

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Insulin-Like-Growth Factor-Binding-Protein 7: An Antagonist to Breast Cancer

Tania Benatar, Yutaka Amemiya,  
Wenyi Yang and Arun Seth

*Division of Molecular and Cellular Biology, Sunnybrook Research Institute,  
Department of Anatomic Pathology, Sunnybrook Health Sciences Centre,  
Department of Laboratory Medicine and Pathobiology,  
University of Toronto, ON,  
Canada*

## 1. Introduction

### 1.1 The insulin-like growth factor (IGF) system

The insulin-like growth factor (IGF) system has been shown to have an integral role in normal growth and development, and in the pathophysiology of various cancers. The IGF system is comprised of a series of circulating ligands (IGF-1, IGF-2), transmembrane receptor tyrosine kinases (IGF-1R, IGF-2R, and the insulin receptor (IR), high affinity ligand-binding proteins (IGFBP1-6), IGFBP proteases, and several low affinity IGFBP-related proteins (IGFBP-rp1 to 10) that work in unison to regulate cell growth [1].

There are two key circulating ligands, IGF-1 and IGF-2, which share approximately 50% structural homology with insulin[2]. IGF-1 is produced primarily in the liver in response to circulating levels of growth hormone(GH) [3]. IGF-1 and IGF-2 are highly homologous small peptide hormones of approximately 7 kDa molecular mass, which are important mitogens that affect cell growth and metabolism [2]. IGFs interact with specific cell surface receptors, designated type I and type 2 IGF receptors, and can also interact with insulin receptor (IR).

The type I IGF receptor (IGF-1R) is a transmembrane heterotetramer consisting of 2 extracellular alpha subunits and two intracellular beta subunits linked by disulfide bonds (fig 1). The intracellular component of IGF-1R has intrinsic tyrosine kinase activity that requires ligand binding for activation [4]. The IGF-1R and the IR share approximately 60% homology which allows them to form hybrid receptors [5]. As a result of this homology, IGF-1R can be activated not only by IGF-1 but also IGF-2 and insulin, although the affinity of IGF-1R for IGF-2 and insulin is approximately 10 fold and 1000 fold lower than for IGF-1, respectively [6]. The type 2 IGF receptor (IGF-2R), which is identical to the cation-independent mannose-6-phosphate receptor, binds IGF-2 with 500 fold increased affinity over IGF-1[7]. IGF-2R does not bind insulin. Most of the biological activity of IGF-2 is thought to be mediated through binding IGF-1R[7]. IGF-2 is known to function primarily as a scavenger receptor, regulating circulating IGF-II levels through internalization and degradation [7].

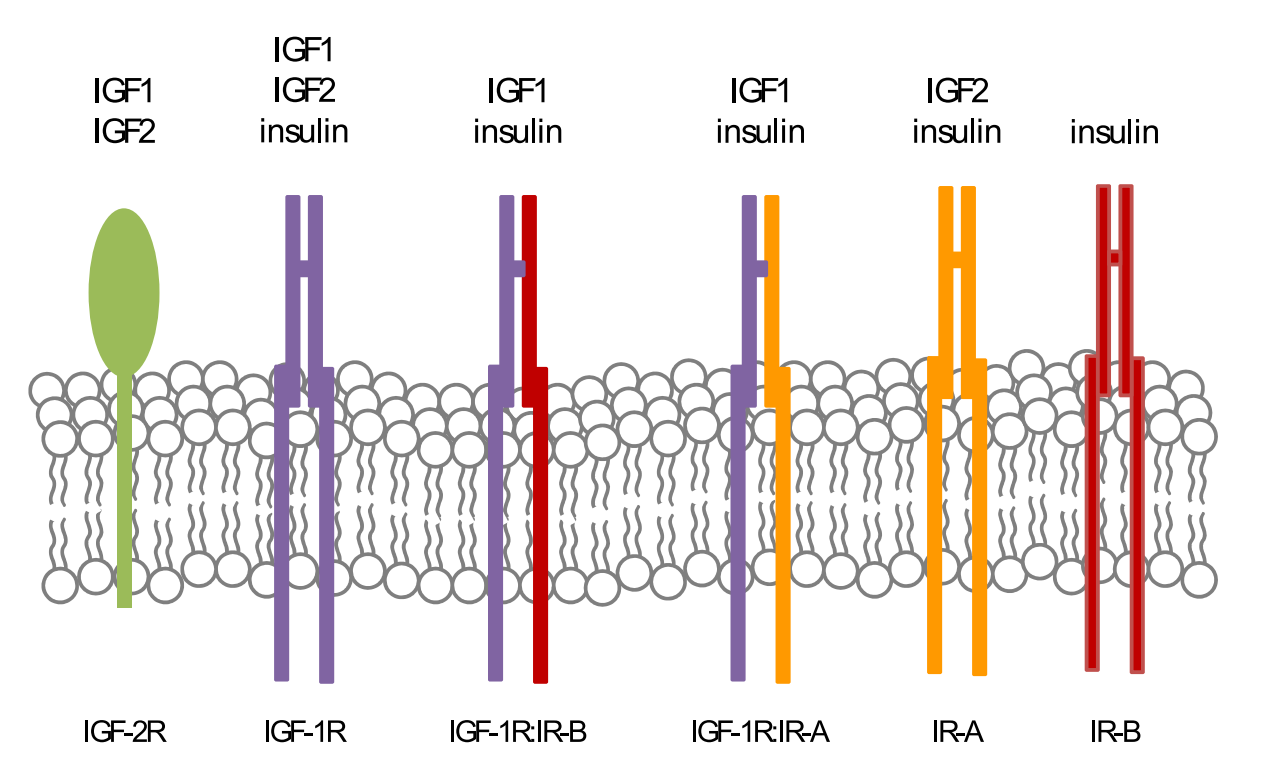


Fig. 1. Cell surface receptors for IGFs and insulin. Illustration of the different transmembrane receptors and ligands of the IGF system. Purple represents the alpha and beta subunit of IGF-1R; red represents the alpha and beta subunit of the IR-B; orange represents the alpha and beta subunit of the IR-A ; green represents the IGF-2R. The potential ligand(s) is shown above the respective receptor.

