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Review

Roles of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) in Glioblastoma

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Abstract. *Insulin-like growth factor binding protein-2 (IGFBP-2) is reported to be a modulator of the action of insulin-like growth factors (IGFs), whereas IGF-independent effects of IGFBP-2 on cellular proliferation, apoptosis, and mobility have been revealed not only during the embryonic state but also in the pathological state of cancer. As well as other types of carcinomas, overexpression of IGFBP-2 has been reported in glioblastoma multiforme (GBM), the most malignant brain tumor characterized by rapid growth, extensive invasiveness, and angiogenesis. However, it remains unclear how IGFBP-2 is involved in the malignant features of GBM. This review outlines the IGF-dependent and -independent functions of IGFBP-2 and focuses on the roles of IGFBP-2 in the progression of GBM.*

Insulin-like growth factor binding proteins (IGFBPs) constitute a family of six circulating proteins that bind insulin-like growth factor (IGF)-I and -II with high affinity. The sequence and structure of IGFBP-2 were determined as a protein with extensive conservation of the distribution of cysteine residues of IGFBP-1 (1). The human *IGFBP-2* gene is localized to chromosome 2 region q33-q34 near the *IGFBP-5* gene. The characteristics of the six IGFBPs are summarized in Table I. IGFBP-2 is predominantly expressed in the early post-implantation epiblast, the apical ectodermal ridge, the progenitors of spleen and liver cells, and the fetal astroglial cells. After birth, the expression of IGFBP-2 decreases significantly, although IGFBP-2 is the second most abundant IGFBP in serum (Table I) (2-8).

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IGFBP-2 has been considered as an inhibitory factor of IGF actions, particularly of IGF-II (9, 10). Indeed, transgenic mice overexpressing IGFBP-2 under the control of cytomegalovirus promoter demonstrated significant reduction of body weight gain (11-13). However, *IGFBP-2* knockout mice showed only decreased spleen size during the early postnatal stages and increased circulating levels of several other IGFBPs in adults, suggesting functional redundancy in the IGFBP family (14, 15). A rather paradoxical role of IGFBP-2 in IGF signaling in which IGFBP-2 promoted the IGF function has also been reported. For example, binding of the IGFBP-2/IGF complex to cellular surface proteoglycan may result in concentrated IGFs on the cellular surface thus enhancing their actions (9, 10, 16-18). In this context, IGFBP-2 serves as a cell surface reservoir of IGFs in order to ensure the efficient availability of IGFs in the pericellular microenvironment (9, 10, 16-18). Integrin-mediated functions of IGFBP-2 have been also suggested. Finally, an IGF-independent function of IGFBP-2 in cellular signaling has been suggested due to its cytosolic or perinuclear localization (19), which may modulate cellular growth and apoptotic signals (20, 21). Therefore, the function of IGFBP-2 could be complex depending on the cell type and cellular microenvironment. In tumor biology, significantly elevated expression of IGFBP-2 has been reported in various malignancies such as prostate (22-24), ovarian (25-27), colon (28), breast (29) and gastric cancer (30, 31), sarcomas (32, 33) and glioma (2, 34). Moreover, IGFBP-2 can be a prognostic marker of breast (35), prostate (36) and ovarian cancer (37), and leukemia (38). These lines of evidence suggest that IGFBP-2 might somehow be involved in tumorigenesis and tumor progression.

Recent studies of gene expression microarrays have revealed specific overexpression of IGFBP-2 in glioblastoma multiforme (GBM), a highly malignant glioma, indicating that IGFBP-2 may play an important role in the malignant progression of glioma (39-43). In this review, we summarize current concepts regarding

