

Review

Functional roles and clinical values of insulin-like growth factor-binding protein-5 in different types of cancers

Gökçe Güllü*, Sevgi Karabulut* and Mustafa Akkiprik

Abstract

Insulin-like growth factor-binding proteins (IGFBPs) are critical regulators of the mitogenic activity of insulin-like growth factors (IGFs). IGFBP5, one of these IGFBPs, has special structural features, including a nuclear transport domain, heparin-binding motif, and IGF/extracellular matrix/acid-labile subunit-binding sites. Furthermore, IGFBP5 has several functional effects on carcinogenesis and even normal cell processes, such as cell growth, death, motility, and tissue remodeling. These biological effects are sometimes related with IGF (IGF-dependent effects) and sometimes not (IGF-independent effects). The functional role of IGFBP5 is most likely determined in a cell-type and tissue-type specific manner but also depends on cell context, especially in terms of the diversity of interacting proteins and the potential for nuclear localization. Clinical findings show that IGFBP5 has the potential to be a useful clinical biomarker for predicting response to therapy and clinical outcome of cancer patients. In this review, we summarize the functional diversity and clinical importance of IGFBP5 in different types of cancers.

Key words Cancer, IGFBP5, structure, function, proteolysis, clinical importance

The insulin-like growth factor (IGF) signaling pathway plays a crucial role in regulating cell growth, differentiation, and apoptosis. All these actions are critical events for normal cell physiology and in carcinogenesis^[1]. The IGF axis consists of two peptide growth factors (IGF-I and IGF-II)^[2,3], two IGF receptors (IGFIR and IGFIIR)^[4,5], a family of six IGF-binding proteins (IGFBP1–6)^[6–11], a group of IGFBP-related proteins that bind IGFs with low affinity, and IGFBP proteases^[12,13].

IGFs, first defined in 1957, are growth factors that have both metabolic and mitogenic activities and activate a tyrosine kinase pathway via binding to IGFIR and IGFIIR on cell surfaces^[14–21]. IGF-binding proteins bind IGFs with high affinity, regulating their activity by prolonging their half-life and circulation turnover^[14] and by

controlling their binding to IGF receptors either positively or negatively^[21], thus directly affecting the IGF signaling pathway. The actions of IGFBPs are modulated by IGFBP proteases that depend on activators and inhibitors^[13].

IGFBPs may inhibit mitogenesis, differentiation, survival, and other IGF-stimulated events by sequestering IGFs away from the IGFIR^[14]. IGFBPs also function independently of the IGF signaling pathway via interacting with proteins other than IGFs, being cleaved, binding their own membrane receptors, and localizing both extracellularly and intracellularly^[22]. Furthermore, IGFBP3, IGFBP5, and IGFBP6 are capable of translocating to nucleus via different entry mechanisms, and subcellular localization of IGFBPs can affect their cellular functions^[23–26].

IGFBP5, the most conserved member of the IGFBP family in all vertebrates, has been shown to regulate cell growth, determine cell fate, and play a role in the metastatic process in cancer development. On the other hand, this protein has recently been deemed a molecular biomarker for predicting response to therapy and clinical outcome^[27] in patients with cancers such as retinoblastoma^[28], glioblastoma^[29], adenocarcinoma^[30],

Authors' Affiliation: Department of Medical Biology, School of Medicine, D.Marmara University, Istanbul 34468, Turkey.

Corresponding Author: Mustafa Akkiprik, Department of Medical Biology, School of Medicine, Marmara University, Tibbiye Cad., No. 49, 34668 Haydarpaşa/Istanbul, Turkey. Tel: +90-216-4144737; Fax: +90-216-3480848; Email: akkiprik@yahoo.com.

* These two authors contributed equally to this paper.

doi: 10.5732/cjc.011.10405

breast cancer^[31], non-functioning pituitary adenoma^[32], prostate cancer^[33], and estrogen receptor-positive breast cancer^[34]. IGFBP5 has been associated with various types of cancers as a cancer promoter or cancer repressor protein^[35-39]. The expression level and functional differences of IGFBP5 in distinct cancer types and in the same tissue type show that there are many mysteries that remain to be solved about the protein. The present article is a review of literature reports on the function and regulation of IGFBP5, as well as its role in cancer development and progression and clinical and prognostic importance.

IGF-dependent or IGF-independent Functions of IGFBP5

The IGF system plays crucial roles in biological processes such as proliferation^[38], differentiation^[15], invasion^[40], tumor expansion^[41], migration^[42], and survival. It can be used to predict clinical outcome^[43,44], diagnosis^[45], endocrine responsiveness^[46], cancer progression^[47] or inhibition of cancer growth^[48], and apoptosis^[49]. The ligands of the IGF system, IGF-I and IGF-II, mediate the growth and development of organisms. They bind to IGFIR and initiate multiple cellular phenotypes^[50], induce cell proliferation^[51], suppress apoptosis^[52-54], and promote differentiation^[55]. IGFBP5s regulate the functions of IGFs by binding to conserved amino terminal domains of IGFs^[56,57], and this action can extend the half-life of IGFs or restrict their function^[33].

IGFBP5, expressed in both normal and cancer tissues^[58], regulates the growth and development of tissues and cells^[59], such as myoblasts^[60], thyroid carcinoma cells^[61], uterine leiomyomata cells^[62], neural cells^[63], and muscle-derived tumor rhabdomyosarcoma cells^[64]. IGFBP5 has both IGF-dependent and IGF-independent effects^[65-68].

IGFs are deemed as cell survival factors that are up-regulated in tumorigenesis and their inhibition by IGFBPs induce cell death^[69]. However, IGFBP5 acts as a survival factor during myogenesis via an IGF-independent mechanism^[59]. Overexpression of wild-type *IGFBP5* (*wtIGFBP5*) decreased muscle IGFIR phosphorylation, probably via binding to and restricting the function of IGF-I. IGFBP5 has also been found to increase Akt phosphorylation through an IGFIR-independent mechanism^[67]. Both transient overexpression and administration of exogenous IGFBP5 in a breast cancer cell line caused cell cycle arrest and apoptosis^[70].

Tripathi *et al.*^[67] examined IGF-independent effects of IGFBP5 during development using mice that express mutant *IGFBP5* (*mutIGFBP5*), which does not bind IGFs. They found that overexpression of *wtIGFBP5* resulted in increased total and free serum IGF-I, but overexpression of *mutIGFBP5* did not change serum IGF-I concen-

trations. Furthermore, despite being highly expressed in the murine model, *mutIGFBP5* had an undetectable effect on the concentration of members of the IGF axis in the circulation relative to *wtIGFBP5*. In addition, overexpression of *wtIGFBP5* rescued the lethal phenotype of mice carrying the maternal *Igf2r*-null allele, but *mutIGFBP5* did not. Mice expressing *wtIGFBP5* showed decreased relative phosphorylation of IGFIR. Notably, overexpression of *wtIGFBP5* activated the p38 MAPK pathway in an IGF-independent manner. This is the first study to report IGF-independent actions of IGFBP5 in a mouse model^[67].

IGF-I affects cells in the growth plate through endocrine, paracrine, and autocrine mechanisms^[71-76]. IGF-I also enhances IGF-I-driven chondrocyte proliferation^[77]. *IGFBP5* has been reported to stimulate osteoblast differentiation in an IGF-independent manner^[78]. Kiepe *et al.*^[79] evaluated the expression profile of IGF system components in proliferating and differentiating growth plate chondrocytes using two cell culture models: RCJ3.1C5.18 (RCJ) mesenchymal chondrogenic cells that, without biochemical or oncogenic transformation, do not express IGF-I, and rat chondrocytes of the growth cartilage in primary culture. They found that *IGF-I* and especially *IGFBP5* gene expression was increased during chondrocyte differentiation. To evaluate a possible functional role of *IGFBP5* on chondrocytes, RCJ cells were transfected with a vector containing human *IGFBP5* cDNA, cultured in serum-deprived media, and then treated with or without IGF-I. *IGFBP5*-transfected cells showed higher levels of expression of *IGFBP5* in response to IGF-I treatment than did control cells. *IGFBP5* promotes the IGF-I-enhanced differentiation of RCJ cells but does not promote chondrocyte differentiation on its own. *IGFBP5*-overexpressing cells showed enhanced IGF-I-stimulated phosphorylation of Akt, a member of PI3 kinase (PI3K) pathway, but MAPK/ERK1/2 cascade was down-regulated. This result suggests that IGFBP5 promotes the IGF-I-enhanced differentiation of RCJ cells, especially increases the activity of the PI3K pathway in a specific manner^[79].

The roles of IGFBP5 may differ in various types of malignant cells. For example, overexpression of IGFBP5 may inhibit IGF-I activity in malignant pleural mesothelioma (MPM)^[80]. Consistent with this, IGFBP5 was found to block the migration of OECM-1, a head and neck squamous cell carcinoma (HNSCC) cell line, which is stimulated by IGF-I^[42]. In contrast, IGFBP5 activity is also associated with elevated IGF activity in several tumors^[1]. Thus, IGFBP5 may promote or inhibit IGF activity depending on tumor type.

Importance of differentially expressed IGFBP5 in cancer tissues

IGFBP5 can be up-regulated by either physiologic

(cell differentiation) or pathologic (cancer) processes^[67]. It can also be down-regulated in some cases. Given the nature of its variable expression pattern and effects in both normal and cancer tissues, *IGFBP5* is considered neither a tumor suppressor nor an oncogene.

MPM is an aggressive neoplasm of the serosal lining of the pleural cavity arising from mesothelial cells. *IGFBP5* was weakly expressed in MPM cell lines and tissue samples, though IGF-I was overexpressed in all MPM specimens^[68].

Intrahepatic cholangiocarcinoma (ICC) arises from bile duct cells in the liver^[61]. Nishino *et al.*^[62] evaluated *IGFBP5* RNA expression among ICC, normal liver (NL), chronic liver disease (CLD), hepatocellular carcinoma (HCC), and extrahepatic adenocarcinomas (breast, colon, stomach, ovary, and lung) and found that *IGFBP5* expression was higher in ICC than in HCC, NL, CLD, or adenocarcinomas originating from other organs. On the other hand, Umemura *et al.*^[63] found that expression of *IGFBP5* was affected by gankyrin in HCC samples and that both *IGFBP5* and gankyrin may play oncogenic roles in early stages of human hepatocarcinogenesis. Furthermore, human osteosarcoma cell line (U2OS) and a well differentiated hepatocyte-derived cellular carcinoma cell line (Huh-7) overexpressing gankyrin had a respective 5.2-fold and 1.7-fold increase in *IGFBP5* mRNA levels compared to noncancerous tissues^[63].