Two distinct insulin receptor isoforms have been identified and are known to hybridize with IGF-1R. The insulin receptor isoform A (IR-A), the IR fetal isoform, is generated by alternative splicing through the deletion of exon 11 of the insulin receptor gene whereas the insulin receptor isoform B (IR-B) retains exon 11 [8]. IR-A is the predominant isoform expressed in fetal tissues and cancers with ubiquitous expression, whereas IR-B appears in postnatal life within insulin-target tissues, such as muscle, adipose tissue and kidney [9,10,11]. Data obtained from murine 32D hemopoietic cells demonstrated that IR-A preferentially induces mitogenic and anti-apoptotic signals, whereas IR-B predominantly induces cell differentiation signals [12]. IR-A, but not IR-B, binds IGF-II with high affinity and operates as a second physiological receptor for this growth factor [13]. The two IR isoform half receptors (composed of one alpha and one beta subunit) can heterodimerize, resulting in the formation of either homologous IR-A/IR-A or IR-B/IR-B receptors as well as the hybrid IR-A/IR-B insulin receptors [14](fig 1). Heterodimers can also form between IGF-1R and IR, resulting in the hybrid IGF-1R/IR-A and hybrid IGF-1R/IR-B. Hybrid IGF-1R/IR receptors are believed to mostly bind IGF-1, although they can also bind insulin but with a much lower affinity [15]. The IGF system is also regulated by a group of at least six high affinity ligand-binding proteins, the insulin-like binding proteins (IGFBPs), as well as low affinity ligand-binding proteins (IGFBP-rp1 to 10).

## 2. The IGFBP superfamily

Unlike insulin, IGFs circulate in biological fluids complexed to a family of structurally related binding proteins, called IGF-binding proteins (IGFBPs). The IGFBP superfamily can be subdivided into two groups: the high affinity IGFBPs (IGFBP1 to 6) and the low-affinity IGFBPs (IGFBP7 to 10, and IGFBP-rP5 to 10). [16].

### *High affinity binding proteins (IGFBPs)*

There are, to date, six well characterized mammalian IGFBPs, designated IGFBP-1 through -6. IGFBPs are capable of binding IGF-1 and IGF-2 with higher affinity than their interactions with the IGF-1R, but do not bind to insulin. Some IGFBPs compete for activity of IGFs at the receptor level and antagonize IGF signaling, while others (eg. IGFBP2 and IGFBP5) appear to amplify IGF signaling [17]. Therefore, IGFBPs function not only as carriers of IGFs, thereby prolonging the half-life of the IGFs, but also act as modulators of IGF availability and activity[18]. Apart from their ability to inhibit or enhance IGF actions, all the IGFBPs have been reported to exert distinct biological actions such as cell proliferation, differentiation, migration, angiogenesis and apoptosis through an IGF/IGF-1R-independent manner [19,20,21,22,23].

All six IGFBPs share approximately 35% sequence identity with each other. The primary structures of mammalian IGFBPs appear to contain three distinct domains of roughly similar sizes: the conserved N-terminal domain, the highly variable midregion, and the conserved C-terminal domain. Within their N-terminal domain, all IGFBPs share a common conserved cysteine-rich domain termed IGFBP motif (GCGCCXXC) (fig 2). The IGFBP motif is encoded by a single exon, has overall similar topology and is only present in vertebrates [19]. Ten to 12 of the 16-20 cysteines found in the prepeptides are located within this domain. In IGFBP1-5 these 12 cysteines are fully conserved, whereas 10 of the 12 cysteines are invariant in IGFBP6 [19]. The midregion is believed to act structurally as a hinge between the N and C terminal domains. Posttranslational modifications (glycosylation, phosphorylation) of the IGFBPs has been found only in the midregion so far. The C-termini of IGFBPs, like the N-terminal domain, are highly conserved, and contain the remaining 6 of the total 16-20 cysteines. The primary sequence of all members of the IGFBP family surrounding the last 5 cysteines is strikingly similar (~40%), implying that the tertiary structure of the C-terminal domain should be almost identical. Interestingly, the amino acid sequences embracing these last 5 cysteines share 37% similarity with the thyroglobulin-type-1 domain, a structural motif occasionally employed as an inhibitor of proteases [19,24]. It has been hypothesized that the N and C-terminal domains are capable of acting independently of each other based on the fact that the cysteines within each of the conserved regions are even numbered, and that proteolytic cleavage products of IGFBPs contain either the C or N-terminal regions. Indeed, disulphide linkages have been shown to form typically within each conserved domain, rather than between domains[25,26]. All the IGFBPs are encoded by 4 exons, except IGFBP3 which has an extra exon, exon 5, that is not translated. The striking observation is the correlation between these IGFBP exons and the three protein domains of IGFBPs. The N-terminal domain is encoded within exon 1 in all of the IGFBPs, as is the 5' untranslated region and a few amino acids of the midregion. Exon 2 encodes the nonconserved midregion. Both exons 3 and 4 encode for the conserved C-terminal domain. The containment of the N-terminal domain within one exon, combined with the ability to bind IGFs, supports the concept of an IGFBP superfamily [27,19].



Fig. 2. Partial amino acid sequence alignment of human IGFBP-1 to 10, and IGFBP-rP5-rP10. The consensus IGFBP motif which relates all of these sequences as a family is boxed. Consensus cysteine residues are shown in red. The matrixptase consensus site sequence for cleavage is indicated in blue. Alignment was performed using the Clustalw2 sequence alignment program (European Bioinformatics Institute; <http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Small gaps were introduced to optimize alignment. Nomenclature for the IGFBP7-IGFBP15, IGFBP-rPs: IGFBP7, IGFBP-rP1;mac25/TAF/PSF1; IGFBP8, IGFBP-rP2, CTGF; IGFBP-rP3,NovH; IGFBP-rP4, Cyr61; IGFBP-rP5, L56/HtrA; IGFBP-rP6, ESM-1; IGFBP-rP7, WISP-2/CTGF-L; IGFBP-rP8, WISP-1; IGFBP-rP9, WISP-3; IGFBP-rP10, Bono1.

Low affinity binding proteins (IGFBP-rPs)

Upon comparison of the IGFBP N-terminus in other cysteine-rich proteins, another group of proteins that were structurally related to the IGFBP family were identified, IGFBP-related proteins (IGFBP-rPs). Based on sequence alignment, the N-terminal domains of the IGFBP-rPs have significant similarities to the IGFBPs (40-57%) within their N-terminal domains, conserving all of the 12 cysteines within the N-terminal domain, including the consensus IGFBP motif. Past the N-terminus, the similarities decrease significantly to less than 15%. Unlike the IGFBPs, the IGFBP-rPs do not contain the thyroglobulin-type 1 domain at the C-terminus [28]. Their low affinity for IGFs together with their conserved structural homology to the IGFBP family suggested that these IGFBPs may have unique biological properties independent of their capacity to bind IGF. The first protein proven to be functionally related to the IGFBPs was IGFBP-rP1(IGFBP7)[29,30]. A group of highly related, cysteine-rich proteins were subsequently identified as part of the IGFBP-like family, termed the CCN family of proteins, including connective tissue growth factor (CTGF)[16], *nov* (nephroblastoma overexpressing) oncogene [31],*cyr61* [32], and three genes (WISP-1, WISP-2, and WISP-3) that are upregulated in Wnt-1-transformed cells and are aberrantly expressed in human colon tumors [33]. HtrA (IGFBP-rP5) refers to a family of serine proteases who's main functions are protein quality control, and have been implicated in



tumour suppression and in the control of proliferation, migration and neurodegeneration (reviewed in [134]). IGFBP-rP10 (Bono1), the most recently identified member of the IGFBP family, with the highest homology to IGFBP7 at the amino acid level (42.2%), has been shown to be involved in the proliferation of osteoblasts during bone formation and bone regeneration [135]. This chapter will preferentially focus on IGFBP7.