Table I. *Summary of the IGFBP family.*

	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-5	IGFBP-6
Gene location	7p13-p12	2q33-q34	7p13-p12	17q12-q21.1	2q33-q36	12q13
Gene size (kbp)	5.3	31.0	9.0	14.0	23.0	4.7
mRNA size (kbp)	1.6	1.4	2.6	2.2	6.3	1.0
Molecular weight (Mature protein)	25 kDa	31 kDa	43-45 kDa	24 kDa	29 kDa	28-30 kDa
Amino acids (aa, Mature protein)	234, 216	289	274	237	252	213
Serum concentration	2-15 nM	2-15 nM	100 nM	2-15 nM	2-15 nM	2-15 nM
RGD motif	(+)	(+)				
Nuclear localization signal			(+)		(+)	
Phosphorylation site	(+)		(+)		(+)	
N-Glycosylation			(+)	(+)		
O-Glycosylation					(+)	(+)

References: 2-8, Entrez Gene (http://www.ncbi.nlm.nih.gov/entrez/ query.fcgi?db=gene) and GeneCards (http://www.genecards.org/ index.shtml). RGD: arginine-glycine-aspartic acid.

IGFBP-2 in the context of malignant phenotypes of GBM, particularly focusing on the IGF signaling-independent roles of IGFBP-2.

Structure–Function Relationship of IGFBP-2 Protein

The precursor of IGFBP-2 is 36 kDa (328 amino acid residues) in size and consists of a signal peptide (40 amino acid residues) and three distinct regions: the highly conserved *N*-terminal region (IGFBP homolog domain, amino acid residues 43-136), the highly conserved C-terminal region with thyroglobulin type 1 repeat (amino acid residues 229-309), and the mid-region with multiple cleavage sites. The *N*- and *C*-terminal domains are cysteine-rich and globular, and both of them have IGF-binding properties that could modulate the IGF/IGF receptor interactions (44). Some reports have emphasized an importance of the *N*-terminal domain by mutagenesis experiments (45) and by iodination protection study (46), while others have described the *C*-terminal region of IGFBP-2 as playing a key role in the binding to IGFs by mutagenesis experiments (47, 48) and by nuclear magnetic resonance spectroscopy (49).

Notably, the *C*-terminal domain contains an arginine-glycine-aspartic acid (RGD) motif and can bind to integrins and glycosaminoglycans. In fact, integrin-mediated functions of IGFBP-2 have been suggested through the RGD motif. IGFBP-2 acts cooperatively with integrin $\alpha_v\beta_3$ and negatively regulates IGF-I-mediated growth and migration of breast cancer cells (50). In contrast, the binding of IGFBP-2 to $\alpha_5\beta_1$ integrin induces outside-in signaling that eventually results in enhanced cellular mobility and reduced phosphorylation of focal adhesion kinase (FAK) and MAP kinase (51, 52). Thus the role of integrins/IGFBP-2 interaction is complex in tumor biology, possibly depending on the cell type. Binding of IGFBP-2 to cell surface proteoglycans has been described in the rat brain olfactory bulb (53), and binding of IGFBP-2 to the extracellular matrix plays an important role in proliferation, migration, and invasion of neuroblastoma cells (54). Heparin binding of the *C*-terminal domain of IGFBP-2 has also been reported (55). On the other hand, it has been reported that the complex formation of IGF, IGFBP and vitronectin, which is a ligand of integrin α_v, plays an important role in IGF I-stimulated cellular migration (56).

IGFBPs are cleaved at specific sites by various proteinases such as serine proteinases including thrombin and prostate specific antigen (human tissue kallikrein 3) (57-59) and matrix metalloproteinase (MMP) (60-62). The cleavage of IGFBPs might be an important regulatory mechanism of pericellular IGF function as the proteolysis of IGFBPs would result in the release of IGF from the IGFBP/IGF complex. In the IGFBP-2 protein, the mid-region includes four major cleavage sites (63). Both positive and negative regulations of IGF function by the cleavage of IGFBP-2 have been suggested (9, 10, 16-18, 64). In addition, since the proteolytic fragment of IGFBP-3 has a direct effect on apoptosis (65), the fragment of IGFBP-2 may have some bioactivities.