IGFBP5 expression is associated with the degree of cell differentiation in different cancers^[64,65]. Recently, Boers *et al.*^[65] showed that *IGFBP5* expression was strongly up-regulated during hepatic stellate cell transdifferentiation *in vitro* and during the development of liver fibrosis *in vivo*. In their current study^[66], they determined that *IGFBP5* extended the survival of human hepatic stellate cell line, LX2, a model for partially activated hepatic stellate cells, but did not affect cell proliferation. *IGFBP5* induced collagen type I, alpha 1 (COL1A1) by 70%, tissue inhibitor of metalloproteinase (TIMP) vmetallopeptidase inhibitor 1 (TIMP1) by 70%, and matrix metallopeptidase 1 by 100% in LX2 cells, suggesting that *IGFBP5* may be a fibrotic marker^[66].

Microarray studies have revealed that *IGFBP5* is overexpressed in pancreatic adenocarcinoma^[48]. When transfected into PANC-1 pancreatic cancer cells, *IGFBP5* inhibited cell growth in serum-free culture and acted as a tumor suppressor. In contrast, *IGFBP5* did not affect the growth of BxPC-3 cells when it was transfected into the cells. Additionally, pancreatic cancer PaC cells can overexpress autocrine growth factors^[67] and overexpression of these growth factors and their receptors is associated with increased tumorigenicity in pancreatic cancer^[68]. BxPC-3 cells overexpressing *IGFBP5* exhibited high DNA replication and cell numbers in the absence of serum, but the opposite phenotype was

observed in PANC-1 cells. In addition, *IGFBP5* promoted cell cycle in BxPC-3 cells but led to G₂/M arrest in PANC-1 cells^[35]. These findings support the notion that *IGFBP5* has cell-specific and environment-specific effects. Thus, *IGFBP5* has cell- and environment-specific effects and its individualized effects must be considered for all cells and cancer types

IGFBP5 is also expressed in ovarian cancers^[89] and may play a role in the development of high-grade serous carcinoma of the ovaries. Wang *et al.*^[90] have revealed that undifferentiated carcinoma, serous carcinoma, and transitional cell carcinomas have significantly higher expression levels of *IGFBP5* when compared to clear cell or mucinous carcinomas^[90]. Mice bearing tumors generated by injection with SKOV3 ovarian cancer cells experienced decreased tumor growth after treatment with recombinant *IGFBP5*^[38]. This result emphasizes the role of *IGFBP5* on tumor suppression.

Cervical carcinoma (CC) is a common cancer of the female reproductive system. Development of cervical intraepithelial neoplasia (CIN) and CC from normal cervical tissue is a gradual process, and the occurrence and development of these diseases is related with persistent human papilloma virus infection. Hou *et al.*^[64] demonstrated that *IGFBP5* levels were highest in CIN samples (91.9%), followed by in normal cervical tissues (71.4%), and lowest in CC tissues (45%). In CC samples, *IGFBP5* is negatively correlated with lymph node metastasis, CC progression, and high differentiation. *IGFBP5* expression is also negatively correlated with clinical stage in CC. The samples in early stage show increased *IGFBP5* expression, whereas those in late stage show decreased *IGFBP5* expression^[64]. In contrast, *IGFBP5* is detected in squamous cell cervical cancer and associated with progression of squamous cell carcinoma at a preneoplastic stage. Hence, *IGFBP5* may be a marker for cancer progression in the cervical epithelium^[47].

IGFBP5 is expressed in the mammary and breast tissues of mammals^[91,92], and it is thought to be necessary for normal mammary gland involution and to possibly regulate mammary gland morphogenesis in response to hormone stimulation^[93]. In addition, *IGFBP5* has also been found to have diverse effects in breast cancer cells. *IGFBP5* inhibits cell growth when transiently expressed in the MDA-MB-231 and Hs578T breast cancer cell lines. Both stable and adenovirus-mediated expression of *IGFBP5* in these cell lines result in a significant decrease in DNA synthesis, but only adenovirus-mediated transfection of *IGFBP5* cause G₂/M arrest compared with vector controls^[70]. As suggested by an *in vivo* model, plasma levels of *IGFBP5* may be correlated with tumor size. In breast tumor-bearing mice,

tumor size increases along with *IGFBP5* levels. Plasma levels of *IGFBP5* are 1.5-fold higher in tumor-bearing mice than in non-tumor-bearing mice^[41]. Nevertheless, Li *et al.*^[44] found no significant correlation between the mRNA level of *IGFBP5* and tumor size, clinical stage, or nuclear grade. They determined that *IGFBP5* mRNA levels were up-regulated in breast cancers relative to normal breast tissues. They also found a positive correlation between *IGFBP5* mRNA levels and the status of hormone receptors, including estrogen receptor (ER) and progesterone receptor (PR), and a negative link between *IGFBP5* mRNA levels and distant metastasis or lymph node status^[44]. In contrast, Hao *et al.*^[19] revealed that IGFBP5 protein expression was elevated in lymph node metastasis samples compared with primary breast tumor samples. These findings suggest that *IGFBP5* is an important player of breast cancer pathogenesis. *IGFBP5* could be a useful marker of cancerous tissue and metastasis, but it could have diverse effects on growth of cancer cells depending on cell type and expression method.

In addition to being up-regulated, IGFBP5 is down-regulated in several cancers, including MPM^[80], and in some immortal cell lines^[94]. Also, IGFBP5 is overexpressed in invasive non-functioning pituitary adenomas (NFPAs)^[32]. IGFBP5 expression may also vary between subclasses of the same cancer, as observed in glioblastomas^[95]. IGFBP5 levels in colorectal cell lines (DLD-1, HCT116, SW837, HT-29, and SW48) were 10-fold lower than those in normal human fibroblasts^[58]. Similarly, IGFBP5 expression was elevated in glial cells co-cultured with retinoblastoma cells^[28]. IGFBP5 expression was also higher in adenocarcinoma than in normal mucosa^[30].

IGFBP5 expression patterns are also impacted by the cellular microenvironment and protein constituency. A hypoxic microenvironment contributes to tumor development, and hypoxia-inducible factor-1 α (HIF-1 α) plays a role in this process. To understand the molecular effects of hypoxia and HIF-1 α , the Human Genome U133A Array was used to determine the gene expression profile of NCI-H446 small cell lung cancer cells cultured in a hypoxic environment after transfection with Ad5-HIF-1 α or Ad5-siHIF-1 α . In addition to other genes, *IGFBP5* is up-regulated at both the mRNA and protein levels in the cells transfected with HIF-1 α ^[96]. IGFBP5 expression can also be regulated by other proteins. In a study comparing colonic mucosa specimens from 12 colorectal cancer patients and 10 healthy controls, Fos, the v-Fos FBJ murine osteosarcoma viral oncogene homolog, was found to activate IGFBP5 in colorectal cancer but down-regulate it in normal colon tissue^[97].

Migration, differentiation, and metastasis effects of IGFBP5 in cancer

The effects of IGFBP5 on the biological activity of cells can change depending on several factors^[98,99]. The protein interacts with many molecules that impact its effects on cellular characteristics^[38]. Among its numerous effects in cells, IGFBP5 can regulate migration and differentiation^[42,100,101]. In some cell lines or tissues, it can enhance migration^[42,99], differentiation, and cell attachment, whereas it can block cell motility, induce retention of cell morphology^[100], and reinforce adhesion^[42] in others.

Cell migration, motility, and attachment to the extracellular matrix (ECM) are important features for cancer progression and metastasis process. ECM components like vitronectin (VN)^[102,103] are important for cell migration and attachment to ECM^[104,105]. The stimulatory effect of IGF-I:IGFBP5:VN complexes on cell migration has been shown in skin keratinocytes and MCF-7 breast cancer cells^[106,107]. Furthermore, elevated levels of IGFBP5 have been associated with breast cancer metastasis^[108]. IGFBP5 expression is higher in metastatic breast carcinomas with axillary lymph node involvement than in primary breast carcinoma^[19]. IGFBP5 was also found to be elevated in lymph node metastasis samples than in primary tumor samples^[19]. Consistent with this, Wang *et al.*^[40] found that expression of IGFBP5 was higher in T1 invasive breast carcinoma than in benign breast epithelium^[40]. Moreover, Hs578T breast cancer epithelial cells treated with IGFBP5 and IGF-I show reduced cell attachment^[99]. These findings suggest that IGFBP5 stimulates cell migration in breast cancer. Similarly, treatment with recombinant IGFBP5 protein or transfection with IGFBP5 plasmid decreases growth, stimulates migration, and reduces adhesion of HNSCC cells, and this effect is IGF-independent^[42]. Therefore, both endogenously expressed or exogenously added IGFBP5 induces the same effect. Thus, IGFBP5 is an effective regulator of cell migration and adhesion and may control these processes through an IGF-independent pathway.

We have mentioned positive effects of IGFBP5 on cell migration, but IGFBP5 also has opposite effects on cell motility. Vascularization is important for metastasis as it mediates cell spreading. IGFBP5 specifically inhibits vascular endothelial growth factor-induced endothelial cell proliferation and, thus, effectively suppresses the formation of blood vessels *in vitro* and *in vivo*^[38]. Protein kinase C was reported to promote IGFBP5-mediated cell adhesion in Hs578T breast cancer cells^[99]. In a recent study, *wildIGFBP5* and *mutIGFBP5* were transfected to MDA-MB-435 breast cancer cells and this study revealed a negative role of IGFBP5 on cell motility^[23]. Also, a nuclear localization signal (NLS) mutant form of

IGFBP5 is generated by site-directed mutagenesis. *mutIGFBP5* and *wtIGFBP5* were transfected into MDA-MB-435 breast cancer cells to generate stable clones overexpressing either *mutIGFBP5* or *wtIGFBP5*. Cells overexpressing *mutIGFBP5* has significantly higher proliferation and migration rates than do cells overexpressing *wtIGFBP5*. Cellular localization analyses have revealed that NLS mutations cause an accumulation of the protein in cytoplasm^[23]. These results indicate that subcellular localization of *IGFBP5* affects the growth and migration of breast cancer cells. Thus, cytoplasmic accumulation of *IGFBP5* could induce cell growth and motility, which could impact cancer development and progression^[23]. *IGFBP5* has also been found to inhibit IGF-I-induced proliferation and migration of smooth muscle cells^[109].

IGFBP5 has been reported to promote activation and migration of peripheral blood mononuclear cells (PBMCs) and these effects on migration were induced via the MAPK pathway and independent of IGF-I. Hence, *IGFBP5* showed a chemoattractant activity^[110]. Abrass *et al.*^[65] found that *IGFBP5* stimulated the migration of rat mesangial cells in an IGF-independent and RGD-independent manner, and McCaig *et al.*^[99] revealed that *IGFBP5* alone reduced cell adhesion that increased after co-administration of *IGFBP5* and IGF-I. These results indicate that *IGFBP5* could induce migration of cells in an IGF-I-dependent or -independent manner and IGF-I treatment could reverse the activity of *IGFBP5*. Since migration of cancer cells is an important point for metastasis, a great attention should be paid to interaction of *IGFBP5* and IGF-I in metastasis and invasion of cancer.

