The Bmi-1/NF- κ B/VEGF story: another hint for proteasome involvement in glioma angiogenesis?

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Abstract Angiogenesis is an essential process for sustaining tumor growth, particularly in cancer cell types with rapid proliferation, including malignant glioma. Bmi-1 is a transcriptional regulator of the polycomb group involved in repression of gene expression by altering the state of chromatin at specific promoters. Bmi-1 overexpression was previously implicated in glioma tumorigenesis, proliferation, self-renewal, apoptotic resistance and invasiveness. In a recent study, Jiang et al. (PLoS One 8:e55527, 2013) have revealed the involvement of Bmi-1/NF- κ B/VEGF pathway in promoting glioma cell-mediated tubule formation and migration of endothelial cells and neovascularization both in vitro and in vivo. NF- κ B inhibition reversed these effects, supporting a role for Bmi-1 in glioma angiogenesis. Given the intimate association of Bmi-1 and NF- κ B with the ubiquitin-proteasome system, a better understanding of protein turnover in angiogenic signaling, discussed here, provides novel implications for anti-angiogenic treatment strategies in gliomas.

Keywords Bmi-1 · NF- κ B · VEGF · Glioma · Ubiquitin-proteasome system · Proteasome inhibitor

A hallmark of malignant gliomas which is considered an indispensable regulatory process for their uninterrupted vascular supply and growth is angiogenesis (Brat and Mapstone 2003; Jensen 2009). There are two major mechanisms used by glioma cells to trigger angiogenesis, which involve either hypoxia-dependent or hypoxia-independent downstream molecular events (Kaur et al. 2004; Argyriou et al. 2009).

The first, hypoxia-driven mechanism, results from an inherent feature of malignant gliomas, particularly glioblastoma, to develop necrosis of “pseudopalisading” morphology, thus rendering the creation and maintenance of a hypoxic micro-environment essential for tumor neovascularization and further expansion (Zagzag et al. 2000; Rong et al. 2006). Central to this process is hypoxia-inducible family of transcription factors (HIF), predominantly exerting its actions via HIF-1 α , which is rapidly degraded by the 26S proteasome under normoxic conditions, but becomes stabilized in hypoxia (Kallio et al. 1999). HIF-1 function relevant to promotion of angiogenesis consists of the transcriptional induction of VEGF via a HIF-1 α binding site in VEGF promoter (Machein and Plate 2000), but also comprises activation of VEGF receptors (VEGFR), metalloproteases (MMPs), plasminogen activator inhibitor (PAI), transforming growth factors α and β , angiopoietin and Tie receptors, endothelin-1, inducible nitric oxide synthase, adrenomedullin, and erythropoietin (Kaur et al. 2005). In hypoxic state, attenuated hydroxylation and acetylation of HIF-1 α reduce its affinity for the E3-Ub ligase von Hippel Lindau (pVHL) and thus its proteasomal degradation. Further, deSUMOylation in hypoxia leads to HIF-1 α stabilization and activation, adding another level of control (Kaur et al. 2005; Cheng et al. 2007). There exists a crosstalk between activation and inhibition of Ub-dependent and Ub-independent proteasome-mediated degradation processes in HIF-1 regulation, with proteins like heat shock protein 90 (Hsp90) mediating this link (Cheng et al. 2007).

The second mechanism is largely part of genetic changes in the context of acquisition of an aberrant growth phenotype, hence it mainly concerns most growth factors known to play a role in gliomagenesis, including VEGF, PDGF, EGF, FGF, HGF, signaling via the intracellular phosphatidylinositol 3-kinase (PI3K) or mitogen-activated protein kinase (MAPK) pathways (Kaur et al. 2004). HIF-independent angiogenesis may also be promoted by the induction of IL-8 gene via a, AP-

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1- and NF- κ B-dependent mechanism, again implicating the ubiquitin-proteasome system (UPS) in this process, not only considering its role in NF- κ B nuclear translocation, but also the repressive activity exerted by the proteasome-substrate, tumor suppressor protein ING4 on NF- κ B-mediated IL-8 transcriptional activation (Brat et al. 2005). Another established promoter of glioma angiogenesis, necrosis formation, has also been linked to UPS as NF- κ B constitutive activity levels were found higher in glioblastomas with necrosis whereas expression of a mutant I κ B α inhibited necrosis formation and retarded tumor growth, in part through down-regulation of tissue factor (TF) expression (Xie et al. 2008). Finally, accumulating evidence seems to establish an interrelation of NF- κ B and HIF-1 in terms of their upregulation in both hypoxic and normoxic environments (van Uden et al. 2008; Scortegagna et al. 2008) which further complicates the already numerous intersections of UPS with this malignancy.

Bmi-1 encodes a 37 kDa polycomb group protein (PcG) from human chromosome 10p11.23 that serves as the key regulatory component of the PRC1 complex (polycomb regulatory complex-1), a modulator of chromatin structure and transcription regulator of several genes (Cao et al. 2011). Bmi-1 expression has been linked with self-renewal and maintenance of both normal and cancer stem cells. Bmi-1 expression is elevated in many types of cancers, and experimental silencing of Bmi-1 protein levels causes apoptosis and/or senescence in tumor cells in vitro and increases their sensitivity to cytotoxic agents (Cao et al. 2011). Regulation of Bmi-1 expression and function is controlled at multiple levels, including gene amplification, transcriptional, post-transcriptional, and post-translational levels (Cao et al. 2011).