Enhanced Expression of IGFBP-2 and its Proposed Roles in GBM

Overexpression of IGFBP-2 mRNA in GBM was first discovered by Fuller *et al.* (2) using gene expression profiling. This finding was confirmed by subsequent studies showing increased IGFBP-2 expression along with progression of the tumor grade in gliomas (5, 34, 39-42) and extended to other tumor types (5). Increased amounts of IGFBP-2 protein have also been reported in GBM (66) and

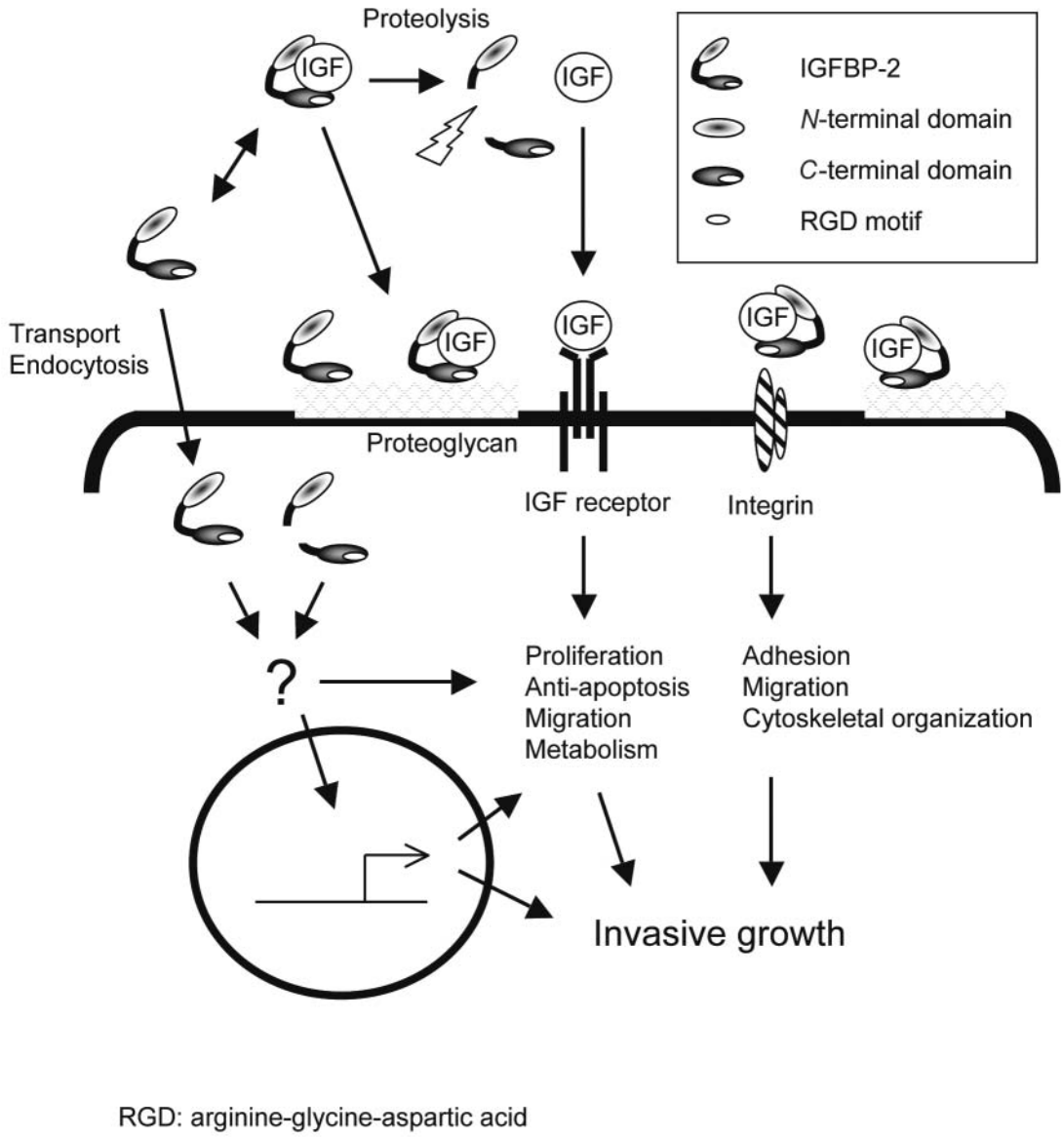

Figure 1. *Proposed mechanisms by which IGFBP-2 induces invasive growth of GBM cells. The proteolytically cleaved IGFBP-2 fragment shows decreased affinity to IGF and releases IGF to induce IGF receptor-mediated signaling. RGD-dependent cell surface association has been shown to result in outside-in signaling mediated by integrin. RGD-independent binding to the cell surface proteoglycan has also been reported, which may modulate cellular function. Moreover, cytosolic and nuclear import of IGFBP-2 is likely to occur, suggesting the existence of additional IGF-independent functions of IGFBP-2 within GBM cells.*

IGFBP-2 was found to be consistently elevated in the CSF of patients with malignant CNS tumors (67). However, little is known regarding the molecular functions of IGFBP-2 in GBM. Since binding of IGFs by IGFBP-2 has growth- and/or migration-inhibitory effects, other mechanisms must be taken into account when the correlation between IGFBP-2 expression and malignant phenotypes of GBM is examined. Some proposed mechanisms which may underlie IGFBP-2-mediated malignant phenotypes of tumor cells (Figure 1) include: (i) RGD-dependent or independent