IGFBP5 has been shown to regulate the differentiation of neuroblastoma cells. LAN-5 neuroblastoma cells differentiate towards a neuronal phenotype and have elevated expression of *IGFBP5* when treated with *all-trans* retinoic acid (RA) at micromolar concentrations^[111]. RNA interference (RNAi)-mediated knockdown of *IGFBP5* prevents neuroblastoma cells from differentiating towards a neuronal phenotype as evidenced by the absence of detectable neuronal markers and neurofilaments after treatment with RA for different durations. Compared to controls, co-treatment with recombinant *IGFBP5* and RA rescues differentiation of cells in which *IGFBP5* expression was knocked down. Complete inhibition of *IGFBP5* caused death in LAN-5 cells^[98].

IGFBP5 is the major protein secreted by skeletal muscles, and its expression is induced during muscle differentiation^[112]. *IGFBP5* inhibits skeletal muscle cell differentiation via binding IGF receptors and thereby blocking IGF action^[100]. In contrast, the addition of purified bone-derived human *IGFBP5* to muscle cells enhances differentiation^[101]. Ren *et al.*^[113] revealed that

the expression of IGF-II and *IGFBP5* was elevated during myogenic differentiation and found that knockdown of *IGFBP5* impaired myogenic differentiation of C2C12 mouse myoblast cells. In these studies, *IGFBP5* induced myogenic differentiation via IGF-II. *IGFBP5* was found to be overexpressed in activated hepatic stellate cells, so it may be a marker for hepatic stellate cell activation^[65]. *IGFBP5*-overexpressing cells have high levels of osteocalcin, which is an indicator of advanced, differentiated osteoblast cells^[114]. Thus, Schneider *et al.*^[115] suggested that *IGFBP5* may promote osteoblast cell differentiation despite not finding a direct effect of *IGFBP5* on osteocalcin expression.

Cell survival and apoptotic effects of *IGFBP5* in cancer

There are numerous studies on the role of *IGFBP5* in survival and apoptosis of both normal and cancer cells. Butt *et al.*^[116] showed that *IGFBP5* activated caspase-8 and caspase-9, causing apoptosis through Bcl-2 in the intrinsic apoptotic pathway in MDA-MB-231 breast cancer cells. *IGFBP5*-expressing MDA-MB-231 cells had elevated levels of JUN N-terminal kinase (JNK), which sensitized the cells to tumor necrosis factor- α (TNF α). Similar to its effects in MDA-MB-231 cells, endogenous *IGFBP5* inhibits the proliferation of MDA-MB-435 breast cancer cells^[23], and *IGFBP5* has been reported to inhibit cell growth and cause G₂/M arrest in human breast cancer and PANC-1 pancreatic cancer cells^[35,70,116]. These findings shed light on the tumor suppressor and antiproliferative properties of *IGFBP5*. Notably, *IGFBP5* do not induce apoptosis but decreased the number of cells in human hepatocellular carcinoma^[83].

Other studies have focused on the antiapoptotic role of *IGFBP5*. In normal cells like myoblasts, *IGFBP5* has been reported to inhibit apoptosis during cell differentiation^[69]. A study of RNAi-mediated *IGFBP5* knockdown in neuroblastoma cells revealed that loss of *IGFBP5* resulted in inhibition of cell growth. In these cells, *IGFBP5* shows both IGF-dependent and IGF-independent effects that are related with cell proliferation. Suppression of *IGFBP5* expression provoked apoptotic morphology, such as hypodiploid DNA content and activation of caspase-3 and caspase-7^[98].

Senescence may contribute to the antiapoptotic effects of *IGFBP5*. The role of *IGFBP5* in cellular senescence was first described by Kim *et al.*^[117]. The knockdown of *IGFBP5* in old human primary endothelial cells triggered anti-aging effects. On the other hand, overexpression of *IGFBP5* in young cells results in aging. *IGFBP5* induces senescence via p53 in human umbilical vein endothelial cells (HUVEC). Phosphorylation of p53 at serine 6 and serine 15 is done by

IGFBP5. Notably, excess IGF-I has been found in old cells compared to young cells, but this trend is not observed for IGF-II^[117].

The expression pattern of IGFBP5 in different types of cancers reflects its role in cell proliferation. IGFBP5 could contribute to survival effects in breast cancer cells via repressing the mitochondria-independent apoptosis pathway^[118], and induce the anti-apoptotic effects of IGF-I in prostate cancer cells^[119]. Retinoic acid inhibits the growth of HPV-negative CC cells by inducing the IGFBP5 expression^[120]. Thus IGFBP5 is an important player of CC cell growth, but definite mechanism is unknown. As described previously, IGFBP5 induces the response to IGF-I stimulation in prostate cancer cells whereas reduces the response in osteosarcoma cells^[121]. In HUVEC cells, IGFBP5 inhibits proliferation, but this effect could be reversed by silencing *IGFBP5* gene expression^[38]. IGFBP5 was found to enhance DNA replication and cell proliferation in BxPC-3 cells after serum starvation^[35]. As indicated above, there are numerous studies on the effects of IGFBP5 on cell proliferation, some of which show a negative effect of IGFBP5 on proliferation, but some show positive effect; however, the exact molecular mechanisms that cause these effects are not determined yet.

Association of IGFBP5 with Signaling Pathways

Most tumors have irregular activation of signaling pathways. IGFBP5 is involved in many signaling pathways that regulate the biological functions of cells.

Using transgenic mice overexpressing *wtIGFBP5*, Kuemmerle *et al.*^[122,123] showed that up-regulation of IGFBP5 reduced IGFIR phosphorylation, but this did not affect the p-Akt level in myogenic cells. Overexpression of IGFBP5 may activate the p38 MAPK pathway^[122], and IGF-I may use the same pathway to stimulate IGFBP5 expression^[123]. IGFBP5 binding to its receptor induced the p38 MAPK and ERK1/2 pathway activation, thereby stimulating growth and IGF-I secretion in human intestinal muscle cells. Moreover, IGF-I stimulated IGFBP5 expression via the PI3K pathway during Schwann cell differentiation^[124].

Kiepe *et al.*^[78] used two specific pharmacologic inhibitors of the p42/44 MAPK pathway, U0126 and PD098059, to evaluate IGF-I-induced *IGFBP5* mRNA expression in rat growth plate chondrocytes. Both inhibitors were capable of suppressing phosphorylation of ERK1/2 but did not alter total ERK1/2 concentration in chondrocyte cell lysates. Inhibition of the p42/44 MAPK pathway by U0126 did not change *IGFBP5* mRNA expression. Similarly, co-incubation of IGF-I with U0126

did not affect IGF-I-induced *IGFBP5* mRNA expression. However, co-incubation of IGF-I with the PI3K inhibitor LY294002 abolished the stimulatory effect of IGF-I on *IGFBP5* mRNA expression. Thus, IGF-I stimulates *IGFBP5* mRNA expression via the PI3K pathway in rat growth plate chondrocytes^[78]. Along the same line, other studies revealed that *IGFBP5* mRNA expression induced by IGF-I is mediated via the PI3K pathway in vascular smooth muscle cells^[125] and primary Schwann cells^[126]. In contrast, Xin *et al.*^[127] found that IGF-I-induced *IGFBP5* expression occurs via the p42/44 MAPK pathway in rat intestinal smooth muscle cells, and Kuemmerle^[123] revealed that *IGFBP5* mRNA expression was induced by IGF-I via the p42/44 MAPK and PI3K pathways. Moreover, IGF-I-induced *IGFBP5* expression was reported to occur via the MAPK pathway in rat intestinal smooth muscle cells^[127] and via the PI3K and MAPK pathways in mammary fibroblasts and human intestinal smooth muscle cells^[123,128].

Kuemmerle^[123] suggested that IGFBP5 stimulates cell growth and IGF-I secretion via the MKK3/6-p38 and Ras-Erk1/2 pathways, and found that a positive feedback mechanism links IGF-I and IGFBP5 in human intestinal muscle cells. IGF-I, via interacting with its cognate receptor that was facilitated by IGFBP5, activated the PI3K and Erk1/2 pathways that mediate enhanced proliferation and secretion of IGFBP5^[122,123]. In turn, IGFBP5, via interacting with its cognate receptor, activates the p38 MAPK and Erk1/2 pathways^[124].

Johnson *et al.*^[35] transfected full-length human IGFBP5 into two pancreatic cancer cell lines, BxCPC-3 and PANC-1. They found that p-Akt levels were elevated after serum deprivation in BxCPC-3 cells expressing IGFBP5, but Akt phosphorylation was reduced in PANC-1 cells expressing IGFBP5. When BxCPC-3 cells were treated with PI3K inhibitor, Akt phosphorylation was not induced. After treatment with MEK1/2 inhibitor, they found that ERK1/2 phosphorylation was 2.6-fold higher in BxPC-3 cells expressing IGFBP5 than in primary BxPC-3 cells and that IGFBP5-mediated growth was inhibited when the MAPK pathway was blocked with MEK1/2 inhibitor. These results suggested that the MAPK pathway is necessary for IGFBP5-enhanced cell growth after serum deprivation. In addition, pathway analysis revealed that ERK1/2-independent pathways contribute to the increase in Akt phosphorylation associated with IGFBP5 and that inhibition of the MAPK or PI3K pathway in IGFBP5-expressing PANC-1 cells may shift signaling to the other pathway^[35].

Recent studies have indicated new molecules and approaches relevant to pathways that regulate IGFBP5. Erlik *et al.*^[129] showed that parathyroid hormone (PTH) induced IGFBP5 protein expression in UMR106-01 osteosarcoma cells and found that activation of cyclic

AMP, protein lipase C α , and protein kinase C δ increased *IGFBP5* mRNA level^[129]. They also found that PTH mediates the activation of *IGFBP5* gene transcription in osteoblast cells^[130]. Also, Akt was phosphorylated through IGFIR phosphorylation, which was induced by IGFBP5^[131].