*IGFBP7 overview*

The gene for human IGFBP7 is localized to chromosome 4q12-13 [34]. The mouse homolog shares 87.5% nucleotide identity and 94.4% similarity with human IGFBP7 [35]. IGFBP7 amino acid sequence has an overall 40-45% similarity and 20-25% identity to IGFBPs. The protein is produced as a precursor of 282 amino acids, which is processed to a mature 27 kD protein of 256 amino acids with one N-glycosylation site resulting in a secreted mature protein of 33 kD [16,30,27]. Structurally, the region of similarity of IGFBP7 to IGFBPs is confined to the N-terminal domain, encompassing the common IGFBP motif in a region containing 11 out of the 12 conserved cysteines [36](fig 2). Another domain found within the

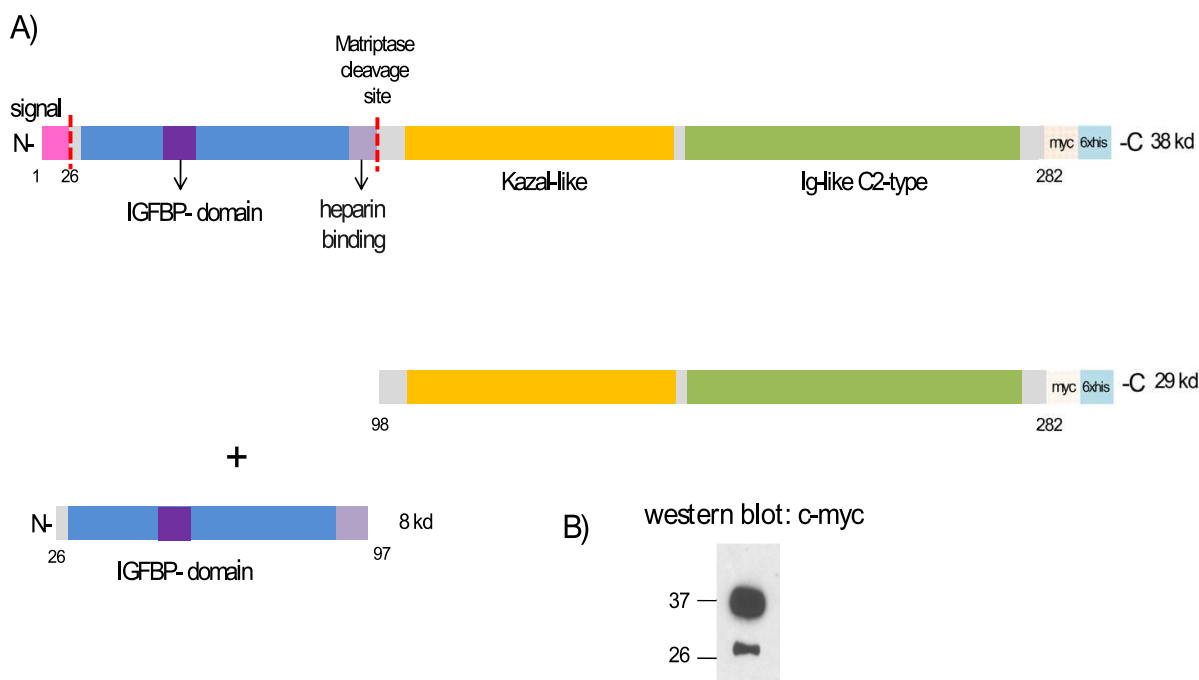


Fig. 3. Processing of recombinant IGFBP7 protein. A) Full length IGFBP7 protein is shown beginning with the signal sequence in red, which is cleaved off upon secretion from the cell. The N terminal contains the consensus IGFBP domain (dark purple), and the heparin binding domain (light purple). Kazal-like motif is shown in yellow and the Ig-like C2 domain is indicated in green. As a result of overexpression through the pSec-Tag2B plasmid, the protein is tagged in our system with *myc* and *his* at the C terminal, as shown in light pink and blue, respectively. Matriptase cleavage site is C terminal to the heparin binding domain between amino acid 97 and 98. Cleavage results in the production of 2 fragments, the N terminal portion (8 kD) and the C terminal 29 kD fragment. B) Western blotting of conditioned medium from MDA-MB-468 overproducing breast cancer cell line with anti-*myc* antibodies produces 2 bands, corresponding to the predominant large 38 kD protein, and the minor 29 kD cleaved protein.

N-terminus is a heparin sulfate binding site, consisting of 20 amino acid residues including 7 basic amino acids, which allows weak cell adhesion by interacting with cell surface-associated heparin sulfate proteoglycans [37](fig 3). Immediately adjacent to the N terminal domain is a stretch of 30-45 amino acid residues that has 30% similarity to the Kazal family of serine proteinase inhibitors, including the human pancreatic secretory trypsin inhibitor [38]. This domain, known as a KI domain, is also found in follistatin, leading to the hypothesis that IGFBP7 was a follistatin-like protein [35]. IGFBP7 can be proteolytically cleaved to a two-chain form by the type II membrane-bound serine proteinase, matrilysin [39](fig 3). Cleavage occurs between K(Lys)97 and A(Ala)98, resulting in a 26 kD protein comprised of the C-terminal domain, and an 8 kD peptide corresponding to the N-terminal domain [40](fig 2,3). Cleavage results in almost a complete loss of both insulin/IGF-1 binding activity, while increasing cell adhesion activity [40].

#### *IGFBP7-interacting proteins*

Four groups independently identified the human IGFBP7 protein. One of these groups cloned the mac25 cDNA from normal leptomeningial and mammary epithelial cells, with expression of IGFBP7 decreased in the corresponding tumor cells [36,34]. The protein was shown to be able to bind IGFs, albeit with much lower affinity than IGFBPs [30]. During that same period, two other proteins were purified and characterized that were subsequently shown to be identical to the protein encoded by *mac25*. First, tumor adhesion factor (TAF) was isolated from the conditioned media of a human bladder carcinoma cell line, and promoted cell adhesion activity [41]. Second, prostacyclin-stimulating factor (PSF) was isolated from the conditioned media of human diploid fibroblasts [42]. It was so termed due to its ability to stimulate prostacyclin production in endothelial cells, but not in patients with diabetes mellitus [43,44]. Finally, T1A12 was identified by subtractive cDNA cloning using RNAs from a normal breast epithelial cell line Hs578Bst and the breast cancer cell line Hs587T [45].