cell surface association of IGFBP-2, which in turn mediates outside-in signaling; (ii) effective use of the "IGF reservoir" function of IGFBP-2 by excess proteinase activities of GBM cells, eventually resulting in concentrated IGF molecules in the pericellular microenvironment; (iii) stimulation of cell proliferation and/or migration in an IGF-independent manner, in which additional functions of intracytoplasmic and intranuclear IGFBP-2 may be critically involved.

There have been a number of studies that suggest potentially important roles of IGFBP-2 in GBM cells. IGFBP-2 and vascular endothelial growth factor (VEGF) are concomitantly up-regulated in GBM cells showing pseudopalisading along the necrotic area, a characteristic histological feature of GBM, suggesting a possible link of IGFBP-2 to angiogenesis (41). Engineered overexpression of IGFBP-2 in GBM cells enhanced cellular invasive capability (52, 68) and up-regulated invasion-enhancing proteins such as MMP-2 (68). We performed a knockdown study using a shRNA retrovirus vector specific for IGFBP-2, and found that the knockdown of IGFBP-2 significantly reduced invasion of GBM cells (69). A similar finding was also reported in ovarian cancer cells by using a neutralizing antibody (70) and siRNA (26). Therefore, it is conceivable to hypothesize that IGFBP-2 is critically involved in the invasive growth of GBM cells. However, the precise mechanism by which IGFBP-2 acts at the cellular level remains to be elucidated, though IGFBP-2/integrin-mediated outside-in signaling may, at least partly, be responsible for the IGFBP-2-mediated malignant phenotype in GBM cells (51, 52).