Taken together, these studies suggest that IGF-I and IGFBP5 have complex molecular mechanisms that require further investigation. Figure 1 shows possible molecular interactions of IGFBP5. Any dysregulation in these pathways could cause deterioration of cellular

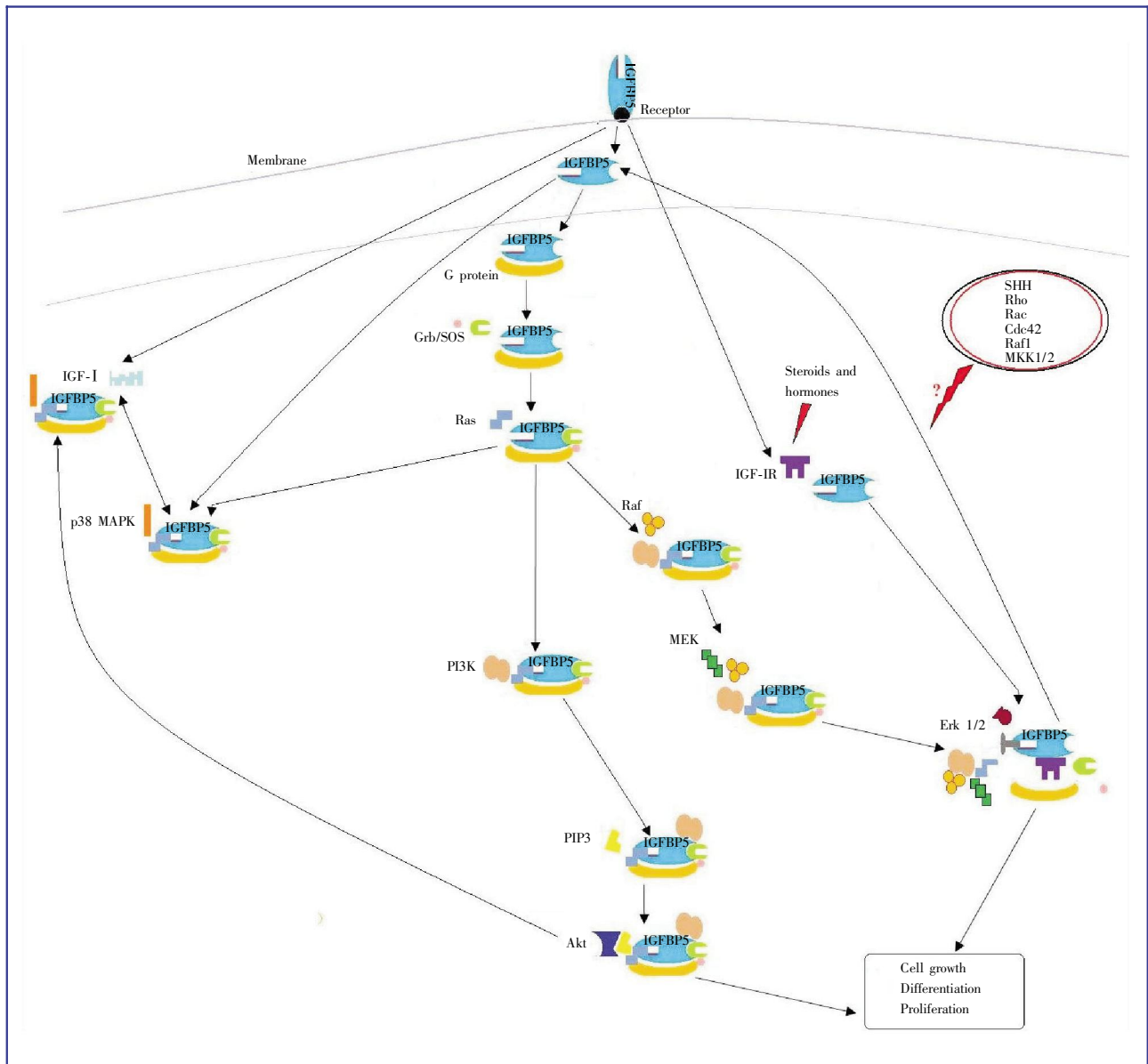


Figure 1. IGFBP5 enters cells through its own surface receptor or by freely diffusing into the cytoplasm. Together with G-proteins, IGFBP5 can induce phosphorylation of Erk1/2 and p38 MAPK. IGFBP5 can act independently of IGF-I to stimulate proliferation and up-regulate IGF-I production through Ras-dependent activation of Grb-Sos-Mek-Erk1/2 and p38 MAPK. Activation of these pathways stimulates proliferation as well as IGFBP5 production. IGFBP5 and IGF-I secretion are linked by a positive feedback mechanism that reinforces their individual effects on cell growth. The PI3K-Akt pathway is also activated by IGFBP5 and IGFIR. Expression of IGFIR is stimulated by steroid and other hormones, resulting in an increase in Erk1/2 pathway activation, which in turn stimulates IGFBP5 production and proliferation. This suggests a positive feedback loop between IGFBP5 and Erk1/2. In some cancer cell lines, the small GTPases Rac, Rho, and Cdc42 are requisite co-factors in Ras-dependent Raf activation and subsequent activation of Erk1/2. IGFBP5-induced growth mediated by the Erk1/2 or p38 MAPK pathways has been shown to involve these small GTPases, as well as Raf1, MKK1/2, and SHH, but the molecular interactions of these proteins remains unclear.

functions such as proliferation, growth, and migration, leading to aggressiveness and metastatic properties in cancers.

Prognostic and Clinical Importance of IGFBP5 in Cancer

IGFBP5 was observed to have a clinical importance in various cancers such as breast cancer^[27,31,34], ovarian cancer^[46], glioblastoma^[29], and NFPA^[32], and it was also found to be a useful marker for diagnosis and differentiation of CC and CIN^[84]. Although *IGFBP5* mRNA was found to be overexpressed in invasive NFPAs and not in non-invasive NFPAs, this differential expression was not confirmed at the protein level.

IGFBP5 expression has been found to correlate with prognosis, clinical outcome^[36], survival^[31], response to therapy^[34], and drug resistance^[27], either negatively or positively. IGFBP5 is one of a group of genes that has been shown to predict the prognosis of primary breast tumors in a dynamic manner^[27] and reverse tamoxifen resistance^[31]. In addition, lower expression levels of IGFBP5 are reportedly associated with shorter overall survival after tamoxifen (TAM) therapy^[31]. IGFBP5 expression was also found to predict response to exemestane therapy in ER-positive (ER⁺) breast cancer^[34] and to be associated with poor clinical outcome and development of metastatic or recurrent disease in patients with malignant fibrous histiocytomas (MFH), pleomorphic sarcomas, and not otherwise specified (NOS) sarcomas^[36].

Because the expression levels of estrogen-regulated genes have been considered potential predictive markers for endocrine therapy for breast cancer, the expression of *IGFBP4*, an estrogen-induced gene, and *IGFBP5*, an estrogen-repressed gene, was analyzed in human breast cancer tissues with quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR). *IGFBP4* and *IGFBP5* mRNA levels were found to be positively correlated with ER and PR status and negatively correlated with HER2 overexpression. IGFBP4 expression was also found to be an independent prognostic factor for disease-free survival in ER⁺ breast cancers. Analysis of 116 patients with ER⁺ breast cancer revealed that higher levels of *IGFBP4* mRNA or lower levels of *IGFBP5* mRNA in tumors were associated with better prognosis and disease-free survival^[43].

To evaluate the clinical significance of IGFBP5 in breast cancer, mRNA expression levels have been analyzed in normal breast tissues, primary tumors, and lymph node metastases using RT-PCR. *IGFBP5* mRNA expression was found to be positively correlated with

invasion of axillary lymph nodes and the status of hormonal receptor, and it was also found to be associated with poor outcome of breast cancer in patients with positive lymph nodes and negative ER status^[44]. Tissue microarray and immunohistochemical analysis of 164 cases of T1 breast carcinoma, either with or without axillary lymph node involvement, revealed that IGFBP5 and IGFBP2 might serve as useful markers of lymph node metastasis in small invasive breast carcinomas^[40].

Taylor *et al.*^[27] assessed the gene expression changes after TAM treatment in an ER⁺ estrogen-responsive breast cancer xenograft model to predict endocrine sensitivity. ZR-75-1 breast cancer cells were implanted into female nu/nu mice, which were separated into two treatment groups: TAM (the experimental group) or estradiol (E2) (the control group). Microarray analysis was used to assess gene expression changes at days 0, 1, 2, 4, 7, and 14, and hierarchical clustering identified 6 time-related gene expression patterns (Set 1–6). These 6 sets of genes were separated into three groups by early/transient responses, continuous/late responses, and variable responses. The continuous/late response group contained Set 5 and Set 6 genes, which have been down-regulated and up-regulated, respectively, in response to therapy. *IGFBP5* and trefoil factor 3 (*TFF3*), Set 6 and Set 5 genes, respectively, exhibit a continuous/late response to treatment. The study of pretreatment and post-treatment samples of 28 patients revealed that the protein expression change in IGFBP5, TFF3 or both was significantly associated with change in tumor volume. Higher *IGFBP5* gene expression was observed in patients with poor prognosis, but there was no significant difference at day 1^[27]. Four data sets representing a total of 404 patients have revealed that the genes most differentially expressed on days 2, 4, and 7 after treatment with TAM were able to predict prognosis. The authors speculated that early/transient expression changes are more likely to be causative and primary events for tumor volume decrease, whereas continuous/late expression changes may be consequential and secondary to the volume changes^[132]. These results also suggest that IGFBP5 may be a good biomarker for outcome after TAM treatment.

Because mouse mammary gland involution resembles a wound healing response with suppressed inflammation^[133] and because inflammation is also associated with tumor development^[134], Stein *et al.*^[135] hypothesized that the wound healing gene expression signature may predict metastasis formation and survival. An independent cancer data set was used to test this hypothesis. Day 3 of mouse mammary gland involution (*Inv3*) and cluster 5 (C5) genes showed the strongest

predictive power for metastasis and survival. When Hierarchical Ordered Partitioning and Collapsing Hybrid (HOPACH) clustering method was applied, the mouse mammary gland involution *Inv3/C5* signature could not cluster the data set into good or poor survival. However, when hierarchical clustering was used, the *Inv3/C5* signature was a good predictor of overall and metastasis-free survival. The authors concluded that the gene signature was possibly a combination of weaker markers making its predictive power dependent on the clustering method used in different data sets^[136]. Based on this finding, we conclude that results of the previous study^[27], which identified 6 time-related gene expression patterns in response to TAM treatment using hierarchical clustering, depend on the clustering method.

It was observed that IGFBP5 warrants reversing TAM resistance both *in vivo* and *in vitro*^[31]. *IGFBP5* knockdown in MCF7 human breast cancer cells has been shown to induce TAM resistance *in vitro* due to concomitant loss of ER α expression and signaling. TAM-resistant MCF7 cells, which were selected from cultures, have also been found to have a reduced IGFBP5 expression. Both TAM-resistant cells and *IGFBP5*-knockdown cells could be resensitized to TAM by treatment with exogenous recombinant IGFBP5 protein. *In vivo*, TAM resistance of a mouse tumor xenograft model generated from *IGFBP5*-knockdown MCF7 cells was also reversed by treatment with recombinant IGFBP5 protein. These findings have been verified by IGFBP5 immunohistochemical staining in a cohort of tumor samples from 153 patients with breast cancer, indicating that low IGFBP5 expression was associated with shorter overall survival after TAM therapy^[31].

