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Review

Insulin-like Growth Factor-binding Protein 2 in Tumorigenesis: Protector or Promoter?[1]

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Introduction

The IGF[3] system is composed of the two peptide ligands (IGF-I and IGF-II), six high-affinity IGF-binding proteins (IGFBP-1 to IGFBP-6), and two IGF receptors (IGF-IR and IGF-IIR), of which IGF-IR confers most of the biological actions of IGFs (1, 2). The actions of IGFs may be modulated by the IGFBPs in either a positive or negative way, depending on tissue type and physiological/pathological status (3). Furthermore, IGF-independent effects of IGFBPs have been described (4).

IGFBP-2 mRNA is already expressed in preimplantation embryos (5), and expression continues at high levels in many tissues during embryonic and fetal development (6, 7). In the postnatal period, IGFBP-2 is the second most abundant IGFBP in the circulation and is present in various other biological fluids and tissues of many vertebrate species (reviewed in Refs. 8 and 9).

Serum IGFBP-2 levels are elevated in many acute or chronic nonphysiological situations, such as shock, fasting, hypoxemia, or after traumata, suggesting complex regulation of IGFBP-2 expression. Furthermore, elevated serum IGFBP-2 concentrations are associated with reduced growth of mice selected for low body weight (10). Recently, quantitative trait loci affecting the serum activity of IGFBP-2 have been mapped in a segregating pedigree of phenotypically different mouse strains (11).

Targeted inactivation of the *IGFBP-2* gene in mice resulted only in subtle phenotypic consequences, eventually due to functional compensation by other IGFBPs which were found to be up-regulated in this model (12–14). In contrast, transgenic mice overexpressing IGFBP-2 under the control of the CMV promoter displayed significantly reduced body weight gain, demonstrating that IGFBP-2 is a negative regulator of normal somatic growth, most probably by sequestering IGFs from their receptors (15–17). The inhibitory effect of IGFBP-2 was even more pronounced when the CMV-IGFBP-2 transgene was crossed into a transgenic mouse model with high GH and IGF-I levels, further supporting the concept of IGFBP-2 being an inhibitor of growth (18).

On the other hand, IGFBP-2 is overexpressed in many malignancies and is often correlated with an increasingly malignant status of the tumor, pointing to a potential involvement of IGFBP-2 in tumorigenesis. However, the mechanisms by which IGFBP-2 might affect tumorigenesis are largely unclear.

This review summarizes current concepts of IGFBP-2 actions in the context of tumor growth, most of which have been deduced from IGFBP-2-treated or -transfected tumor cell lines.

IGFBP-2 Levels in Patients with Cancer: Tumor Marker and/or Prognostic Factor?

Increased serum IGFBP-2 levels were found in patients suffering from malignancies of the lung (19), colon (20, 21), adrenal glands (22), ovary (23, 24), prostate (25), and CNS (26); in patients with lymphoid tumors (27–29); in patients with non-islet cell tumor hypoglycemia (8, 30–32); and in children suffering from Wilms' tumor (33), but not in endometrial cancer patients (34, 35).

Serum IGFBP-2 concentrations in combination with carcinoembryonic antigen determinations were highly prognostic for metastasis and recurrence of colon cancers, and their use in cancer surveillance was suggested (36, 37). Nevertheless, increased serum IGFBP-2 levels before the clinical manifestation of cancer correlated with decreased colon cancer risk, as shown by a recent prospective study (38).

In ovarian cancer patients, serum IGFBP-2 levels were positively correlated with the ovarian tumor marker CA 125 (24).

Serum IGFBP-2 levels of patients with prostate carcinoma were elevated (Fig. 1*A*) and significantly correlated with the levels of PSA (Fig. 1*B*). Therefore, serum IGFBP-2 measurement might be useful in patients with low serum PSA levels and for monitoring the prostate tumor burden (25, 39, 40). In addition, higher tissue levels of IGFBP-2 are present in hormone-refractory prostate cancers as compared with benign prostate glands or primary prostate cancers (Ref. 41; Fig. 1*C*). However, serum IGFBP-2 levels are not indicative of an increased prostate cancer risk because serum IGFBP-2 levels are normal before the diagnosis of prostate cancer, as shown by a recent prospective study (42). Also, in postoperative serum samples from prostate cancer patients, IGFBP-2 concentrations were of no prognostic value with respect to recurrence of the tumor (43).

IGFBP-2 was consistently elevated in the CSF of patients with malignant CNS tumors, whereas in patients with peripheral tumors, normal IGFBP-2 levels were found in the CSF (26). This observation led to the suggestion of CSF IGFBP-2 as a specific marker for CNS tumors.

Tissue levels of IGFBP-2 were low in well-differentiated hepatoblastoma or normal livers but high in poorly differentiated hepatoblastoma, and it was suggested that IGFBP-2 might serve as a marker of tumor differentiation (44). The liver is known to produce and

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[3] The abbreviations used are: IGF, insulin-like growth factor; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; FAK, focal adhesion kinase; GH, growth hormone; HIF-1α, hypoxia-inducible factor 1α; IGFBP, insulin-like growth factor-binding protein; IGF-IR, insulin-like growth factor I receptor; IGF-IIR, insulin-like growth factor II/mannose 6-phosphate receptor; PLG, plasminogen; PSA, prostate-specific antigen; RGD, arginin-glycine-aspartic acid; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor.