Upstream and Downstream Factors of IGFBP-2-induced Signaling

The expression level of IGFBP-2 varies considerably, showing high levels during embryonic and fetal development. It is also altered in various pathological conditions. However, it remains unclear how the expression of IGFBP-2 is controlled. Hormonal regulation of expression has been reported in myoblasts and also in the hypothalamus of female rats (71, 72). In the adult brain, IGFBP-2 expression is associated not only with tumors but also with hypoxia, regeneration and trauma (73-75). At the cellular level, the expression of IGFBP-2 is influenced by the functional status of phosphatase and tensin homolog on chromosome ten (PTEN), a known tumor suppressor having an important role in glioma progression (76). The expression level of PTEN is inversely correlated with that of IGFBP-2 in GBM and prostate carcinomas, and serum IGFBP-2 level may be a potential biomarker of PTEN status and the activation of the phosphatidylinositol-3-phosphate kinase (PI3K)-Akt pathway (77). Although PTEN is a negative regulator of IGFBP-2 expression, a recent report has indicated that the expression of PTEN itself is down-regulated by excess IGFBP-2 (78), suggesting that the balance between PTEN and IGFBP-2 may have a critical role in tumor cell biology. The p53 tumor suppressor has also been found to be a transcription factor regulating the expression of IGFBP-2 and therewithal to be influenced by it (79). Regarding the hypoxia-induced expression of IGFBP-2, hypoxia inducible factor 1α (HIF1α), which is induced by IGF, appears to be required for the enhanced expression of IGFBP-2 (80). Analyses of the promoter region of the *IGFBP-2* gene suggest that there are many different signaling pathways explaining the enhanced expression of IGFBP-2 in tumors (5). It lacks a TATA box, but contains GC-rich sequences and therefore putative Sp1-binding sites. Other possibly important regions contain activating enhancer binding protein 4 (AP-4) and nuclear factor-κB (NF-κB) binding sites. Moreover, portions responsible for dexamethasone-induced transcriptional activity and IGF-induced stimulation of transcription have also been reported (5).

To date, little is known regarding the downstream targets of IGFBP-2 in tumor cells. In order to identify the molecules related to IGFBP-2-induced signaling, recent studies have utilized real-time PCR analyses of patients' samples or gene expression profiling of cultured GBM cells undergoing forced expression of IGFBP-2 (2, 41, 42, 68). The results suggest that expression of IGFBP-2 is correlated with the expression of invasive growth-associated genes in GBM such as *MMP-2, VEGF, fibronectin, thrombospondin* and *integrin* α_5 and α_6 (2, 41, 42, 68). The extracellular signal regulated kinase (ERK1/2) and the stress activated protein kinase/*c*-Jun *N*-terminal kinase (SAPK/JNK) pathway have also been shown to be involved in IGFBP-2-induced proliferation of ovarian cancer cells (70). It should be noted that IGFBP-3, a major IGFBP in the serum, can be transferred into the nucleus and has a direct effect on gene transcription (81). Intracytoplasmic or intranuclear localization has been also reported for IGFBP-2 (19-21), indicating the possible existence of a functional interaction between IGFBP-2 and intracellular signaling molecules or transcriptional factors in tumor cells. However, transcriptional activity of IGFBP-2 is not proven. In lung epithelial cells, it has been shown that IGFBP-2 forms a complex with the cyclin-dependent kinase inhibitor p21CIP1/WAF1 in the cytoplasm during growth inhibition (20). An IGFBP-2 binding protein, invasion inhibitory protein-45 (IIp45), has been identified as a negative regulator of IGFBP-2-mediated invasion of GBM cells (82). It interacts with IGFBP-2 through the RGD motif, interferes with IGFBP-2/integrin interaction, and eventually inhibits the expression of transcription factor NF-κB (82).

Taken altogether, IGFBP-2 is not only a modulator of IGF/IGF receptor signaling, but also a pleiotropic factor which has important effects on cellular proliferation, motility, interactions with the extracellular matrix and transcription. Possible molecular interactions of IGFBP-2 in GBM cells are shown in Figure 2.

Conclusion

Various basic and clinical research has revealed overexpression of IGFBP-2 in GBM and its pleiotropic