IGFBP5 expression is not only associated with TAM; it is also associated with exemestane therapy response in ER⁺ breast cancers^[34]. Fifteen postmenopausal patients over 70 years who were diagnosed with primary ER⁺ breast cancer were treated daily with exemestane for 6 months. Before and after treatment, paraffin block sections of 15 paired tumors were analyzed with immunohistochemistry. Patients who responded to therapy showed higher expression of HER2 or IGFBP5 in both the cytoplasm and nuclei. However, cytoplasmic expression of IGFBP5 was increased in post-treatment sections, regardless of treatment response^[34]. These results suggest that the expression of IGFBP5 can be influenced by exemestane treatment and that nuclear IGFBP5 expression level is likely a predictor of response.

IGFBP5 expression levels also have some diagnostic significance for cervical carcinomas^[84]. cDNA microarray and immunohistochemistry analysis of squamous cell carcinomas (SCCs) and their adjacent

normal squamous epithelia revealed that IGFBP5 mRNA and protein levels were significantly decreased in SCCs. Premalignant CIN lesions and advanced CIN3 lesions showed significantly weaker or negative staining for IGFBP5 compared to normal squamous epithelia, suggesting a role for IGFBP5 in cancer progression in cervical epithelia^[47]. The expression levels of IGFBP5 and cellular Fas-associated death domain-like interleukin-1 β -converting enzyme (FLICE)-like inhibitory protein (cFLIP) were measured in CC, CIN, and normal cervical tissue samples by RT-PCR and immunohistochemistry. Stages II and III CIN tissues had the highest IGFBP5 protein expression, and CC samples had decreased protein levels relative to controls. The *IGFBP5* mRNA levels in CC and CIN samples, relative to the controls, were higher and lower, respectively. There is no detectable expression of cFLIP protein or mRNA in normal cervical tissues. However, a positive correlation was found between the degree of pathologic change and expression levels of cFLIP protein and mRNA. These results indicate that IGFBP5 expression is up-regulated during CIN progression and down-regulated in invasive CC. Therefore, IGFBP5 and cFLIP may be useful markers for diagnosing and differentiating CIN and CC^[84].

Serial analysis of gene expression (SAGE) of HCC and ICC cDNA libraries revealed distinct gene expression patterns for HCC and ICC^[82]. Gene expression was validated with real-time RT-PCR prior to comparing the ICC library with the gastric, colon, prostate, and breast cancer libraries. The biglycan (*BGN*), *IGFBP5*, and claudin-4 (*CLDN4*) genes were identified as ICC-specific markers. Immunohistochemistry analysis of 74 samples revealed that *CLDN4* was highly expressed in ICC. Discrimination analysis was done with randomly selected 53 samples from the total 74 samples and showed an efficient receiver operating characteristic (ROC) curve with an area under curve (AUC) value of 0.987. Confirmation of the discrimination analysis was completed with the remaining 21 samples and the Z-score was found to be positive for all ICC samples. These results indicate that a combination of the *BGN*, *IGFBP5*, and *CLDN4* genes could be used to distinguish ICC from HCC or metastatic adenocarcinoma, but a validation using a larger cohort is needed to confirm this result.

Tissue microarray and immunohistochemistry analysis of normal ovarian surface epithelia and high-grade ovarian carcinomas of different histological types revealed that IGFBP2 and IGFBP5 are overexpressed in high-grade carcinomas, suggesting a role of IGFBP5 in the development of high-grade ovarian carcinomas. However, survival analysis revealed no correlation between survival and IGFBP5 expression^[90].

Down-regulation of IGFBP5 and IGFBP3 and up-regulation of IGFBP4 has been reported in the ER⁺, estrogen-responsive ovarian cancer cell line PE04 after exposure to E2. Decreased expression of IGFBP5 and IGFBP3 was found to be reversed by TAM treatment. Use of ER α -specific and ER β -specific agonists revealed that changes produced by ER α -specific agonist were very similar to those produced by E2, suggesting that ER α is the main modulator of IGFBP expression. Semi-quantitative immunohistochemistry analysis of paraffin-fixed sections obtained from ovarian cancer patients treated with letrozole revealed that the expression of IGFBP5 and IGFBP3 was reduced but IGFBP4 expression was increased in nonprogressive (CA125 reduction or a minimal increase of <50%) tumors. Multivariate logistic regression analysis reveals that IGFBP3 is the most powerful predictor of lack of stable disease and ER α status was found to add power to the prediction. Combined expressions of ER α plus IGFBP4 and ER α plus IGFBP5 also have predictive power, but they are inferior to IGFBP3 alone. The combinations of IGFBP4 plus ER α and IGFBP5 plus ER α are superior to IGFBP3 alone but are still inferior to the combination of IGFBP3 with ER α ^[46].

IGFBP5 shows clinical importance in HNSCC. Hung *et al.*^[42] identified the suppressive effects of IGFBP5 on the tumorigenesis of HNSCC. In addition, a functional polymorphism in the *IGFBP5* promoter was recently found to be associated with risk of late-stage HNSCC. In this study, which included 1082 patients with HNSCC and cancer-free controls, differential binding of transcription factor activator protein 1 (AP-1) to *IGFBP5* promoter with the 1195C variant was associated with risk of late-stage HNSCC when compared to the T variant genotype. Thus, this polymorphism could be a marker for susceptibility to late-stage HNSCC^[136]. However, there are limited studies on the polymorphisms of *IGFBP5*, and further study is needed to evaluate polymorphic differences of *IGFBP5* in different cancers.

Recently, Shersher *et al.*^[137] used immunobead assays to measure serum levels of IGFBP5 in non-small cell lung cancer patients with different nodal status and metastatic progression. They found that low serum IGFBP5 levels correlated strongly with a positive nodal status and disease recurrence and also predicted poor recurrence-free survival^[137]. These results, along with previous findings, suggest that IGFBP5 is an important player of carcinogenesis in various types of cancers and could be a strong biomarker for identifying non-small cell lung cancer progression and patient outcome.

IGFBP5 is involved in carcinogenesis, treatment, and diagnosis processes either negatively or positively depending on the type of tissue or cell and its micro-

environment. Some inconsistent findings summarized above could be due to the complexity of the IGF pathway and process of carcinogenesis. It is not clear that if *IGFBP5* is a tumor suppressor or an oncogene, a predictor of good prognosis or bad prognosis, biomarker of survival or disease progression.

Conclusions

IGFBP5 is a critical member of the IGF system. It binds to IGFs, interacts with cell surface and matrix components, and becomes modified post-translationally, leading to altered cell proliferation, apoptosis, survival, and migration. Despite a great deal in secondary structural analyses, the three-dimensional structure of the protein has not yet been determined. Further investigations will provide insight into its extensive potential use in medicine. IGFBP5 localizes in both the cytoplasm and nucleus, and its localization affects cell growth, migration, and proliferation properties directly and indirectly by inducing the nuclear uptake of other proteins. Because IGFBP5 has tissue-specific and cell-specific effects, further studies should take this into consideration. Furthermore, we speculate that IGFBP5 could have individual effects depending on the activities and expression patterns of its interacting proteins. Because cytoplasmic expression increases in post-treatment sections of breast cancer samples^[34] and overexpression of the NLS mutant form in breast cancer cells causes higher proliferation and migration rates^[23], we hypothesize that proteins that interact with IGFBP5 can cause the protein to accumulate in the cytoplasm. For these reasons, IGFBP5 needs to be investigated in terms of its function as well as its direct and indirect binding partners. This will reveal its potentially extensive and significant use in cancer prognosis, differential diagnosis, and treatment. A hypothetical comparative model for IGFBP5 in breast cancer and normal tissue is shown in Figure 2.

The inhibitory and stimulatory affects of IGFBP5 are thought to proceed via the MAPK, PI3K, Ras-Erk1/2, and MKK3 pathways. To determine exact molecular mechanism and properties of IGFBP5, molecular interactions with proteins in these pathways should be examined. Identifying these critical components is expected to increase the prognostic and clinical significance of IGFBP5, thereby serving to overcome obstacles concerning the prediction of clinical outcome, drug resistance, and response to therapy. Because IGFBP5 has different effects on normal and cancer tissues, drug development related to IGFBP5 may result in cancer cell-specific treatment strategies.

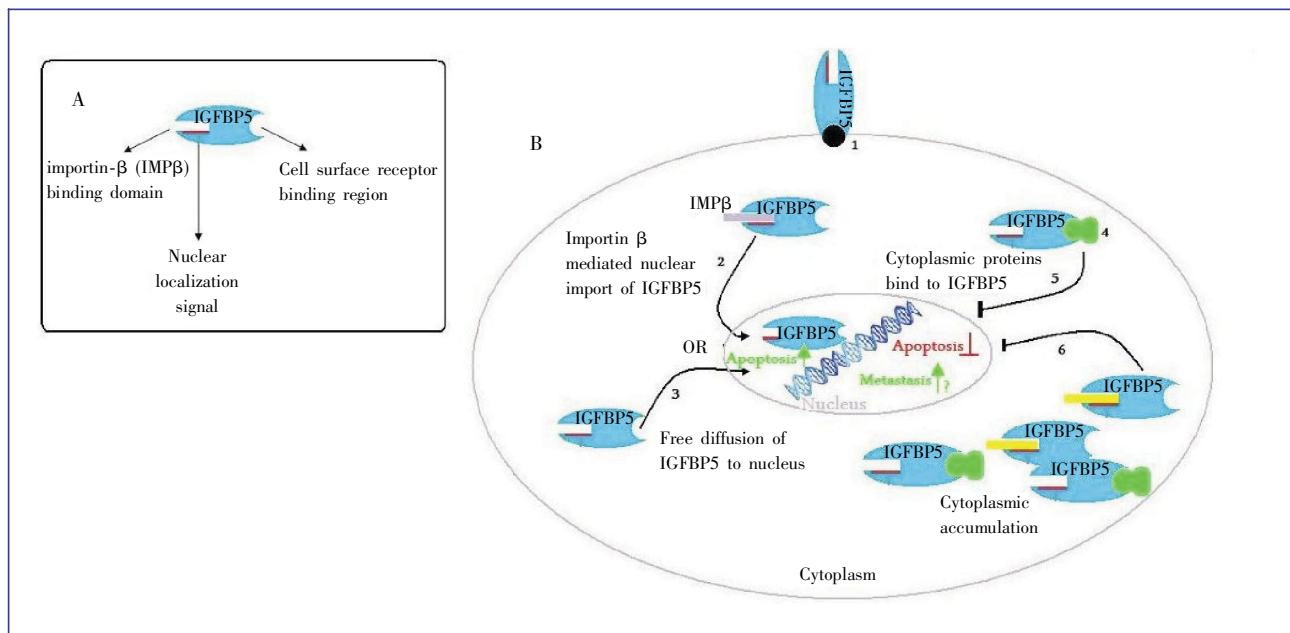


Figure 2. Schematic view of IGFBP5 and possible regulation in cell proliferation networks. A, IGFBP5 enters the cell, probably by binding to a cell surface receptor through its receptor-binding domain. The NLS sequence directs the protein to the nucleus, where it is transported across the nuclear membrane by importins. B, IGFBP5 enters the cell through its potential cell surface receptor (1). The protein is imported into the nucleus by importins (2) or by free diffusion (3), thereby inducing apoptosis and suppressing cell growth and proliferation. IGFBP5 can also interact with cytoplasmic proteins (4), which blocks the NLS sequence or changes the three-dimensional structure of the protein (5 and 6), thereby preventing nuclear uptake and causing IGFBP5 to accumulate in the cytoplasm. Cytoplasmic accumulation of IGFBP5 decreases or blocks apoptosis and may cause metastasis in some cancers.