The ability of IGFBP7 to bind both IGF-1 and IGF-2, albeit with lower affinity than IGFBPs, led to its renaming as IGFBP7 [30]. However, IGFBP7 is unique amongst its family members in that it can bind insulin with high affinity, whereas IGFBPs 1-6 can only bind insulin with low affinity. This ability of IGFBP7 is due to the exposure of the insulin binding site at the amino terminal region due to lack of conserved cysteine residues in the C-terminal end, which are important for IGF binding by IGFBPs [46,47]. IGFBP7 can compete with insulin receptors for binding of insulin, thus preventing insulin-stimulated autophosphorylation of the insulin receptor  $\beta$  subunit [47]. IGFBP7 also contains a 'follistatin module' in its protein sequence, and has been shown to bind activin, a member of the TGF- $\beta$  superfamily of growth factors [48]. Activin and its receptors are associated with growth modulation in glandular organs. Specifically, when activin signaling is disrupted or lost in normal mammary cells, malignant progression is potentiated, as demonstrated by the global decrease in the abundance of activin and its receptors in high grade breast cancer [49].

Another binding partner is type IV collagen. IGFBP7 co-localizes with type IV collagen in the vascular basement membrane [29]. IGFBP7 also can bind to cell surface-associated heparin sulfate proteoglycans, specifically, syndecan-1 [40]. IGFBP7 has also been shown to bind certain CC chemokines, specifically, RANTES, SLC, and the CXC chemokine, IP-10 [50].

#### *Expression*

IGFBP7 is found in some biological fluids, such as serum, urine, CSF and amniotic fluid [51]. In normal human adult sera, the median IGFBP7 was 21.0  $\mu\text{g/liter}$ . IGFBP7 is expressed in a

variety of normal tissues including heart, spleen, ovary, small intestine and colon [52]. Immunohistochemistry performed on normal human tissues showed a ubiquitous intense staining of peripheral nerves, smooth muscle cells, including those from blood vessel walls, gut, bladder, breast and prostate. Cilia from the respiratory system, epididymis, and fallopian tube also demonstrated intense positive staining. Most endothelial cells were seen to be positive, whereas fat cells, plasma cells and lymphocytes were negative. Specific IGFBP7 expression was limited to certain cell types in the kidney, adrenal gland and skeletal muscle [52]. IGFBP7 has also been shown to play a role in endometrial physiology. IGFBP7 expression is increased in the receptive versus prereceptive endometrium, and rises sharply again in late luteal phase. The protein was localized at the apical part of the luminal and glandular epithelium, as well as in stromal and endothelial cells [53]. Strong expression of IGFBP7 has also been seen in high endothelial vessels (HEV)[50].

#### *Oncogene induced senescence*

Normal cells have a limited proliferative lifespan, after which they enter a state of irreversible growth arrest. This process, originally observed by Hayflick and Moorhead and called replicative senescence, is believed to result in human cells from telomere shortening as a consequence of cell division [54,55]. This was thought to be a failsafe mechanism preventing the expansion of aged cells[56]. Almost three decades ago, it was observed that normal cells are refractory to oncogene transformation [57]. Ectopic expression of the oncogene H-RAS<sup>G12V</sup> in normal fibroblasts induced senescence that was later shown to be telomere-independent, representing another type of senescence triggered by oncogenes, called oncogene-induced senescence (OIS)[58,59]. OIS, together with oncogene-induced apoptosis, has been suggested to act as a true barrier to cancer, once cellular damage is inefficiently repaired[56,60]. OIS can be triggered by activated oncogenes like BRAF<sup>E600</sup> or RAS<sup>V12</sup> or by the loss of tumor suppressor proteins, like PTEN or NF1[61,62,63]. OIS is often characterized by the upregulation of the CDK inhibitors p15<sup>INK4B</sup>, p16<sup>INK4A</sup>, and p21<sup>CIP1</sup>, as well as by an increase in senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -Gal) activity [64,65]. Acute inactivation of certain genes, such as Rb or p53, can reverse OIS [66,67,68]. A typical example of OIS occurs in melanocytic nevi, which are benign skin lesions that rarely progress to melanoma [69,70]. Nevi are growth arrested and display classical hallmarks of senescence, including expression of SA- $\beta$ -Gal, and the cell cycle inhibitor, p16<sup>INK4A</sup> [62,71,72]. Activating BRAF mutations account for up to 82% of melanocytic nevi [73]. Senescent cells secrete a broad spectrum of factors, primarily involved in IGF and TGF- $\beta$  signaling, ECM remodeling and inflammation [74,75,76,77,78]. Together, these secreted factors are referred to as the Senescence-Messaging Secretome (SMS) or the Senescence-Associated Secretory Phenotype (SASP) [79,78]. IGFBP7 has been identified as one of these factors responsible for the establishment and/or maintenance of OIS [34,75].

### **3. IGFBP7 as tumor suppressor in various cancers**

IGFBP7 has been shown to be a tumor suppressor in a variety of solid cancers (summarized in Table 1). Its expression is lost upon progression to more aggressive cancer types. Loss of expression is associated with poor prognoses. Reexpression or exposure of cancer cell lines to IGFBP7 results in either senescence or apoptosis, and when these IGFBP7-expressing cell lines are xenografted in mice, tumor growth is inhibited.



Breast cancer

IGFBP7 has been shown to be a tumor suppressor in breast cancer. IGFBP7 was identified as one of the genes overexpressed in senescent human mammary epithelial cells (HMEC) (10 fold higher than quiescent cells of the same origin), and which was upregulated in normal mammary epithelial cells by all-*trans*-retinoic acid [34,80]. We cloned the gene for IGFBP7 by subtractive hybridization from the Hs568T breast cancer cell line and found IGFBP7 to be downregulated in primary breast cancer tissues. In normal breast tissue, IGFBP7 protein expression is concentrated in the cytoplasm of luminal epithelial cells, in ducts and acini of normal and benign primary breast tissues as well as other luminal, normal human cellular structures, suggesting an important role for IGFBP7 in the maintenance of normal breast and tissue architecture in general [45].

Cancer type		Down-regulated	Up - regulated	IGFBP7 Introduction	Effect	Reference
Breast	MCF-7			Overexpressed	G0-G1 arrest Senescence	[86]
	MDA-MB-468	pERK1/2		Overexpressed	↓ Tumour-genicity ↓ Growth	[85]
	Xenograft-MDA-MB-468			Overexpressed	and migration	[85]
	MDA-MB-231		pp38 p53, p21	Exogenous Protein	↓ Cell growth ↑ Senescence ↑ Apoptosis	Manuscript submitted
Colorectal	SW620, COLO205, HT29			5-Aza-dc	↓ Cell migration/i nvasion	[93]
	RKO, CW2	E-cadherin B-catenin pRB	p53	Overexpressed	G1 arrest Senescence	[95]
	DLD-1				↓ Anchorage indepe- ndent growth ↑ Cell adhesion	[98]
	Xenograft-DLD-1			Overexpressed	↓ Tumour-genicity	[98]
	Xenograft-HT29, SW620			Exogenous protein	↓ Tumour-genicity	[89]
Hepatocellular	PLC/PRF/5	SMARCB1 BNIP3L p27	pERK1/2 cyclin D1 cyclin E	shRNA targeting IGFBP7 mRNA	IFNα resistance ↑ Cell growth ↓ Apoptosis	[106,117]
Melanoma	Nevi	pERK1/2	RKIP	Exogenous protein	Senescence	[75]
	Cell line		BNIP3L		Apoptosis	[75]
	Xenograft				Apoptosis	[75]
	Metastatic			Intervenous protein injection	Growth inhibition	[89]
	Murine metastatic	VEGF	Caspase-3	Intra-tumoral plasmid injection	Apoptosis	[90]