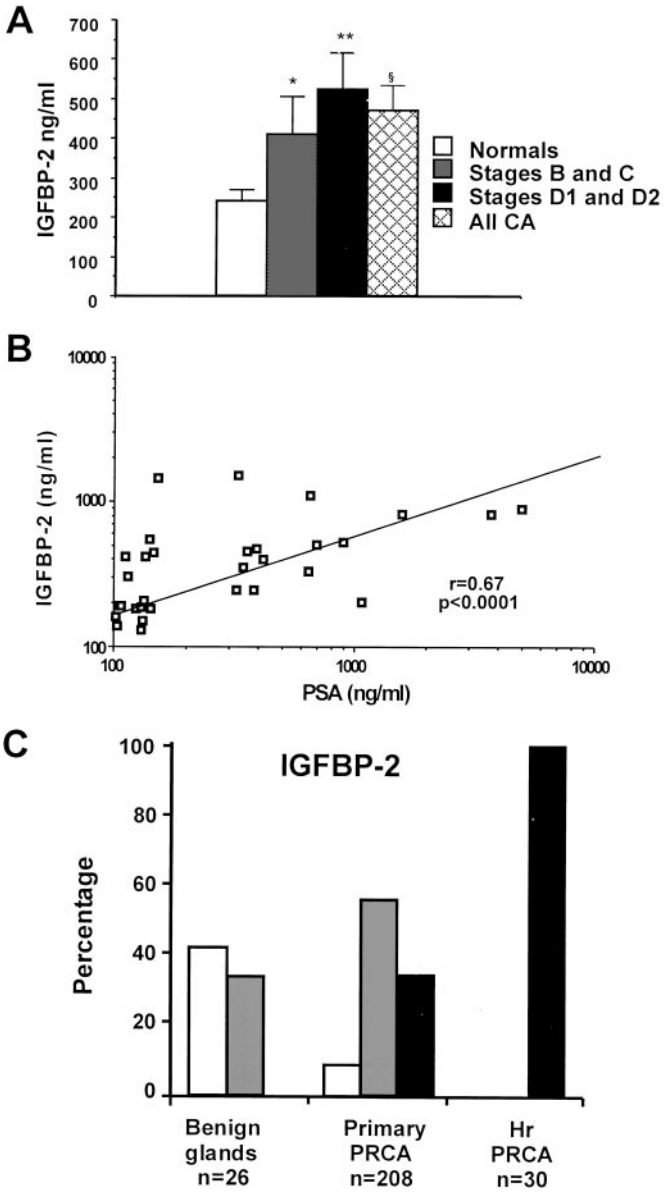

Fig. 1. *A*, serum IGFBP-2 concentrations in different stages of prostate cancer. *Normals*, age-matched controls ($n = 16$); *Stages B and C*, early clinical stages of prostate cancer; *Stages D1 and D2*, late clinical stages of prostate cancer; *All CA*, prostate cancer stages B, C, D1, and D2 ($n = 32$). Reproduced with permission from Cohen *et al.* (Ref. 25; © The Endocrine Society). *B*, correlation of serum IGFBP-2 concentration to serum PSA levels ($n = 32$). Reproduced with permission from Cohen *et al.* (Ref. 25; © The Endocrine Society). *C*, frequency distribution of IGFBP-2 expression during progression to hormone-refractory prostate cancer (*Hr*) as measured by immunohistochemistry on a prostate cancer tissue microarray (*PRCA*, primary untreated prostate cancer; *white*, *gray*, or *black bars*: weak, moderate, or strong immunoreactivity, respectively). Reproduced with permission from Bubendorf *et al.* (Ref. 41; © Oxford University Press).

secrete high amounts of IGFBP-2. Therefore, high tissue levels of IGFBP-2 do not necessarily indicate increased expression of IGFBP-2 but might also indicate altered secretion or even uptake of IGFBP-2.

Control of IGFBP-2 Expression

The regulation of IGFBP-2 expression and abundance is highly complex and influenced by multiple hormones and growth factors (reviewed in Ref. 3). Known hormonal regulators of IGFBP-2 expression include GH (45–47), IGF-I (48–51), IGF-II (52, 53), human chorionic gonadotropin (54–56), follicle-stimulating hormone (57), TGF-β (58, 59), interleukin 1 (60), estradiol (61), glucocorticoids (62–64), and insulin (49, 65, 66).