Acknowledgments

This work was supported partly by grants (SBAG-111S161 to MA) from the Scientific and Technological Research Council of Türkiye (TUBITAK) and

(SAG-C-YLP-210311-0048 to MA) from the Research Foundation of Marmara University (BAPKO).

Received: 2011-10-25; accepted: 2012-01-15.

References

- [1] Akkiprik M, Feng Y, Wang H, et al. Multifunctional roles of insulin-like growth factor binding protein 5 in breast cancer. *Breast Cancer Res*, 2008,10:212.
- [2] Salmon Jr WD, Daughaday WH. A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage *in vitro*. *Lab Clin Med*, 1957,49:825–836.
- [3] Daughaday WH, Parker KA, Borowsky S, et al. Measurement of somatomedin-related peptides in fetal, neonatal, and maternal rat serum by insulin-like growth factor (IGF) I radioimmunoassay, IGF-II radioreceptor assay (RRA), and multiplication-stimulating activity RRA after acid-ethanol extraction. *Endocrinology*, 1982,110:575–581.
- [4] Rechler MM, Nissley SP, King GL, et al. Multiplication stimulating activity (MSA) from the BRL 3A rat liver cell line: relation to human somatomedins and insulin. *Supramol Struct Cell Biochem*, 1981,15:253–286.
- [5] Rosenfeld RG, Hintz RL. Characterization of a specific receptor for somatomedin C (SM-C) on cultured human lymphocytes: evidence that SM-C modulates homologous receptor concentration. *Endocrinology*, 1980,107:1841–1848.
- [6] Baxter RC, Martin JL. Binding proteins for insulin-like growth factors in adult rat serum: comparison with other human and rat binding proteins. *Biochem Biophys Res Commun*, 1987,147:408–415.
- [7] Binkert C, Landwehr J, Mary JL, et al. Cloning, sequence analysis and expression of a cDNA encoding a novel insulin-like growth factor binding protein (IGFBP-2). *EMBO J*, 1989,8:2497–2502.
- [8] Brinkman A, Groffen C, Kortleve DJ, et al. Isolation and characterization of a cDNA encoding the low molecular weight insulin-like growth factor binding protein (IBP-1). *EMBO J*, 1988,7:2417–2423.
- [9] Martin JL, Willetts KE, Baxter RC. Purification and properties of a novel insulin-like growth factor-II binding protein from transformed human fibroblasts. *J Biol Chem*, 1990,265:4124–4130.
- [10] Shimasaki S, Shimonaka M, Zhang HP, et al. Identification of five different insulin-like growth factor binding proteins (IGFBPs) from adult rat serum and molecular cloning of a novel IGFBP-5 in rat and human. *J Biol Chem*, 1991,266:

- 10646–10653.
- [11] Shimonaka M, Schroeder R, Shimasaki S, et al. Identification of a novel binding protein for insulin-like growth factors in adult rat serum. *Biochem Biophys Res Commun*, 1989,165:189–195.
- [12] Oh Y, Nagalla SR, Yamanaka Y, et al. Synthesis and characterization of insulin-like growth factor-binding protein (IGFBP)-7. Recombinant human mac25 protein specifically binds IGF-I and -II. *J Biol Chem*, 1996,271:30322–30325.
- [13] Rajah R, Katz L, Nunn S, et al. Insulin-like growth factor binding protein (IGFBP) proteases: functional regulators of cell growth. *Prog Growth Factor Res*, 1995, 6:273–284.
- [14] Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev*, 1995,16:3–34.
- [15] Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. *Mol Pathol*, 2001,54:133–137.
- [16] Kim JJ, Accili D. Signalling through IGF-I and insulin receptors: where is the specificity? *Growth Horm IGF Res*, 2002,12:84–90.
- [17] Renehan AG, Harvie M, Howell A. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and breast cancer risk: eight years on. *Endocr Relat Cancer*, 2006,13:273–278.
- [18] Sachdev D, Yee D. The IGF system and breast cancer. *Endocr Relat Cancer*, 2001,8:197–209.
- [19] Hao X, Sun B, Hu L, et al. Differential gene and protein expression in primary breast malignancies and their lymph node metastases as revealed by combined cDNA microarray and tissue microarray analysis. *Cancer*, 2004,100:1110–1122.
- [20] Morgan DO, Edman JC, Standing DN, et al. Insulin-like growth factor II receptor as a multifunctional binding protein. *Nature*, 1987,329:301–307.
- [21] Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev*, 2002,23:824–854.
- [22] Mohan S, Baylink DJ. IGF-binding proteins are multifunctional and act via IGF-dependent and -independent mechanisms. *Endocrinol*, 2002,175:19–31.
- [23] Akkiprik M, Hu L, Sahin A, et al. The subcellular localization of IGFBP5 affects its cell growth and migration functions in breast cancer. *BMC Cancer*, 2009,9:103.
- [24] Iosef C, Gkourasas T, Jia CY, et al. A functional nuclear localization signal in insulin-like growth factor binding protein-6 mediates its nuclear import. *Endocrinology*, 2008,149:1214–1226.
- [25] Bhattacharyya N, Pechhold K, Shahjee H, et al. Nonsecreted insulin-like growth factor binding protein-3 (IGFBP-3) can induce apoptosis in human prostate cancer cells by IGF-independent mechanisms without being concentrated in the nucleus. *J Biol Chem*, 2006,281:24588–24601.
- [26] Schedlich LJ, Le Page SL, Firth SM, et al. Nuclear import of insulin-like growth factor-binding protein-3 and -5 is mediated by the importin beta subunit. *J Biol Chem*, 2000,275:23462–23470.
- [27] Taylor KJ, Sims AH, Liang L, et al. Dynamic changes in gene expression *in vivo* predict prognosis of tamoxifen-treated patients with breast cancer. *Breast Cancer Res*, 2010,12:R39.
- [28] Xu XL, Lee TC, Offor N, et al. Tumor-associated retinal astrocytes promote retinoblastoma cell proliferation through production of IGFBP-5. *Am J Pathol*, 2010,177:424–435.
- [29] Santosh V, Arivazhagan A, Sreekanthreddy P, et al. Grade-specific expression of insulin-like growth factor-binding proteins-2, -3, and -5 in astrocytomas: IGFBP-3 emerges as a strong predictor of survival in patients with newly diagnosed glioblastoma. *Cancer Epidemiol Biomarkers Pre*, 2010,19:1399–1408.
- [30] Femia AP, Luceri C, Toti S, et al. Gene expression profile and genomic alterations in colonic tumours induced by 1,2-dimethylhydrazine (DMH) in rats. *BMC Cancer*, 2010,10:194.
- [31] Ahn BY, Elwi AN, Lee B, et al. Genetic screen identifies insulin-like growth factor binding protein 5 as a modulator of tamoxifen resistance in breast cancer. *Cancer Res*, 2010,70:3013–3019.
- [32] Galland F, Lacroix L, Saulnier P, et al. Differential gene expression profiles of invasive and non-invasive non-functioning pituitary adenomas based on microarray analysis. *Endocr Relat Cancer*, 2010,17:361–371.
- [33] Beattie J, Allan GJ, Lochrie JD, et al. Insulin-like growth factor-binding protein-5 (IGFBP-5): a critical member of the IGF axis. *Biochem J*, 2006,395:1–19.
- [34] Yamashita H, Takahashi S, Ito Y, et al. Predictors of response to exemestane as primary endocrine therapy in estrogen receptor-positive breast cancer. *Cancer Sci*, 2009,100:2028–2033.
- [35] Johnson SK, Haun RS. Insulin-like growth factor binding protein-5 influences pancreatic cancer cell growth. *World J Gastroenterol*, 2009,15:3355–3366.
- [36] Daigeler A, Klein-Hitpass L, Stricker I, et al. Malignant fibrous histiocytoma—pleomorphic sarcoma, NOS gene expression, histology, and clinical course. A pilot study. *Langenbecks Arch Surg*, 2010,395:261–275.
- [37] Naciff JM, Khambatta ZS, Thomason RG, et al. The genomic response of a human uterine endometrial adenocarcinoma cell line to 17alpha-ethynyl estradiol. *Toxicol Sci*, 2009,107:40–55.
- [38] Rho SB, Dong SM, Kang S, et al. Insulin-like growth factor-binding protein-5 (IGFBP-5) acts as a tumor suppressor by inhibiting angiogenesis. *Carcinogenesis*, 2008,29:2106–2111.
- [39] de Bont JM, van Doorn J, Reddingius RE, et al. Various components of the insulin-like growth factor system in tumor tissue, cerebrospinal fluid and peripheral blood of pediatric medulloblastoma and ependymoma patients. *Int J Cancer*, 2008,123:594–600.
- [40] Wang H, Arun BK, Wang H, et al. IGFBP2 and IGFBP5 overexpression correlates with the lymph node metastasis in T1 breast carcinomas. *Breast J*, 2008,14:261–267.
- [41] Pitteri SJ, Faca VM, Kelly-Spratt KS, et al. Plasma proteome profiling of a mouse model of breast cancer identifies a set of up-regulated proteins in common with human breast cancer cells. *J Proteome Res*, 2008,7:1481–1489.
- [42] Hung PS, Kao SY, Shih YH, et al. Insulin-like growth factor binding protein-5 (IGFBP-5) suppresses the tumorigenesis of head and neck squamous cell carcinoma. *J Pathol*, 2008,214:368–376.
- [43] Mita K, Zhang Z, Ando Y, et al. Prognostic significance of insulin-like growth factor binding protein (IGFBP)-4 and IGFBP-5 expression in breast cancer. *Jpn J Clin Oncol*, 2007,37:575–382.
- [44] Li X, Cao X, Li X, et al. Expression level of insulin-like growth factor binding protein 5 mRNA is a prognostic factor for breast cancer. *Cancer Sci*, 2007,98:1592–1596.
- [45] Liu Y, Sun W, Zhang K, et al. Identification of genes differentially expressed in human primary lung squamous cell carcinoma. *Lung Cancer*, 2007,56:307–317.
- [46] Walker G, MacLeod K, Williams AR, et al. Insulin-like growth factor binding proteins IGFBP3, IGFBP4, and IGFBP5 predict endocrine responsiveness in patients with ovarian cancer. *Clin Cancer Res*, 2007,13:1438–1444.
- [47] Miyatake T, Ueda Y, Nakashima R, et al. Down-regulation of insulin-like growth factor binding protein-5 (IGFBP-5): novel marker for cervical carcinogenesis. *Int J Cancer*, 2007,120:2068–2077.
- [48] Johnson SK, Dennis RA, Barone GW, et al. Differential expression of insulin-like growth factor binding protein-5 in pancreatic adenocarcinomas: identification using DNA microarray. *Mol Carcinog*, 2006,45:814–827.
- [49] Kulik G, Klippel A, Weber MJ. Antiapoptotic signalling by the