Cancer type		Down-regulated	Up - regulated	IGFBP7 Introduction	Effect	Reference
Prostate	M12			Overexpressed	↑ Doubling time ↓ Apoptosis sensitivity Epithelial Morphology change ↓ Colony formation	[102]
	M12 xenograft			Over-expressed	↓ Tumour size	[102]
Thyroid	N1M1	pERK1/2	p53 p21 cleaved PARP	Over-expressed	Apoptosis ↓ Cell migration	[105]
	N1M1			Over-expressed	Reduced tumour growth	[105]

Table 1. IGFBP7 as a tumor suppressor in various cancer models. Summarized data from six different cancers, showing the effect of overexpression or inhibition of IGFBP7 on cancer cell growth both in vivo and in vitro, as well as signaling pathways affected.

Expression of IGFBP7 decreases with breast cancer progression. Normal breast tissues had very high IGFBP7 protein levels, such as luminal epithelial cells of normal lobules and ducts, as well as in benign proliferation of ducts consistent with fibroadenoma [45]. By immunohistochemical staining, IGFBP7 expression was detected in all normal and benign patient samples examined, with particularly strong staining in luminal epithelial cells of normal ducts, and acini or endothelial cells of blood vessels [81]. Intermediate to weak IGFBP7 staining was evident in hyperplastic breast tissue and DCIS specimens [81]. In addition, IGFBP7 was significantly upregulated in low grade ductal carcinoma in situ (DCIS) relative to high grade DCIS, as judged by CDNA microarray analysis. In invasive breast tumors, immunohistochemical analysis revealed that IGFBP7 is downregulated at the protein level [45]. IGFBP7 is downregulated in some breast tumors by loss of heterozygosity (LOH), and is also reduced by promoter methylation, both of which lead to increased tumor incidence and poor overall survival [45,82,83]. When DNA extracted from microdissected breast tissues was used with a microsatellite marker based method to determine allelic loss of the IGFBP7 locus in paired normal and invasive breast tissues, 50% of the informative samples from 30 matched pairs of normal and breast tumor tissues showed allele-specific LOH suggesting that the IGFBP7 gene was inactivated by deletions in at least a portion of each tumor [45]. A thoroughly characterized group of 106 invasive breast samples was surveyed using the tumor tissue microarray technique and immunohistochemistry [84]. Approximately 40% of tumors have low or no IGFBP7 staining suggesting that the gene or gene product was inactivated in a subset of invasive breast cancer samples [84]. Low IGFBP7 was associated with high cyclin E expression, retinoblastoma protein (pRb) inactivation, poorly differentiated tumors and higher stage. There was a significantly impaired prognosis for patients with low IGFBP7-expressing tumors. IGFBP7 also showed an inverse correlation with proliferation (Ki-67) in ER<sup>-</sup> tumors [84].

IGFBP7 expression was examined in 32 primary patient breast tumors and matched metastatic counterparts (fig 4). Low levels of IGFBP7 expression were found in 25/32 primary tumors. Approximately half of these tumors had lower levels of IGFBP7 in their metastatic tumors compared to the matched primary tumor, indicating that loss of IGFBP7 confers a selective growth advantage for metastatic lesions [85].

In order to investigate the growth of human breast cancers in an in vivo model, 7 human primary tumors were implanted into human bone grafts under the right flank of human-bone NOD/SCID mice. Only triple negative breast tumors grew in these mice (table 2). One of the triple negative primary breast tumors was serially transplanted more than five times. Each serial transplant resulted in increased tumor uptake and shorter growth rate. The tumor latency was decreased by approximately half after the first re-implantation. Examination of IGFBP7 expression revealed that each serial transplant resulted in lower levels of IGFBP7 expression by qRT-PCR [85](fig 4). Comparing the xenografted tumor to the original primary patient tumor revealed an increase in the anti-human specific proliferation marker, Ki67 ( $42.03 \pm 8.87$  to  $53.3 \pm 3.6$ ). These results again confirmed an inverse correlation between IGFBP7 expression and breast tumor growth as well as aggressiveness of the tumor.