In gliomas, Bmi-1 overexpression partly emanates from gene amplification (Häyry et al. 2008). Also, reduction of microRNA (miR)-128 expression, which is a direct negative transcription regulator of the Bmi-1 mRNA 3'-untranslated region, further contributes to Bmi-1 upregulation (Godlewski et al. 2008). With regard to post-translational modifications, EGF-induced Akt activation results in Bmi-1 phosphorylation, rendering the protein resistant to proteasomal degradation, and promoting its nuclear accumulation and oncogenic activity (Kim et al. 2011). In contrast, p38 MAPK activation may block the Akt-dependent Bmi-1 stabilization and accelerate proteasome-mediated degradation of Bmi-1 (Kim et al. 2011).

The documentation of an active Bmi-1/NF- κ B pathway that leads to VEGF-dependent neo-angiogenesis in glioma cells and xenografts, by Jiang et al. adds a new variable to the landscape of transformations induced by this key protein forging the aggressive phenotype of glioma (Jiang et al. 2013). They have previously shown that Bmi-1 renders apoptotic resistance to glioma cells through reduction of activated caspase-3 and PARP, and induction of Bcl-xL, all resulting from activation of the canonical NF- κ B pathway (Li et al. 2010). They have also correlated the activation of

Bmi-1/NF- κ B pathway, with the NF- κ B target gene product MMP-9, inducing migration and invasiveness of glioma cells, and replicated their findings in glioma tumor samples displaying increased MMP-9 expression (Jiang et al. 2012). In their present work, they found that induced expression of Bmi-1 augmented, whereas knockdown of Bmi-1 reduced the ability of glioma cells to promote tubule formation and migration of endothelial cells and neovascularization in chicken chorioallantoic membrane. They also reproduced these effects in vivo, in orthotopically transplanted human gliomas. They explain these effects by Bmi-1 overexpression-mediated induction of NF- κ B transcriptional activity, resulting in increased production of its target-gene product, VEGF-C, whereas it was abrogated after Bmi-1 knockdown or specific NF- κ B inhibition (Jiang et al. 2013).

The use of proteasome inhibitors has recently been proposed as an alternative anti-angiogenic treatment, based on their negative effect on HIF-1 resulting from: a) inhibition of HIF-1 α carboxy-terminal activation domain (CAD) (Kaluz et al. 2006; Birle and Hedley 2007), b) inhibition of HIF-1 co-activator CBP/p300 recruitment by the asparaginyl hydroxylase FIH-1 (factor inhibiting HIF-1) (Shin et al. 2008), c) stabilization of the transcriptional cofactor CITED2 (Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2) which competes with HIF-1 α for binding to CBP/p300 (Shin et al. 2008), and d) inhibition of intracellular signaling pathways that contribute to either increased stability and translation of HIF-1 α , such as the Ras/Mek/Erk and PI3K/Akt/mTOR pathways (Kaur et al. 2005; Blum et al. 2005). As a result of this regulation, it is not surprising that loss of PTEN, which is a frequent feature of primary glioblastomas, facilitates IGF-1- and HIF-1-regulated angiogenic gene expression via enabling Akt activation of HIF-1 α activity (Zundel et al. 2000). In addition, inhibition of NF- κ B nuclear accumulation and activation is a long-known critical effect of proteasome inhibitors, central to many of their antitumor effects, including inhibition of angiogenic signals (D'Alessandro et al. 2009).

Intriguingly, Bmi-1 levels were also found to be reduced by treatment with a proteasome inhibitor in vitro and this effect was associated with reduced cell proliferation, G2 cycle arrest, and increased apoptosis (Balasubramanian et al. 2012). This Bmi-1 downregulation was accompanied by a compensatory increase in the level of mRNA encoding proteasome protein subunits in response to proteasome inhibitor treatment and an increase in proteasome activity. The increase in proteasome subunit levels was in turn associated with increased nuclear respiratory factor 1 (Nrf1) and Nrf2 levels, as knockdown of Nrf1 attenuated the proteasome inhibitor-dependent increase in proteasome subunit expression and restored Bmi-1 expression (Balasubramanian et al. 2012).

Although there is currently no specific Bmi-1 inhibitor, the observations of Jiang et al. regarding the angiogenic

properties of Bmi-1 in glioma, combined with the reported inhibitory effect of proteasome inhibition on angiogenic signals at multiple levels, including direct downregulation of Bmi-1, and inhibition of NF κ B downstream, further support the rationale for targeting the proteasome as a viable strategy against glioma angiogenesis. Thus, the results of ongoing combinations of bortezomib, which is the only proteasome inhibitor in clinical use so far, with anti-angiogenic targeted agents, are eagerly awaited. Finally, evaluating a putative predictive role for Nr1f1 might be worth testing in future trials, as a means to assess efficiency of proteasome inhibition with respect to Bmi-1-driven angiogenesis.

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