- insulinlike growth factor I receptor, phosphatidylinositol 3-kinase and Akt. *Mol Cell Biol*, 1997,17:1595–1606.
- [50] Ferry RJ Jr, Katz LE, Grimberg A, et al. Cellular actions of insulin-like growth factor binding proteins. *Horm Metab Res*, 1999,17:1595–1606.
- [51] Flier JS, Usher P, Moses AC. Monoclonal antibody to the type I insulin-like growth factor (IGF-I) receptor blocks IGF-I receptor-mediated DNA synthesis: clarification of the mitogenic mechanisms of IGF-I and insulin in human skin fibroblasts. *Proc Natl Acad Sci USA*, 1986,83:664–668
- [52] Rodriguez-Tarduchy G, Collins MK, Garcia I, et al. Insulin-like growth factor-I inhibits apoptosis in IL-3–dependent hemopoietic cells. *J Immunol*, 1992,149:535–540.
- [53] Harrington EA, Bennett MR, Fanidi A, et al. c-Myc–induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO J*, 1994,13:3286–3295.
- [54] O'Connor R, Kauffmann-Zeh A, Liu Y, et al. Identification of domains of the insulin-like growth factor I receptor that are required for protection from apoptosis. *Mol Cell Biol*, 1997,17:427–435.
- [55] Valentinis B, Romano G, Peruzzi F, et al. Growth and differentiation signals by the insulin-like growth factor 1 receptor in hemopoietic cells are mediated through different pathways. *J Biol Chem*, 1999,274:12423–12430.
- [56] Imai Y, Moralez A, Andag U, et al. Substitutions for hydrophobic amino acids in the N-terminal domains of IGFBP-3 and -5 markedly reduce IGF-I binding and alter their biologic actions. *J Biol Chem*, 2000,275:18188–18194.
- [57] Zeslawski W, Beisel HG, Kamionka M, et al. The interaction of insulin-like growth factor-I with the N-terminal domain of IGFBP-5(J). *EMBO J*, 2001,20:3638–3644.
- [58] Reichling T, Goss KH, Carson DJ, et al. Transcriptional profiles of intestinal tumors in Apc (Min) mice are unique from those of embryonic intestine and identify novel gene targets dysregulated in human colorectal tumors. *Cancer Res*, 2005,65:166–176.
- [59] Cobb LJ, Salih DA, Gonzalez I, et al. Partitioning of IGFBP-5 actions in myogenesis: IGF-independent anti-apoptotic function. *J Cell Sci*, 2004,117:1737–1746.
- [60] James PL, Stewart CE, Rotwein P. Insulin-like growth factor binding protein-5 modulates muscle differentiation through an insulin-like growth factor-dependent mechanism. *J Cell Biol*, 1996,133:683–693.
- [61] Stolf BS, Carvalho AF, Martins WK, et al. Differential expression of IGFBP-5 and two human ESTs in thyroid glands with goiter, adenoma and papillary or follicular carcinomas. *Cancer Lett*, 2003,191:193–202.
- [62] Tsibris JC, Segars J, Coppola D, et al. Insights from gene arrays on the development and growth regulation of uterine leiomyomata. *Fertil Steril*, 2002,78:114–121.
- [63] Pera EM, Wessely O, Li SY et al. Neural and head induction by insulin-like growth factor signals. *Dev Cell*, 2001,1:655–665.
- [64] Khan J, Bittner ML, Saal LH, et al. cDNA microarrays detect activation of a myogenic transcription program by the PAX3-FKHR fusion oncogene. *Proc Natl Acad Sci USA*, 1999,96:13264–13269.
- [65] Abrass CK, Berfield AK, Andress DL. Heparin binding domain of insulin-like growth factor binding protein-5 stimulates mesangial cell migration. *Am J Physiol*, 1997,273:F899–F906.
- [66] Gleeson LM, Chakraborty C, McKinnon T, et al. Insulin-like growth factor binding protein-1 stimulates human trophoblast migration by signaling through $\alpha 5\beta 1$ integrin via MAPK pathway. *J Clin Endocrinol Metab*, 2001,86:2484–2493.
- [67] Tripathi G, Salih DA, Drozd AC, et al. IGF-independent effects of insulin-like growth factor binding protein-5 (IGFBP5) *in vivo*. *FASEB J*, 2009,23:2616–2626.
- [68] Andress DL. Insulin-like growth factor-binding protein-5 (IGFBP-5) stimulates phosphorylation of the IGFBP-5 receptor. *Am J Physiol Endocrinol Metab*, 1998,274:E744–E750.
- [69] Baserga R. The IGF-I receptor in cancer research. *Exp Cell Res*, 1999,253:1–6.
- [70] Butt AJ, Dickson KA, McDougall F, et al. Insuline like growth factor-binding protein-5 inhibits the growth of human breast cancer cells *in vitro* and *in vivo*. *J Biol Chem*, 2003,278:29676–29685.
- [71] Ohlsson C, Nilsson A, Isaksson OG, et al. Effect of growth hormone and insulin-like growth factor-I on DNA synthesis and matrix production in rat epiphyseal chondrocytes in monolayer culture. *J Endocrinol*, 1992,133:291–300.
- [72] Olney RC, Wang J, Sylvester JE, et al. Growth factor regulation of human growth plate chondrocyte proliferation *in vitro*. *Biochem Biophys Res Commun*, 2004,317:1171–1182.
- [73] Reinecke M, Schmid AC, Heyberger-Meyer B, et al. Effect of growth hormone and insulin-like growth factor (IGF-I) on the expression of IGF-I messenger ribonucleic acid and peptide in rat tibial growth plate and articular chondrocytes *in vivo*. *Endocrinology*, 2000,141:2847–2853.
- [74] Wang J, Zhou J, Cheng CM, et al. Evidence supporting dual, IGF-I-independent and IGF-I-dependent, roles for GH in promoting longitudinal bone growth. *J Endocrinol*, 2004,180:247–255.
- [75] Wang J, Zhou J, Bondy CA. IGF1 promotes longitudinal bone growth by insulin-like actions augmenting chondrocyte hypertrophy. *FASEB J*, 1999,13:1985–1990.
- [76] Yakar S, Rosen CJ, Beamer WG, et al. Circulating levels of IGF-1 directly regulate bone growth and density. *J Clin Invest*, 2002,110:771–781.
- [77] Kiepe D, Andress DL, Mohan S, et al. Intact IGF-binding protein-4 and -5 and their respective fragments isolated from chronic renal failure serum differentially modulate IGF-I actions in cultured growth plate chondrocytes. *J Am Soc Nephrol*, 2001,12:2400–2410.
- [78] Kiepe D, Ciarmatori S, Hoeflich A, et al. Insulin-like growth factor (IGF)-I stimulates cell proliferation and induces IGF binding protein (IGFBP)-3 and IGFBP-5 gene expression in cultured growth plate chondrocytes via distinct signaling pathways. *Endocrinology*, 2005,146:3096–3104.
- [79] Kiepe D, Ciarmatori S, Haarmann A, et al. Differential expression of IGF system components in proliferating vs. differentiating growth plate chondrocytes: the functional role of IGFBP-5. *Am J Physiol Endocrinol Metab*, 2006,290:E363 – E371.
- [80] Hoang CD, D'Cunha J, Kratzke MG, et al. Gene expression profiling identifies matrix metalloproteinase overexpression in malignant mesothelioma. *Chest*, 2004,125:1843–1852.
- [81] Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis*, 2004,24:115–125.
- [82] Nishino R, Honda M, Yamashita T, et al. Identification of novel candidate tumour marker genes for intrahepatic cholangiocarcinoma. *J Hepatol*, 2008,49:207–216.
- [83] Umemura A, Itoh Y, Itoh K, et al. Association of gankyrin protein expression with early clinical stages and insulin-like growth factor-binding protein 5 expression in human hepatocellular carcinoma. *Hepatology*, 2008,47:493–502.
- [84] Hou XJ, Zhang YZ, Liu X, et al. Expressions of IGFBP-5, cFLIP in cervical intraepithelial neoplasia, cervical carcinoma and their clinical significances: a molecular pathology. *J Exp Clin Cancer Res*, 2009,28:70.
- [85] Boers W, Aarass S, Linthorst C, et al. Transcriptional profiling reveals novel markers of liver fibrogenesis: gremlin and insulin-like growth factor-binding proteins. *J Biol Chem*, 2006,281:16289–16295.
- [86] Sokolovi A, Sokolovi M, Boers W, et al. Insulin-like growth factor binding protein 5 enhances survival of LX2 human