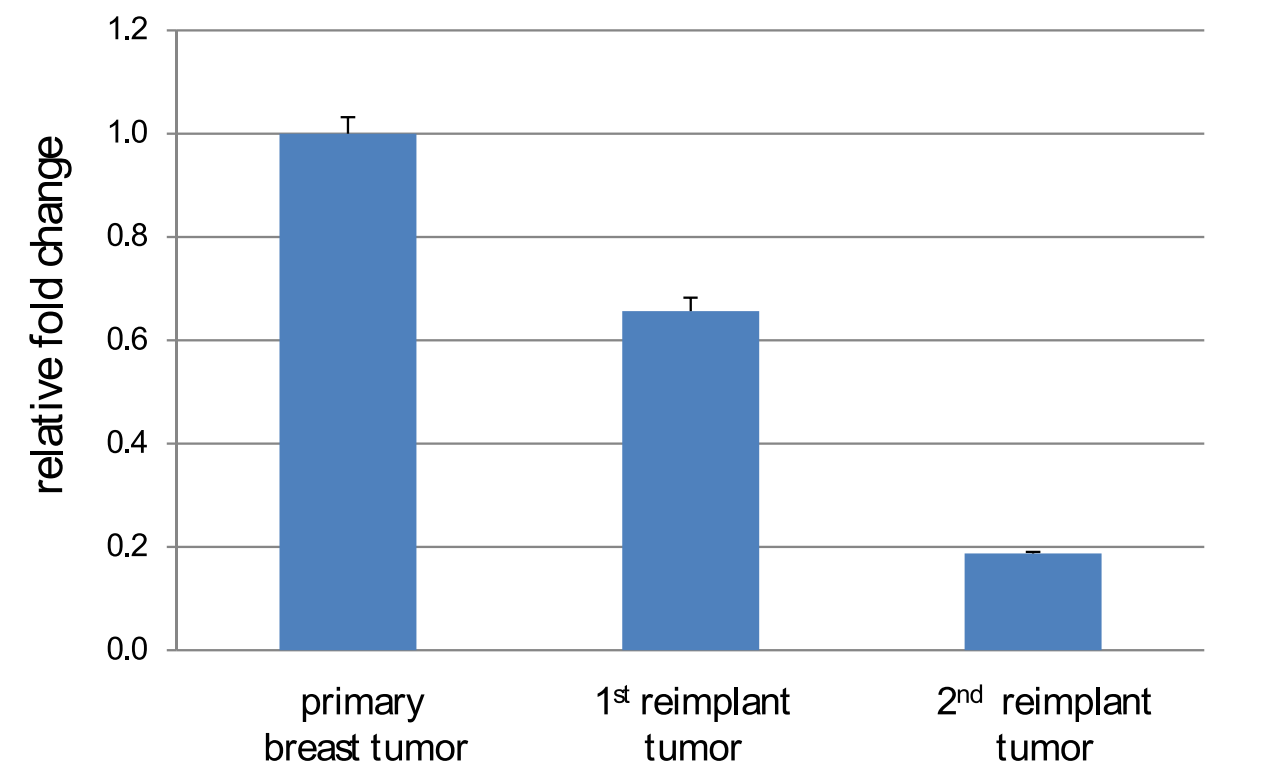


Fig. 4. Expression of IGFBP7 in primary and xenografted patient breast tumors by qRT-PCR. Quantitative PCR of IGFBP7 expression in primary and successively xenografted human breast tumors derived from first and second implantation into NOD/SCID mice. The data represent average values and standard error measurement from two triplicate samples, normalized against  $\beta$ -actin mRNA levels. The relative fold changes of the selected genes are obtained by dividing the expression levels of the re-implanted tumors by the expression levels in the primary patient tumors.

Patient	Age (Years)	Histo- pathological diagnosis	Grading	Estrogen /Progesterone receptor expression	ErB-2 expressio n	Node invasion (number positive/ number harvested)	Growth in hu-bone NOD/ SCID mice
HuP-1	67	Invasive ductal carcinoma	II/III	+/-	+	1/12	-
HuP-2	35	Metaplastic carcinoma	III	-/-	-	0/23	+
HuP-3	40	Invasive ductal carcinoma	II/III	+/+	+	0/2	-
HuP-4	48	Invasive ductal carcinoma	III	-/-	-	0/17	+
HuP-5	81	Invasive ductal carcinoma	II	+/-	-	0/1	-
HuP-6	50	Invasive and In-situ duct carcinoma	II	+/+	-	0/16	-
HuP-7	75	Invasive ductal carcinoma	I	+/+	-	4/15	-

Table 2. Characteristics of the human patient breast tumor tissues engrafted in hu-bone NOD/SCID mice.

The major traits of the engrafted human patient breast tumor samples (patient age, histopathological diagnosis, grading, estrogen/progesterone receptor expression, ErB-2 expression, node invasion) are indicated. The table also shows if the patient tumor samples were able to grow in the hu-bone NOD/SCID mouse model.

In order to transcriptionally characterize the colonization and aggressive behavior of engrafted patient breast tumors, microarray gene expression profiling was performed on breast tumors that were serially transplanted in the human-bone NOD/SCID mice. Genes were identified that were differentially expressed in the xenografted tumors by at least 1.5 fold compared to the primary patient tumors. There were 205 genes found to be differentially regulated in both HuP-2 and HuP-4 bone residing-breast tumors. Of the 129 known genes, 97 were expressed at higher levels and 32 at lower levels in the patient breast tumors colonized in bone. To narrow the spectrum of genes, 14 up-regulating and 18 down-regulating genes with bone colonization potentials are displayed (Table 3). Many of these gene identified have been previously associated with cancer function or metastatic activities such as cell viability, apoptosis and oncogenic transformation. IGFBP7 was identified as one of the genes that were downregulated in the xenografted tumors.

Gene	Fold Differences		Description	Identified cancer involvements
	HuP-2	HuP-4		
Down-regulated				
MTBP	7.11	1.94	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein	p53 regulator, Metastasis and cell proliferation suppressor
PARK7	5.30	3.14	Parkinson disease (autosomal recessive, early onset) 7	Negative regulator of PTEN, cell survival & aggressiveness
TOB1	1.79	6.12	Transducer of ERBB2, 1	Anti-proliferative protein
SDCBP	2.01	5.64	Syndecan binding protein (syntenin)	Cell adhesion & protein trafficking
CD24	2.12	11.29	CD24 molecule	Breast cancer stem cell marker & associated with bone metastasis
IL1R1	4.27	2.44	Interleukin 1 receptor, type I	Mediate cytokine induced immune & inflammatory response
PDLIM5	2.60	1.70	PDZ and LIM domain 5	Negative factor of oncogenic activity in neural tumor
HLA-DRA	2.15	6.33	Major histocompatibility complex, class II, DR alpha	Tumor immunosurveillance
PRKACB	3.11	3.93	Protein kinase, cAMP-dependent, catalytic, beta	Cell proliferation & differentiation
UBE2I	5.55	1.63	Ubiquitin-conjugating enzyme E2I	Suppressing p53 functions via RPA2 activity
IGFBP7	3.82	2.00	Insulin-like growth factor binding protein 7	Tumor suppressor & cell proliferation
ITM2B	1.94	2.28	Integral membrane protein 2B	Cell survival
ADAMT S12	1.61	3.43	ADAM metallopeptidase with thrombospondin type 1 motif, 12	Prevents tumorigenic effect of HGF
SPIN	1.56	3.35	Spindlin	Cell cycle regulation
PTPRF	1.86	2.32	Protein tyrosine phosphatase, receptor type, F	Regulation of epithelial cell-cell contact and cell growth
UHRF1B P1L	2.54	2.34	UHRF1 (ICBP90) binding protein 1-like	Regulate VEGF gene expression & tumor angiogenesis
TRMT5	2.20	1.87	TRM5 tRNA methyltransferase 5 homolog	Methylation
NR3C1	1.62	2.29	Nuclear receptor subfamily 3, group C, member 1)	Signaling and transduction
Up-regulated				
EIF5A	2.78	1.79	Eukaryotic translation initiation factor 5A	Cell viability & senescence
PCNXL2	2.81	1.79	Pecanex-like 2	Tumorigenesis in colorectal carcinoma
CD1C	3.11	1.54	CD1c molecule	Mediate immune responses to tumors
CSF1R	3.71	5.70	Colony stimulating factor 1 receptor	Metastasis & cell invasiveness
RPS5	1.99	1.99	Ribosomal protein S5	Cell differentiation and apoptosis
GOT2	2.10	1.74	Glutamic-oxaloacetic transaminase 2, mitochondrial	Serum GOT correlated with cancer and metastatic disease
RABL4	3.95	1.64	RAB, member of RAS oncogene family-like 4	Ras-related putative GTP-binding protein
RPS14	2.39	2.02	ribosomal protein S14	Haploinsufficiency disease gene
KLF8	1.57	1.53	Kruppel-like factor 8	Oncogenic transformation & EMT, downstream of FAK
DGKQ	1.82	2.28	Diacylglycerol kinase, theta 110kDa	Signal transduction pathways
AP2S1	3.23	1.60	Adaptor-related protein complex 2, sigma 1 subunit	Clathrin adaptor complex associated with plasma membranes