The proximal promoter region of the *IGFBP-2* gene lacks a TATA box but contains GC-rich sequences and therefore putative Sp1 binding sites and is highly conserved in mammalian species (67–71). Recently, Sp1 binding to distinct regions (−778 to −752 and −701 to −687) upstream of the transcription start site has been shown by gel retardation assays (72). In addition, a distal region of the IGFBP-2 promoter has been found to be important for its activity (73). In this distal region, binding of the basic helix-loop-helix transcription factor AP-4 was shown to potently activate *IGFBP-2* gene transcription. By using 1.4 kb from the promoter region upstream of the transcription start site from the rat *IGFBP-2* gene, dexamethasone-induced transcriptional activity of a luciferase reporter gene construct was demonstrated (62). Another promoter study revealed that 633 bp of the IGFBP-2 promoter are sufficient for IGF-induced stimulation of transcription (74). IGF-I also induced expression of HIF-1α, which is involved in the control of IGFBP-2 expression because HIF-1α-deficient embryonic stem cells had reduced IGFBP-2 expression. Notably, the redox-regulated transcription factor nuclear factor κB can bind the IGFBP-2 promoter region at four distinct positions (75). Nuclear factor κB has been suggested to activate IGFBP-2 expression in lung alveolar epithelial cells under oxidative stress. Thus, there are many different signaling pathways explaining the high levels of IGFBP-2 in cancer.

Expression of IGFBP-2 by Tumors and Tumor Cell Lines

Expression of IGFBP-2 by tumors has often been demonstrated, and a positive correlation between the tumor grade and level of IGFBP-2 expression has been described in colon tumors (36, 37, 76), adrenal cancer (22, 77–79), mammary tumors (80–85), ovarian cancer (23, 86, 87), prostate cancer (25, 39, 40, 88–91), CNS tumors (26, 92–95), leukemia (27, 96), hepatoblastoma (44), thyroid cancer (97), and Wilms' tumors (98).

IGFBP-2 is also secreted by cancer cell lines derived from lung (99–102), colon (103–110), adrenal gland (111–114), mammary gland (115–118), ovary (119, 120), prostate (121–123), CNS (124–127), skin (128, 129), thyroid gland (130), pancreas (131, 132), and lymphoid tumors (133, 134). The following sections describe regulatory mechanisms of IGFBP-2 expression in various tumor types.

Colon Cancer. In Caco-2 cells, IGFBP-2 secretion is stimulated by short-chain fatty acids, bacterial products present in the intestine (135). Notably, a connection between histone acetylation and IGFBP-2 secretion was found in this study: both butyrate and trichostatin A, a known inhibitor of histone deacetylase, resulted in increased histone H4 acetylation and IGFBP-2 secretion in Caco-2 cells.

Adrenal Cancer. Normal human adrenocortical cells express and secrete IGFBP-1 to IGFBP-5. Whereas the expression and secretion of IGFBP-1, IGFBP-3, and IGFBP-5 are regulated by adrenocorticotropic hormone and IGFs, the secretion of IGFBP-2 remains unaffected by the different hormones (136). The up-regulation of IGFBP-2 in adrenocortical carcinomas was interpreted as a posttranscriptional mechanism because IGFBP-2 mRNA remained unaltered in the tumors (22). In the human adrenocortical carcinoma cell line NCI-H295R, fibroblast growth factor 2 stimulated cell proliferation and inhibited IGFBP-2 expression as well as maturation of pro-IGF-II to IGF-II (114). This would argue for an inhibitory effect of IGFBP-2 in this model.

Breast Cancer. In a human breast cancer cell line (MCF-7) the addition of exogenous estradiol stimulated secretion of IGFBP-2 (116). This is in contrast to findings in MCF-7/6 cells, which predominantly produce IGFBP-2 and IGFBP-5 and in which estradiol-17β specifically decreased production and secretion of both IGFBPs (118), and to *in vivo* studies where estrogen inhibited both growth and

IGFBP-2 expression of tumors derived from a mammary adenocarcinoma cell line (R32030AC) after injection into rats (83). Reduced secretion of IGFBP-2 was associated with the development of tamoxifen resistance and estrogen independence in human breast cancer cell lines (137). Exposure of different breast cancer cells to IGF-I and retinoic acid resulted in an increased secretion of IGFBP-2 (138–141). The fact that retinoic acid-induced inhibition of bovine mammary tumor cell (MAC-T cells) proliferation was associated with marked accumulation of IGFBP-2 in the conditioned media and in plasma membrane preparations (142) points to a link between the growth-inhibitory effects of retinoic acid and a growth-inhibitory effect of IGFBP-2. Administration of human chorionic gonadotropin to female rats increased *IGFBP-2* and *IGFBP-5* gene expression and reduced IGFBP-3 and IGF-IR mRNA expression in the mammary gland (54).

Female Reproductive Tissue. In endometrial carcinoma cells (HEC-1B), IGF-I induced secretion of IGFBP-2 and IGFBP-3 within 24 h (143). Moreover, in HEC-1B cells and Ishikawa cells, activation of the protein kinase C by phorbol 12-myristate 13-acetate resulted in a reduction of IGFBP-2 mRNA abundance (144). Therefore, a link has been established between protein kinase C signaling and control of IGFBP expression. In the human endometrial carcinoma cell line HEC-1A, platelet-activating factor induced secretion of IGFBP-2 (145). Because platelet-activating factor is known to represent an autocrine growth factor for these cells and because HEC-1A cells are almost insensitive to exogenous IGF-I, IGF-II, or des(1–3) IGF-I, increased IGFBP-2 secretion might have IGF-independent effects in these cells.