- hepatic stellate cells. *Fibrogenesis Tissue Repair*, 2010,3:3.
- [87] Murphy LO, Abdel-Wahab YH, Wang QJ, et al. Receptors and ligands for autocrine growth pathways are up-regulated when pancreatic cancer cells are adapted to serum-free culture. *Pancreas*, 2001,22:293–298.
- [88] Hansel DE, Kern SE, Hruban RH. Molecular pathogenesis of pancreatic cancer. *Annu Rev Genomics Hum Genet*, 2003,4:237–256.
- [89] Conover CA, Hartmann LC, Bradley S, et al. Biological characterization of human epithelial ovarian carcinoma cells in primary culture: the insulin-like growth factor system. *Exp Cell Res*, 1998,238:439–449.
- [90] Wang H, Rosen DG, Wang H, et al. Insulin-like growth factor-binding protein 2 and 5 are differentially regulated in ovarian cancer of different histologic types. *Mod Pathol*, 2006,19:1149–1156.
- [91] Tonner E, Barber MC, Travers MT, et al. Hormonal control of insulin-like growth factor-binding protein-5 production in the involuting mammary gland of the rat. *Endocrinology*, 1997,138:5101–5107.
- [92] Tonner E, Allan G, Shkreta L, et al. Insulinlike growth factor binding protein-5 (IGFBP-5) potentially regulates programmed cell death and plasminogen activation in the mammary gland. *Adv Exp Med Biol*, 2000,480:45–53.
- [93] Ning Y, Hoang B, Schuller AG, et al. Delayed mammary gland involution in mice with mutation of the insulin-like growth factor binding protein 5 gene. *Endocrinology*, 2007,148:2138–2147.
- [94] Lee BP, Rushlow WJ, Chakraborty C, et al. Differential gene expression in premalignant human trophoblast: role of IGFBP-5. *Int J Cancer*, 2001,94:674–684.
- [95] Brennan C, Momota H, Hambardzumyan D, et al. Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. *PLoS One*, 2009,4:e7752.
- [96] Wan J, Ma J, Mei J, et al. The effects of HIF-1 α on gene expression profiles of NCI-H446 human small cell lung cancer cells. *J Exp Clin Cancer Res*, 2009,28:150.
- [97] Wang L, Sun Y, Jiang M, et al. FOS proliferating network construction in early colorectal cancer (CRC) based on integrative significant function cluster and inferring analysis. *Cancer Invest*, 2009,27:816–824.
- [98] Tanno B, Cesi V, Vitali R, et al. Silencing of endogenous IGFBP-5 by microRNA interference affects proliferation, apoptosis and differentiation of neuroblastoma cells. *Cell Death Differ*, 2005,12:213–223.
- [99] McCaig C, Perks CM, Holly MP. Intrinsic actions of IGFBP-3 and IGFBP-5 on Hs578T breast cancer epithelial cells: inhibition or accentuation of attachment and survival is dependant upon the presence of fibronectin. *J Cell Sci*, 2002,115:4293–4303.
- [100] Mukherjee A, Wilson EM, Rotwein P. Insulin-like growth factor (IGF) binding protein-5 blocks skeletal muscle differentiation by inhibiting IGF actions. *Mol Endocrinol*, 2008,22:206–215.
- [101] Ewton DZ, Coolican SA, Mohan S, et al. Modulation of insulin-like growth factor actions in L6A1 myoblasts by insulin-like growth factor binding protein (IGFBP)-4 and IGFBP-5: a dual role for IGFBP-5. *J Cell Physiol*, 1998,177:47–57.
- [102] Nam T, Morales A, Clemmons D. Vitronectin binding to IGF binding protein-5 (IGFBP-5) alters IGFBP-5 modulation of IGF-I actions. *Endocrinology*, 2002,143:30–36.
- [103] Nogita T, Kawashima M. Increased levels of plasma vitronectin in severe psoriatic patients. *Arch Dermatol Res*, 1992,284:315–317.
- [104] Huang X, Wu J, Spong S, et al. The integrin α v β 6 is critical for keratinocyte migration on both its known ligand, fibronectin and on vitronectin. *J Cell Sci*, 1998,111:2189–2195.
- [105] Clemmons DR, Horvitz G, Engleman W, et al. Synthetic α v β 3 antagonists inhibit insulin-like growth factor-I-stimulated smooth muscle cell migration and replication. *Endocrinology*, 1999,140:4616–4621.
- [106] Hyde C, Hollier B, Anderson A, et al. Insulin-like growth factors (IGF) and IGF-binding proteins bound to vitronectin enhance keratinocyte protein synthesis and migration. *J Invest Dermatol*, 2004,122:1198–1206.
- [107] Krickler JA, Towne CL, Firth SM, et al. Structural and functional evidence for the interaction of insulin-like growth factors (IGFs) and IGF binding proteins with vitronectin. *Endocrinology*, 2003,144:2807–2815.
- [108] Nishidate T, Katagiri T, Lin ML, et al. Genome-wide gene expression profiles of breast cancer cells purified with laser microbeam microdissection: identification of genes associated with progression and metastasis. *Int J Oncol*, 2004,25:797–819.
- [109] Parker A, Rees C, Clarke J, et al. Binding of insulin-like growth factor (IGF)-binding protein-5 to smooth-muscle cell extracellular matrix is a major determinant of the cellular response to IGF-I. *Mol Biol Cell*, 1998,9:2383–2392.
- [110] Yasuoka H, Yamaguchi Y, Feghali-Bostwick CA. The profibrotic factor IGFBP-5 induces lung fibroblast and mononuclear cell migration. *Am J Respir Cell Mol Bio*, 2009,41:179–188.
- [111] Cesi V, Vitali R, Tanno B, et al. Insulin-like growth factor binding protein 5 (IGFBP-5): contribution to growth and differentiation of neuroblastoma cells. *Ann NY Acad Sci*, 2004,1028:59–68.
- [112] James PL, Jones SB, Busby WH Jr, et al. A highly conserved insulin-like growth factor-binding protein (IGFBP-5) is expressed during myoblast differentiation. *J Biol Chem*, 1993,268:22305–22312.
- [113] Ren H, Yin P, Duan C. IGFBP-5 regulates muscle cell differentiation by binding to IGF-II and switching on the IGF-II auto-regulation loop. *J Cell Biol*, 2008,182:979–991.
- [114] Stein GS, Lian JB, Owen TA. Bone cell differentiation: a functionally coupled relationship between expression of cell-growth- and tissue-specific genes. *Curr Opin Cell Biol*, 1990,2:1018–1027.
- [115] Schneider MR, Wolf E, Hoeflich A, et al. IGF-binding protein-5: flexible player in the IGF system and effector on its own. *J Endocrinol*, 2002,172:423–440.
- [116] Butt AJ, Dickson KA, Jambazov S, et al. Enhancement of tumor necrosis factor- α -induced growth inhibition by insulin-like growth factor-binding protein-5 (IGFBP-5), but not IGFBP-3 in human breast cancer cells. *Endocrinology*, 2005,146:3113–3122.
- [117] Kim KS, Seu YB, Baek SH, et al. Induction of cellular senescence by insulin-like growth factor binding protein-5 through a p53-dependent mechanism. *Mol Biol Cell*, 2007,18:4543–4552.
- [118] Perks CM, McCaig C, Holly JM. Differential insulin-like growth factor (IGF)-independent interactions of IGF binding protein-3 and IGF binding protein-5 on apoptosis in human breast cancer cells: involvement of the mitochondria. *J Cell Biochem*, 2000,80:248–258.
- [119] Miyake H, Nelson C, Rennie PS, et al. Overexpression of insulin-like growth factor binding protein-5 helps accelerate progression to androgen-independence in the human prostate LNCaP tumor model through activation of phosphatidylinositol 3'-kinase pathway. *Endocrinology*, 2000,141:2257–2265.
- [120] Higo H, Duan C, Clemmons DR, et al. Retinoic acid inhibits cell growth in HPV negative cervical carcinoma cells by induction of insulin-like growth factor binding protein-5 (IGFBP-5) secretion. *Biochem Biophys Res Commun*, 1997,239:706–709.
- [121] Conover CA, Kiefer MC. Regulation and biological effect of endogenous insulin-like growth factor binding protein-5 in human osteoblastic cells. *J Clin Endocrinol Metab*, 1993,76:

- 1153–1159.
- [122] Kuemmerle JF, Bushman TL. IGF-I stimulates intestinal muscle cell growth by activating distinct PI 3-kinase and MAP kinase pathways. *Am J Physiol*, 1998,275:G151–G158.
- [123] Kuemmerle JF. Endogenous IGF-I regulates IGF binding protein production in human intestinal smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol*, 2000,278:G710–G717.
- [124] Kuemmerle JF, Zhou H. Insulin-like growth factor-binding protein-5 (IGFBP-5) stimulates growth and IGF-I secretion in human intestinal smooth muscle by Ras-dependent activation of p38 MAP kinase and Erk1/2 pathways. *J Biol Chem*, 2002,277:20563–20571.
- [125] Duan C, Liimatta MB, Bottum OL. Insulin-like growth factor (IGF)-I regulates IGF-binding protein-5 gene expression through the phosphatidylinositol 3-kinase, protein kinase B/Akt, and p70 S6 kinase signaling pathway. *J Biol Chem*, 1999,274:37147–37153.
- [126] Cheng HL, Shy M, Feldman EL. Regulation of insulin-like growth factorbinding protein-5 expression during Schwann cell differentiation. *Endocrinology*, 1999,140:4478–4485.
- [127] Xin X, Hou YT, Li L, et al. IGF-I, increases IGFBP-5 and collagen 1 (I) mRNAs by the MAPK pathway in rat intestinal smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol*, 2004,286:G777–G783.
- [128] Fleming JM, Brandimarto JA, Cohick WS. The mitogen-activated protein kinase pathway tonically inhibits both basal and IGF-I-stimulated IGF-binding protein-5 production in mammary epithelial cells. *J Endocrinol*, 2007, 194:349–359.
- [129] Erclik MS, Mitchell J. The role of protein kinase C-delta in PTH stimulation of IGF-binding protein-5 mRNA in UMR-106-01 cells. *Am J Physiol Endocrinol Metab*, 2002,282:E534–E541.
- [130] Erclik MS, Mitchell J. Activation of the insulin-like growth factor binding protein-5 promoter by parathyroid hormone in osteosarcoma cells requires activation of an activated protein-2 element. *J Mol Endocrinol*, 2005,34:713–722.
- [131] Maeda H, Yonou H, Yano K, et al. Prostate-specific antigen enhances bioavailability of insulin-like growth factor by degrading insulin-like growth factor binding protein 5. *Biochem Biophys Res Commun*, 2009, 381:311–316.
- [132] Cameron DA, Ritchie AA, Langdon S, et al. Tamoxifen induced apoptosis in ZR-75 breast cancer xenografts antedates tumour regression. *Breast Cancer Res Treat*, 1997,45:99–107.
- [133] Clarkson RW, Wayland MT, Lee J, et al. Gene expression profiling of mammary gland development reveals putative roles for death receptors and immune mediators in post-lactational regression. *Breast Cancer Res*, 2004,6:R92–R109.
- [134] Schedin P, O'Brien J, Rudolph M, et al. Microenvironment of the involuting mammary gland mediates mammary cancer progression. *J Mammary Gland Biol Neoplasia*, 2007,12:71–82.
- [135] Stein T, Salomonis N, Nuyten DS, et al. A mouse mammary gland involution mRNA signature identifies biological pathways potentially associated with breast cancer metastasis. *J Mammary Gland Biol Neoplasia*, 2009, 14:99–116
- [136] Niu J, Huang YJ, Wei S, et al. Association between a functional polymorphism (-1195T>C) in the IGFBP5 promoter and head and neck cancer risk. *Head Neck*, 2010,33:650 – 660.
- [137] Shersher DD, Vercillo MS, Fhied C, et al. Biomarkers of the insulin-like growth factor pathway predict progression and outcome in lung cancer. *Ann Thorac Surg*, 2011,92:1805 – 1811.