PALM	1.58	1.83	Paralemmmin	Cell shape control
RPS8	1.87	1.76	Ribosomal protein S8	Up-regulated in astrocytoma and pancreatic cancer
POLR2J	3.61	2.07	DNA directed RNA polyermase II	Enzyme & transcription

Table 3. Schematic representation of microarray analysis from xenografted tumors compared to primary tumors. To identify genes with bone colonization potential, xenografted tumor tissues were harvested for microarray analysis. Fold changes are obtained by dividing the gene expression levels in the xenografted tumors by the expression levels in the primary patient tumors. 205 genes are at least 1.5 fold differentially expressed in both HuP-2 and HuP4 bone residing breast-tumors compared with their primary patient breast tumors. A representation of genes whose expressions in xenografted tumors were at least 1.5 fold down-regulated from primary patient tumors (18 of 157 genes), or upregulated from primary patient tumors (14 of 48 genes) are shown.

The increased expression of IGFBP7 in senescent versus proliferating normal HMECs [34], prompted the evaluation of potential antiproliferative capabilities of IGFBP7 in breast cancer cells. In order to test this theory, IGFBP7 was overexpressed by retroviral vector in the ER/PR+ IGFBP7- MCF-7 breast cancer cell line. IGFBP7-transduced MCF-7 breast cancer cells showed a significant reduction in cell growth compared to parental IGFBP7 negative MCF-7 cells. When further analyzed, cells had arrested at the G0-G1 phase of cell cycle upon IGFBP7 expression. IGFBP7 was found to induce senescence rather than apoptosis [86].

ER/PR-negative breast cancers are the most aggressive and hardest to treat. In order to examine whether restoration of IGFBP7 could inhibit triple negative breast cancer cell growth, IGFBP7-overexpressing cells were engineered using a pSec-Tag2 plasmid in MDA-MB-468, a triple negative breast cancer line with barely detectable levels of endogenous IGFBP7, that is also tumorigenic in mice [87]. The vector contained a C-terminal *c-myc* epitope for detection with an anti-*myc* antibody, and a polyhistidine (6xhis) tag for rapid purification with nickel-chelating resin and detection with an anti-*his*(C-term) antibody (fig 3). Western blots of conditioned medium from stable IGFBP7-transfectants revealed two bands in response to IGFBP7 staining, a 38 kD band seen also in cell lysates, and a weaker, smaller 29 kD band. N-terminal sequencing revealed that both bands are IGFBP7 gene products [85]. The 38 kD band corresponded to the full length protein minus the signal sequence, whereas the smaller 29 kD band was cleaved after amino acid lys<sup>97</sup>, suggesting cleavage by the enzyme matriptase [39,85](fig 2, 3). IGFBP7 overexpression in MDA-MB-468 cells reduced cell growth and migration compared to parental MDA-MB-468 cells. Similarly, conditioned medium from IGFBP7 overexpressing breast cancer cell lines also lowered the growth of MDA-MB-468 cells. In order to examine the mechanism of IGFBP7-mediated growth inhibition, the effect of IGFBP7 overexpression on the MAP kinase pathway was analyzed. IGFBP7 overexpression inhibited the phosphorylation of MEK-1/2 and ERK-1/2 compared to parental MDA-MB-468 cells [85](fig. 5). These results are consistent with those observed in melanoma studies, whereby IGFBP7 is thought to act through autocrine and paracrine pathways to inhibit BRAF-MEK-ERK signaling resulting in induction of senescence or apoptosis [75].

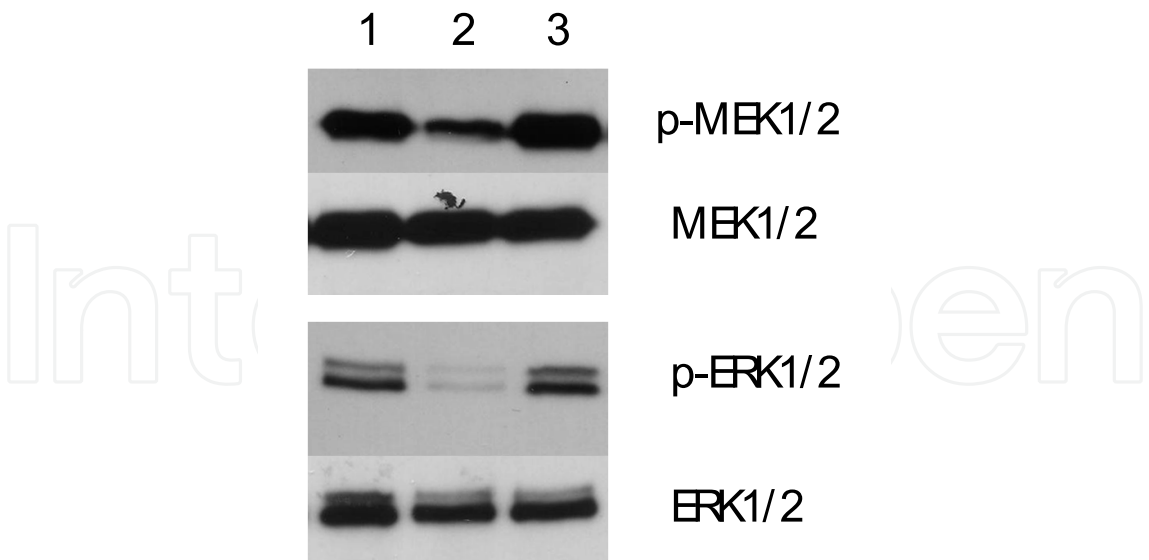


Fig. 5. Effect of IGFBP7 overexpression on the MAP kinase signaling pathway. Western blotting using equal amounts of protein from total cell lysates from MDA-MB-468 (lane 1), MDA-MB-468/IGFBP7 (lane 2), and empty vector control (lane 3) cells were examined by western blotting with antibodies to pERK-1/2,ERK-1/2, pMEK-1/2, and MEK-1/2.

The effects of IGFBP7 mediated growth inhibition were also examined *in vivo*. Parental MDA-MB-468 breast cancer cells and the IGFBP7-overexpressing variant were injected into NOD/SCID or NSG mice. Examination of tumor growth revealed a significant inhibition of tumor growth from the IGFBP7 overexpressing MDA-MB-468 cells (fig 6). Tumors were considerably smaller in the presence of IGFBP7. Immunohistochemistry and qRT-PCR of revealed the expression IGFBP7 in tumors derived from IGFBP7 overexpressing cells, confirming continual production of IGFBP7 *in vivo* during the duration of the experiment, which suggested that IGFBP7 was responsible for tumor growth suppression [85].