Prostate Cancer. In rats, castration or antiandrogen treatment increased gene expression of *IGFBP-2* and other IGFBPs in the prostate (146, 147). It might thus be speculated that androgens represent repressors of IGFBP expression. Overexpression of IGFBP-4 in transfected M12 prostate epithelial cells resulted in enhanced sensitivity to induction of apoptosis and in a reduction of tumor development when injected into nude mice (122). Notably, IGFBP-2 secretion was reduced in all IGFBP-4-transfected clones. Dihydrotestosterone induced IGFBP-2 in conditioned media from LNCaP prostate carcinoma cells by 50% (148).

CNS Tumors. In contrast to other tissues (*e.g.*, breast cancer tissue), retinoic acid consistently decreased IGFBP-2 concentrations in human neuroblastoma cells, indicating tissue-specific regulation and function of IGFBP-2 (149). In C6 glioma cells, expression of IGF-I antisense RNA had a negative effect on cell proliferation but also led to decreased IGFBP-2 expression (127). In contrast, N-*myc* overexpression in SK-N-SH neuroblastoma cells resulted in enhanced expression of IGFBP-2 and other components of the IGF system (150). N-*myc*-overexpressing cells also displayed enhanced proliferative activity and were tumorigenic when injected s.c. into nude mice, whereas control cells did not form tumors in this system. The study demonstrated activation of IGF-IR mRNA transcription by N-*myc* and provided a link between this oncogene and the IGF system.

Leukemia. When leukemic T cells were kept in medium containing 5% FCS, these cells secreted increased amounts of IGFBP-2. Subsequent analysis revealed that endogenous IGF-II stimulates IGFBP-2 secretion and growth of these cells through the IGF-IR (134).

Neuroendocrine Tumors. Expression of the IGF system has been studied in 37 neuroendocrine tumor specimens by reverse transcription-PCR (151). Among all other components, only IGFBP-2 mRNA was consistently present in all tumor samples analyzed, and a tumor-promoting role has been suggested for IGFBP-2.

Effects of IGFBP-2 in Tumorigenesis: Conclusions from *in Vitro* Studies

Inhibitory Effects. Inhibitory effects have been attributed to IGFBP-2 in different cell lines (103, 124, 125, 152) because IGF analogues with reduced affinity for the IGFBPs were more potent than normal IGF-I in stimulating cell proliferation (Fig. 2). Furthermore, the addition of exogenous IGFBP-2 had negative effects on cell proliferation in different IGF-dependent cell culture systems.

In small cell lung cancer cells, soluble IGFBP-2 inhibited IGF-dependent DNA synthesis (153). By cross-linking analysis, membrane-associated IGFBP-2 was found, and IGFs were linked exclusively to IGFBP-2, but not to IGF-IR or IGF-IIR, which are also present in small cell lung cancer cells. It was concluded that both soluble and membrane-associated IGFBP-2 molecules compete with IGF receptors for ligand binding and exert an inhibitory effect on IGF-mediated actions.

In nontransformed IEC-6 cells, exogenous IGFBP-2 resulted in decreased proliferation (154), whereas expression of antisense IGFBP-2 resulted in growth stimulation (155).

In human embryonic kidney fibroblast cells (293 cells), overexpression of mouse IGFBP-2 resulted in IGF-dependent growth inhibition because the addition of exogenous IGF-I or IGF-II completely eliminated the negative effect of IGFBP-2 and because LongR3-IGF-I was more potent than native IGF-I or IGF-II (152).

In PC12 cells (rat pheochromocytoma cells), exogenous IGFBP-2 represented an inhibitor of antiapoptotic action exhibited by IGF-I or IGF-II (111). As a potential mechanism for the inhibition of IGF-induced survival, direct sequestration of the IGFs by IGFBP-2 was presumed.

When C6 glioma cells were transfected with an expression vector for ovine IGFBP-2, clones with low or intermediate IGFBP-2 expression displayed reduced proliferative activity (156). In contrast, clones with the highest levels of IGFBP-2 expression did not show reduced cell proliferation, presumably because of the increased IGF-I expression that was observed in these clones and may have compensated for the negative effect of IGFBP-2 (156).

