%; 15/27) and GBM (56.4%; 31/55), however, lower grade brain tumors where not assessed in this study.[23] Similarly, Abou-Ghazal et al. showed that Stat3 phosphorylation was detected in slightly greater than 50% of AA (53%; 9/17) and GBM (51%; 27/53),[20] while Kohsaka et al. recently observed positive staining for phopsho-Stat3 in the majority of human GBM patients

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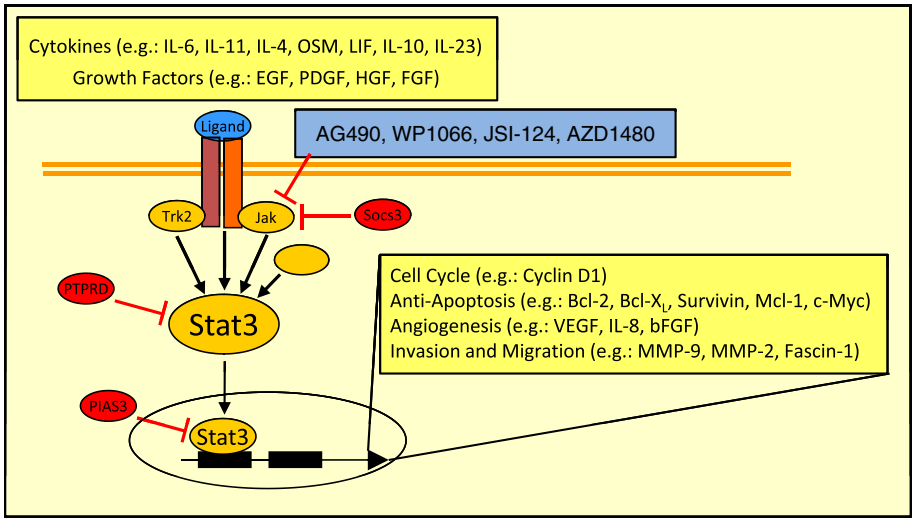

Fig. 1. Schematic of signal transducer and activator of transcription-3 (Stat3) signaling. The binding of cytokine and growth factor to their corresponding receptors leads to downstream activation of Stat3 either directly or through intermediate substrates (Src, Jak, Trk). In turn, Stat3 enters the nucleus where it triggers gene expression of many pro-cancerous proteins involved in cell cycle progression, anti-apoptosis, angiogenesis, migration, and invasion. Regulation of Stat3 activity by protein tryosine phosphatase receptor delta (PTPRD), Socs3 and protein inhibitor of activated Stat3 (PIAS3) is often disrupted in glioblastoma multiforme (GBM) resulting in increased Stat3 signaling. In addition, several inhibitors targeting the Jak/Stat3 pathway (including AG490, WP1066, JSI-124, AZD1480) have been developed and used in pre-clinical GBM models. Red lines with blunt ends indicate inhibitory effects; black lines with arrow heads indicate stimulatory effects. Bcl = B-cell lymphoma, EGF = epidermal growth factor, FGF = fibroblast growth factor, HGF = hepatocyte growth factor, IL = interleukin, LIF = leukemia inhibitory factor, Mcl = myeloid cell leukemia sequence, MMP = matrix metalloproteinase, OSM = oncostatin M, PDGF = platelet-derived growth factor, VEGF = vascular endothelial growth factor.

(83%; 30/36).[21] Although they did not evaluate whether Stat3 phosphorylation increased with tumor grade and thus predicts malignancy of brain tumors, Kohsaka et al. also demonstrated that Stat3 phosphorylation was enhanced in eight out of 14 samples from patients with recurrent GBM following temozolomide treatment compared to prior samples from the same patients when first presenting with GBM.[21] In addition, Stat3 has been shown to be constitutively active in a large percentage of GBM tissue by electrophoretic mobility shift assay and western blot analysis[26,27] and that Stat3 phosphorylation correlated with histopathological grade as determined by western blot analysis.[28]

In contrast, Wang et al. observed that only 9% (4/46) of AA and 9% (6/70) of human GBM samples displayed detectable phospho-Stat3 by immunohistochemistry and that phosphorylated Stat3 did not increase over histological grade.[25] Disparities between this report and others discussed above may have arisen due to variable immunostaining techniques or greater stringency with evaluating positive staining. Interestingly, all five studies used specific phospho-Stat3 antibodies from the same company; however, the study by Wang et al. which observed the lowest percentage of phospho-Stat3 staining used the lowest dilution, despite a similar incubation time (Table 1). Whether this had a bearing on the overall outcome of staining is unclear.

Further clinical evidence suggesting Stat3 may be involved in GBM progression comes from studies evaluating the expression of a Stat3 phosphatase, protein tryosine phosphatase receptor delta (PTPRD) in GBM patient samples.[29,30] Solomon et al. recently identified focal deletions, missense and nonsense mutations within the PTPRD gene in primary GBM samples.[29] In another study, intragenic and homozygous deletions were identified in 41% (89/215) of GBM tumors.[30] Veeriah et al. went on to examine epigenetic changes, finding hyper-methylation of the PTPRD promoter leading to loss of expression of PTPRD in 37% of GBM samples. Importantly, they reported that loss of PTPRD expression occurred at a higher frequency in GBM compared to lower grade gliomas, with loss of PTPRD expression predicting poorer prognosis in GBM patients.[30] To our knowledge, inverse correlative studies examining the relationship of PTPRD expression and Stat3 activation, and the causative role of these signaling alterations, have not been performed using human primary GBM samples (or any other tumor samples). Nevertheless, laboratory studies with GBM cell lines do indicate a clear negative correlation between PTPRD expression and Stat3-mediated tumorigenicity.[30]

The expression of another regulator of Stat3, protein inhibitor of activated Stat3 (PIAS3) which functions as a transcriptional repressor by blocking Stat3 binding to DNA, has also been investigated in GBM patient samples. PIAS3 expression was detected in only 4/35 (11%) GBM samples tested whereas 33/33 (100%) of control non-neoplastic brain samples from patients diagnosed with epilepsy expressed PIAS3.[26] This report went on to show a negative correlation between PIAS3 and phosphorylated Stat3 expression although this was performed in a smaller subset of samples to the 35 GBM samples tested above. Nonetheless, Brantley et al. identified that PIAS expression is frequently reduced and may contribute to enhanced Stat3 activation in GBM.[26]

Finally, additional supporting evidence that Stat3 activity promotes GBM progression is derived from studies evaluating the level of the key Stat3 modulator interleukin-6 (IL-6) ligand within

Table 1
Percentage of positive detected Stat3 phosphorylation in human primary brain tumor samples by histopathological grade

Astrocytoma Grade I	Astrocytoma Grade II	Anaplastic astrocytoma Grade III	Glioblastoma multiforme Grade IV	Reference
–	12.5% (1/8)	8.7% (4/46)	8.6% (6/70)	Wang et al. (2004)[25]
–	–	55.6% (15/27)	56.4% (31/55)	Mizoguchi et al. (2006)[23]
–	0% (0/3)	52.9% (9/17)	50.9% (27/53)	Abou-Ghazal et al. (2008)[20]
27.3% (3/11)	29.3% (7/24)	57.1% (8/14)	66.7% (4/6)	Lo et al. (2008)[22]
–	–	66.7% (2/3)	83.3% (30/36)	Kohsaka et al. (2012)[21]