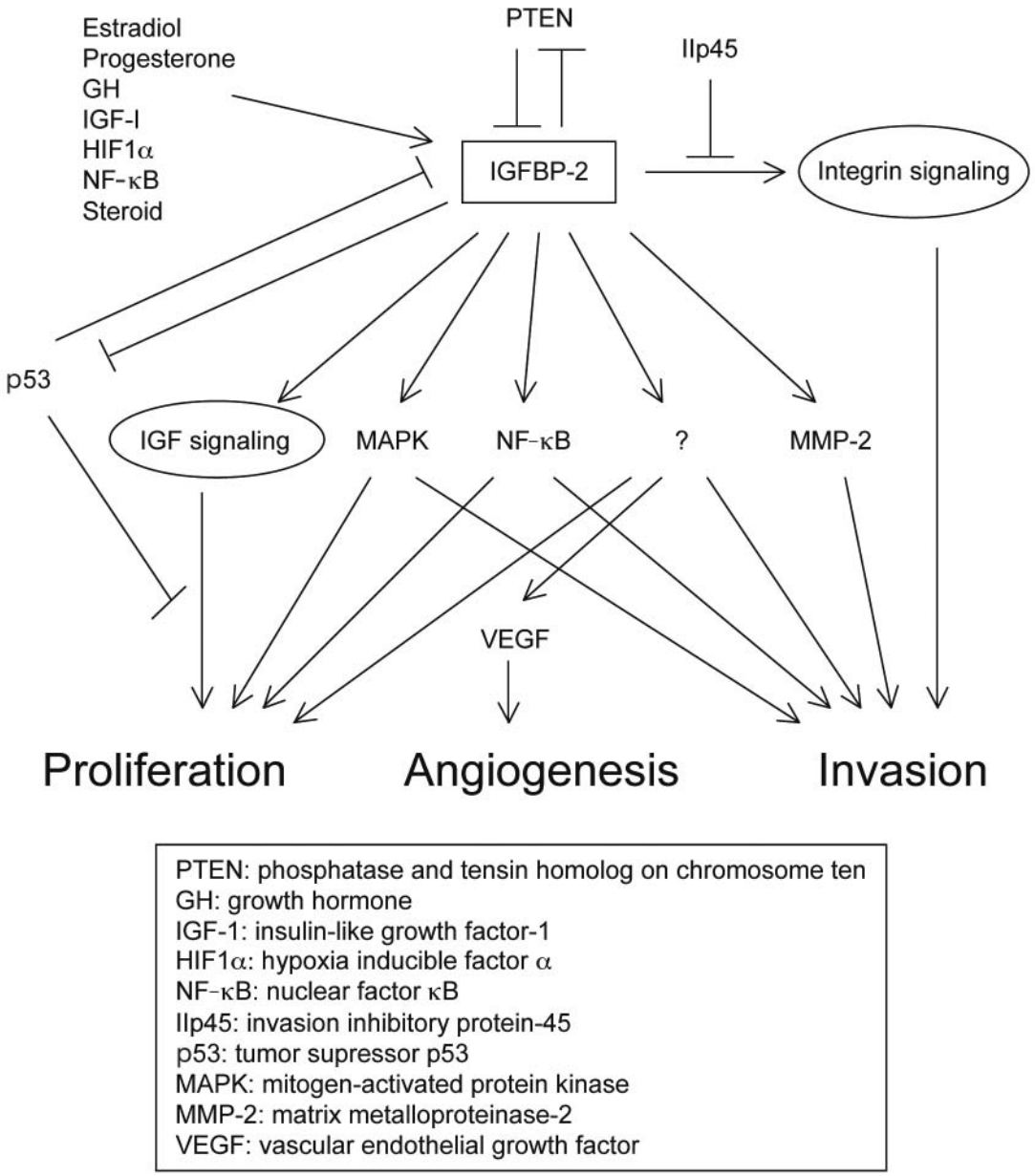

Figure 2. *Possible molecular interactions of IGFBP-2 in GBM cells involved in cellular proliferation, invasion and angiogenesis.*

bioactivities dependent or independent of IGF/IGF receptor signaling. An appropriate understanding of the mechanism underlying the effects of IGFBP-2 in GBM cells is required. Explanation of the biological roles of IGFBP-2 and the regulatory mechanism of IGFBP-2 overexpression in GBM cells will shed light on an important aspect of glioma progression. Moreover, the pathway involved in IGFBP-2-induced malignant phenotypes of GBM cells might represent a potential molecular target or a new therapeutic modality for treatment of GBM and other cancers.

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