Stimulatory Effects on Cell Proliferation. There are a number of *in vitro* studies (*e.g.*, studies using colon, adrenal, and prostate cancer cells or nontransformed osteoblasts) in which a positive association between IGFBP-2 expression and cell proliferation was detected. Notably, in human colon carcinoma cells (Caco-2), a positive correlation between IGFBP-2 levels and cell proliferation has been found (108). Whereas IGFBP-3 and IGFBP-4 clearly correlated with markers of differentiation, IGFBP-2 significantly correlated with prolifer-

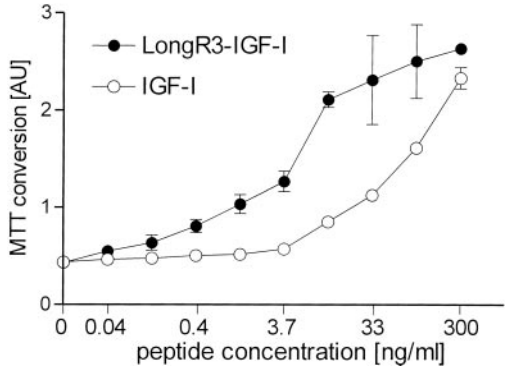

Fig. 2. Growth inhibition in 293 cells stably transfected with an IGFBP-2 expression vector is compensated by using high amounts of IGF-I. LongR3-IGF-I, which is not bound by IGFBP-2, stimulates cell proliferation at much lower concentrations as compared with IGF-I. Reproduced with permission from Höflich *et al.* (Ref. 152; © Federation of European Biochemical Societies).

ative activity in Caco-2 cells. In accordance with our findings, enhanced IGFBP-2 expression in rapidly growing preconfluent Caco-2 cells has also been described in another study (109).

In a model of malignant transformation using prostate cells with different degrees of malignancy (benign prostate cells, SV40 large T antigen-immortalized prostate epithelial cells, P69 cells, and two sublines of P69 cells), secretion of IGFBP-3 to IGFBP-6 decreased as cells became progressively tumorigenic, whereas IGFBP-2 remained stable and represented the predominant IGFBP in the malignant cells (123). Importantly, coincident with increasing malignancy, the cells also became more and more independent of IGF-I, a finding that was consistent with decreasing IGF-IR mRNA expression.

Birnbaum *et al.* (157) performed a cell culture study with nontransformed rat osteoblasts in which they found high mRNA expression for IGFBP-2 in proliferating cells. When differentiation was inhibited, enhanced IGFBP-2 secretion was found. It was concluded that the differentiation status of osteoblasts determines the secretion profile of the IGFBPs.

In Madin-Darby bovine kidney cells, IGFBP-2 was able to stimulate cell proliferation. However, the stimulating effect of IGFBP-2 was dependent on the presence of an unknown factor in platelet-poor plasma (158).

We have studied the effects of elevated IGFBP-2 on mouse adrenocortical tumor cells (Y-1 cells), which do not express detectable levels of IGFBP-2 (159). Overexpression of mouse IGFBP-2 in transfected Y-1 cells resulted in an increase of tumorigenic potential and in altered cellular morphology. IGFBP-2-expressing cell clones proliferated faster and formed more colonies under anchorage-independent conditions as compared with mock-transfected control cells. Because this effect could not be blocked by the addition of the anti-IGF-IR antibody αIR-3 and because Y-1 cells are quite unresponsive to exogenous IGFs, an IGF-independent effect of IGFBP-2 has been assumed in this model. Data also point to a stimulatory role of IGFBP-2 on tumor progression and cell growth in mammary tissue. Notably, exogenous IGFBP-2 and IGFBP-3 had stimulatory effects on IGF-I-induced proliferation of MCF-7 cells, whereas IGFBP-4 and IGFBP-5 had no effects (160).

Recombinant porcine IGFBP-2 was shown to increase both basal and IGF-induced proliferation of porcine endometrial glandular epithelial cells, and both IGF-dependent and -independent mechanisms have been suggested in this model (Ref. 161; Fig. 3*A*). Because the IGF analogue with selective affinity for the IGF-IIR ([Leu^{27}]-IGF-II), alone or in combination with IGF-I, had stimulatory effects on cell proliferation, an involvement of functional IGF-IIR in mitogenic signaling has been presumed. Epidermoid carcinoma cells (KB 3.1) were stably transfected using human IGFBP-2 cDNA (162). After s.c. injection of clones expressing high amounts of IGFBP-2 into nude mice, tumors developed faster and grew larger than they did after the injection of control clones. However, this did not seem to be a direct effect of IGFBP-2 overexpression because increased proliferation of IGFBP-2-overexpressing cell clones was not observed *in vitro*. Interestingly, in the tumors, decreased IGFBP-1 expression and increased IGFBP-3 proteolysis were found, eventually due to an enhanced expression of tissue-type PLG activator and decreased expression of its inhibitor (PLG-activator inhibitor type I). Therefore, increased IGFBP-3 proteolysis might be the mechanism responsible for this enhanced growth of IGFBP-2-overexpressing tumors *in vivo*, and additional studies are required to understand the link between increased IGFBP-2 expression and IGFBP-3 proteolysis.

Potential Mechanisms of IGFBP-2 Action

Interference with Effects of IGF. Several *in vitro* studies (152–154) show that the consequence of IGF ligand binding to IGFBP-2 is an inhibition of the effects of IGF. In contrast, stimulation of the effects of IGF is related in some studies to decreased affinity due to proteolytic cleavage of IGFBP-2 (163, 164), which also supports the concept of IGF-dependent growth-inhibitory effects of IGFBP-2.

Cell Surface Binding. Binding of IGFBP-2 to proteoglycans and to the extracellular matrix or heparin has been demonstrated previously (165, 166). RGD-dependent cell surface association of IGFBP-2 has been investigated in two different tumor cell lines (Ewing's sarcoma A673 and breast cancer cell line Hs578T). In both cell lines, exogenous IGFBP-2 bound to the cell surface in an $\alpha_5\beta_1$ integrin-dependent manner and reduced phosphorylation of FAK and cell adhesion (167, 168). Notably, both RGD-dependent and -independent cell membrane association are known to be related to cell-cell interactions, and therefore an implication of IGFBP-2 for cell adhesion can be assumed.

Proteolysis. Controlled release of the IGFs from IGFBP-2 by specific proteolytic cleavage might represent a mechanism of time- and tissue-specific growth control. Proteolysis of IGFBP-2 has been described in a neuroblastoma cell line (SK-N-SH) upon the addition of PLG (163). In these cells, IGFBP-2 fragments (16 and 20 kDa) with reduced affinity for IGF-II were described, and an increase in proliferative activity has been explained by free IGF-II dissociated from the IGFBP-2 fragments. Because αIR-3, a specific inhibitor of the IGF-IR, blocked PLG-induced cell proliferation, this assumption seems plausible.

Proteolysis of IGFBP-2 has also been described in another neuroblastoma cell line (SK-N-MC; Ref. 169). In these cells, basic fibroblast growth factor induced proteolysis of both membrane-bound and secreted IGFBP-2, giving rise to fragments of 14 and 22 kDa with reduced affinity for IGF-I. Whereas the 22-kDa fragment lost its capability of binding the cell surface, the 14-kDa IGFBP-2 was still able to associate with the cell membrane and bind IGF-I. In neuroblastoma cells, retinoic acid and TGF-β had antagonistic effects on PLG-induced mitosis and proteolysis of IGFBP-2. The addition of

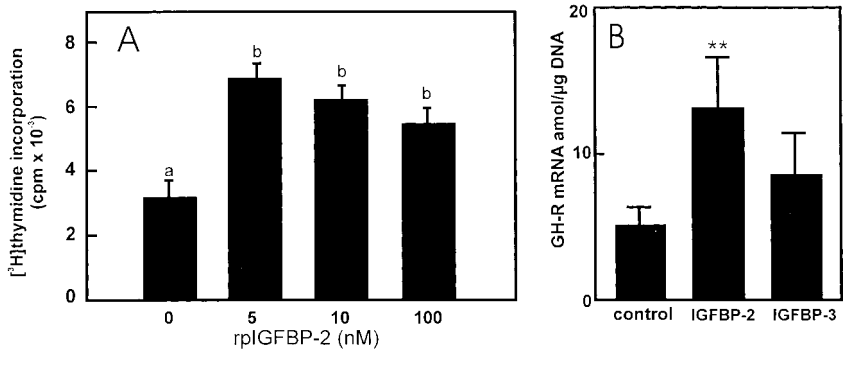

Fig. 3. *A*, stimulation of basal mitogenesis in endometrial cells by increasing amounts of exogenous recombinant porcine IGFBP-2. Reproduced with permission from Badinga *et al.* (Ref. 161; © Society for Endocrinology). *B*, effects of IGFBP-2 and IGFBP-3 on GH receptor mRNA levels in rat osteosarcoma cells. Reproduced with permission from Slootweg *et al.* (Ref. 174); © Society for Endocrinology).

retinoic acid resulted in increased cell proliferation after 24 h, whereas the presence of TGF-β resulted in a reduction of PLG-induced mitosis and proteolysis of IGFBP-2 (163, 170). These results are consistent with studies in which retinoic acid decreased IGFBP-2 mRNA stability (171) or correlated with reduced concentrations of IGFBP-2 in different neuroblastoma cells (149).

Hydrolysis of IGFBP-2 secreted by rat osteosarcoma cells (UMR-106) resulted in IGF-dependent growth stimulation, indicating a growth-inhibitory effect of IGFBP-2 in that cell system (164).

Strong IGFBP-2 proteolytic activity was also found in colon cancer extracts, but not in normal colon extracts (172), and it was speculated that enhanced IGFBP-2 proteolysis might lead to increased levels of free IGFs and thereby confer an advantage for the colon carcinoma cells.

LNCaP and PC-3 prostate carcinoma cell-conditioned media were shown to contain cathepsin D or procathepsin D, respectively (173). Because incubation of cell-conditioned media at 37°C for 24 h at an acidic pH led to the disappearance of all IGFBPs and to increased ^{125}I-IGF-I binding to LNCaP cells, it was speculated that cathepsin D is able to modify the actions of IGF-I by proteolysis of the IGFBPs.

Stimulation of GH Receptor Binding. An IGF-independent mode of action of IGFBP-2 has been described in the rat osteosarcoma cell line UMR-106.01 (174). In this system, exogenous IGFBP-2 as well as IGFBP-3 stimulated GH receptor binding and mitogenesis at high concentrations. IGFBP-2 but not IGFBP-3 significantly increased GH receptor mRNA expression (Fig. 3*B*). IGF-independent GH-mediated effects were suggested because exogenous IGF-I or IGF-II had no effects on cell proliferation.

Potential Functions of IGFBP-2 during Cancer Progression (Apoptosis, Angiogenesis, and Metastasis). By the combined use of cDNA and tissue microarrays, IGFBP-2 has been found to be consistently overexpressed in end-stage hormone-refractory prostate tumors, as discussed previously (41). Interestingly, heat shock protein 27 was coexpressed in 31% of the end-stage tumors, and a potential link between IGFBP-2 and antiapoptotic pathways seems indicated (41). This link had been previously suggested to play a role in the mouse brain for protection and repair of mitral neurons (175). In this study, IGF-I induced expression of bcl-2 and IGFBP-2, and therefore an involvement of IGFBP-2 in two different antiapoptotic pathways was suggested.

Neoangiogenesis is known to play an important role in multistage carcinogenesis, and reactivation of IGFBP-2 expression in high-grade tumors of the CNS (glioblastoma multiforme) but not in lower-grade tumors (astrocytomas, oligodendrocytomas) was accompanied by VEGF expression in advanced cancer (93). Notably, the hypoxia-inducible factor HIF-1α represents a common regulator of VEGF and IGFBP-2 and has been demonstrated to be involved in both VEGF (176) and IGFBP-2 expression (177).

As mentioned above, Schütt *et al.* (168) demonstrated that IGFBP-2 reduced cell adhesion by an integrin-dependent mechanism. An involvement of IGFBP-2 with metastasis has to be demonstrated *in vivo*.

Induction of Antioxidative Mechanisms and Nuclear Localization. Elevated IGFBP-2 expression has been measured after exposing lung epithelial cells to oxidative stress (178, 179). It is therefore tempting to speculate that IGFBP-2 might activate defense mechanisms against oxidative stress and thereby contribute to the highly malignant phenotype of many cancers characterized by overexpression of IGFBP-2. Besnard *et al.* (179) have related induction of IGFBP-2 by oxidative stress to IGFBP-2 translocation to the nucleus (Fig. 4*A*). Our own data complement the presented results because we find cytosolic uptake of IGFBP-2 present in the culture medium by different colon cancer cells (Fig. 4*B*). Although the consequences of the cytosolic import are unknown at present, the findings suggest additional functions of IGFBP-2 within the cell or even in the nucleus.

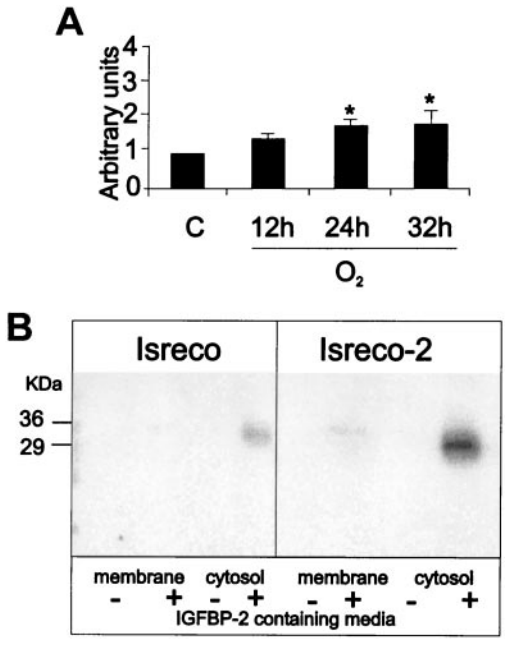

Fig. 4. *A*, IGFBP-2 is present in nuclear extracts from lung adenocarcinoma cells. Hyperoxia results in an increase of IGFBP-2 present in the nuclei. Reproduced with permission from Besnard *et al.* (Ref. 179; © 2001, Elsevier Science). *B*, cytosolic import of IGFBP-2 by different colon carcinoma cell lines. Cells were incubated for 12 h in either conditioned media of CMV-IGFBP-2-transfected 293 cells (152) containing 1.5 μg/ml IGFBP-2 or conditioned medium from nontransfected 293 cells containing no IGFBP-2. Monolayers were washed, harvested using a cell scraper, and centrifuged for 5 min (400 $\times$ *g*); the pellet was dissolved in a lysis buffer (10 mM Tris, 4.2 mM phenylmethylsulfonyl fluoride, and 1 trypsin inhibitor unit/ml aprotinin) and lysed for 1 min on ice using a cell homogenizer (Art, Mühlheim, Germany). Cell debris and nuclei were separated by centrifugation (1000 $\times$ *g*, 10 min, 4°C). The supernatant containing cell membranes and the cytosolic fraction was separated by centrifugation for 30 min at 21,000 $\times$ *g* at 4°C. Equal amounts of proteins (30 μg/lane) were separated by 12% SDS-PAGE, and IGFBP-2 was identified by Western ligand blot analysis using ^{125}I-IGF-II. Signals were detected by a PhosphorImager (Molecular Dynamics, Inc., Krefeld, Germany).

Conclusions and Outlook

IGFBP-2 is a highly sensitive marker of malignant progression in different tumors, although serum IGFBP-2 concentrations have no predictive value for tumor risk in human patients. Several IGFBP-2 *in vitro* and *in vivo* models suggested that binding of IGFs by IGFBP-2 has growth-inhibitory consequences; therefore, other mechanisms must be taken into account when the correlation between IGFBP-2 and tumor growth is examined. To date, four principles of bioactivity have been described for IGFBP-2 (Fig. 5): (*a*) proteolysis of IGFBP-2 is a specific process that gives rise to defined IGFBP-2 fragments with distinct affinity for the IGFs and potential activity on all other levels; (*b*) cell surface association in a RGD-dependent manner has been shown to result in altered FAK phosphorylation and cell adhesive properties, whereas cell surface association via proteoglycans has also been described to occur in a RGD-independent manner; (*c*) IGFBP-2 also stimulated cell proliferation in an IGF-independent manner; and (*d*) cytosolic and/or nuclear import of IGFBP-2 suggests additional functions of IGFBP-2 within the cells.

At present, the most urgent questions are as follows: (*a*) can IGFBP-2 be positioned within the sequence of multistage carcinogenesis; (*b*) what are the factors controlling access of IGFBP-2 to the cytosol and/or the nucleus; (*c*) are there specific target genes induced by IGFBP-2; (*d*) which factors induce *IGFBP*-2 gene expression; and (*e*) what is the significance of RGD-independent cell surface associ-

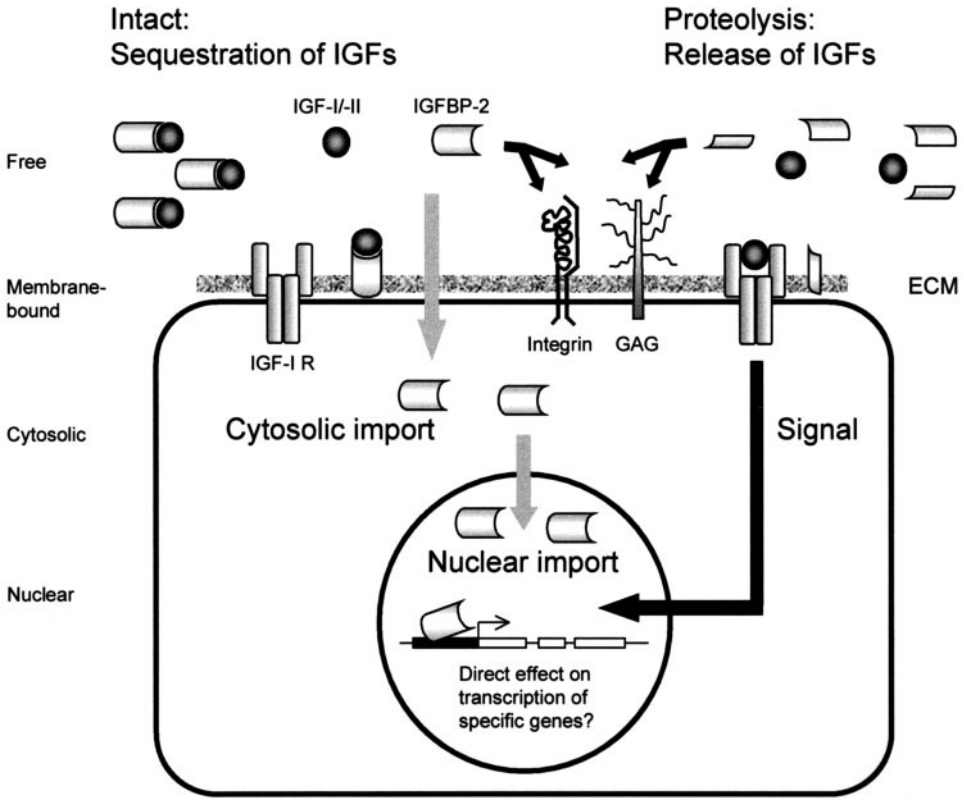

Fig. 5. Potential mechanisms of IGFBP-2 actions. IGF binding prevents mitogenic signaling of the IGFs, whereas after proteolytic cleavage, the IGFs dissociate from IGFBP-2 and transfer mitogenic signals via the IGF-IR. IGFBP-2 and/or proteolytic IGFBP-2 fragments can associate with the cell membrane or the ECM. RGD-dependent and -independent binding has been shown to $\alpha_5\beta_1$ integrins and to proteoglycans, respectively. After RGD-dependent cell surface association, reduced FAK phosphorylation and cell adhesion have been described. Furthermore, IGFBP-2 or IGFBP-2 fragments have been shown to be imported into the cell or even into the nucleus, where additional IGF-independent actions might be exerted (*e.g.*, IGFBP-2 has been shown to stimulate GH receptor expression, GH binding to the cell surface, and cell proliferation). *ECM*, extracellular matrix; *GAG*, glycosaminoglycan.

ation of IGFBP-2? The new methodologies applied at present will certainly lead to an appropriate understanding of the central capacities covered by this versatile and potent IGFBP.

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