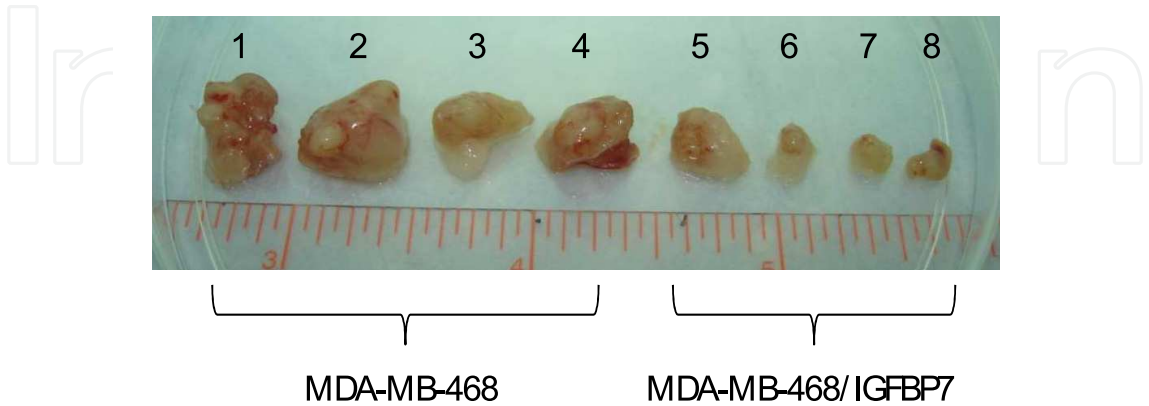


Fig. 6. Effect of IGFBP7 overexpression on breast tumor formation in vivo. 5x10<sup>6</sup> MDA-MB-468 cells or MDA-MB-468/IGFBP7 cells were injected into NSG or NOD/SCID mice. After 36 days, tumors were removed and analyzed.

### *Melanoma*

IGFBP7 was shown to be a tumor suppressor in melanoma studies, in that loss of IGFBP7 expression was critical step in melanoma development [75]. Activating BRAF mutations are found at a high frequency in melanomas (50-70%)[88]. In normal melanocytes, IGFBP7 is expressed at low levels. Following expression of the activating BRAFV600E mutation in melanocytic nevi, IGFBP7 is upregulated and induces senescence [75]. Melanoma cell lines harboring the activating BRAFV600E mutation, did not express IGFBP7, due to epigenetic silencing through promoter methylation of IGFBP7 [75,89]. Upon exposure to IGFBP7, BRAFV600E-positive melanoma cells underwent apoptosis. BRAFV600E expression in melanoma cells results in hyperactivation of the BRAF-MEK-ERK pathway. IGFBP7 treatment blocked cellular proliferation in part through inhibition of this pathway. Specifically, the phosphorylation of MEK by BRAF was prevented by upregulation of the RAF inhibitory protein (RKIP) by IGFBP7 through autocrine/paracrine pathways [75]. The apoptotic pathway induced by IGFBP7 involved the upregulation of BNIP3L, a proapoptotic BCL2 family protein. Furthermore, systemically administered IGFBP7 markedly suppressed the growth of BRAF-positive melanomas in xenografted mice, also through induction of apoptosis [75]. Epigenetic silencing of IGFBP7 is even more pronounced in human metastatic samples [89]. In a mouse model of metastatic melanoma, where mice were injected via tail vein with the highly metastatic BRAFV600E-positive malignant melanoma cells A375M-*Fluc*, IGFBP7 systemic administration suppressed tumor growth and increased survival [89]. Another group demonstrated that intratumoral injection of IGFBP7 in the form of the plasmid, pcDNA3.1-IGFBP7, promoted stable expression of IGFBP7, and suppressed the growth of the murine malignant melanoma cell line, B16-F10, by inducing apoptosis. Caspase 3 levels were increased and VEGF levels were decreased in the pcDNA3.1-IGFBP7 treated group [90].

### *Colorectal cancer*

In the normal colon, IGFBP7 expression varies from the basal compartment to the surface epithelium. Epithelial cells at the surface contain very strong IGFBP7 expression, whereas IGFBP7 staining was much weaker at the crypt base, which indicates that IGFBP7 expression is stronger in the differentiating areas of the colonic epithelium. Interestingly, IGFBP7 expression is actually increased in colorectal cancer. In colon carcinoma, IGFBP7 expression is strongest in the well differentiated colorectal adenocarcinoma, while weakly expressed in poorly differentiated colorectal adenocarcinoma [91]. IGFBP7 expression was correlated with differentiation, low grade tumor, and better prognosis. Cell differentiation and apoptosis are considered a result of normal colonocyte terminal differentiation in vivo. Introduction of IGFBP7 into colon cancer cells induced a more differentiated morphology. Upregulation of several colonic epithelial cell differentiation markers, such as AKP and CEA occurred with reintroduction of IGFBP7 [91]. This study identified IGFBP7 as a potential key marker associated with colon cancer differentiation.

The inhibition of IGFBP7 expression in colon cancer cell lines was shown to be due to aberrant DNA hypermethylation of the CpG island in exon 1 of IGFBP7, specifically in the promoter region [92]. Reactivation of IGFBP7 by 5-aza-dC treatment inhibited colon cancer cell proliferation in a dose dependent manner [93]. Demethylation restored p53-induced IGFBP7 expression[94]. Epigenetic inactivation of IGFBP7 appears to play a key role in tumorigenesis of CRCs with CpG island methylator phenotype (CIMP) by enabling

escape from p53-induced senescence [94]. Cell cycle was arrested, as cells accumulated in G2/M phase. 5-aza-dC treatment also increased the percentage of cells undergoing apoptosis. Cell migration and invasion were also reduced after treatment with 5-aza-dC [93]. The authors argue that demethylation increased the expression of tumor suppressor proteins, specifically IGFBP7, which was involved in the 5-aza-dC induced growth inhibitory effects.

A more direct effect of IGFBP7 as a tumor suppressor in colon cancer was shown in a subsequent study. Colorectal carcinoma cells, RKO and CW2, transfected with pcDNA3.1-IGFBP7 showed reduced proliferation. Cells were arrested in G1 phase of cell cycle (15% increased compared to control cells). The expression of E-cadherin and  $\beta$ -catenin were reduced in IGFBP7-transduced CW2 cells. Migration was not affected. A senescence like phenotype was induced, as judged by increased SA- $\beta$ -Gal activity, together with increased p53 and reduced pRB expression [95]. Cellular senescence is a barrier to cancer, preventing cells from unlimited proliferation [96,97]. This study suggested that IGFBP7 is an important molecule that triggers senescence through two important pathways, the p53-dependent pathway and the p16/p21-pRB pathway [95].

IGFBP7 was also shown to inhibit colon cancer tumor growth. Overexpression of IGFBP7 in the human colon cancer cell line, DLD-1, reduced its tumorigenicity *in vivo* [98]. Anchorage independent growth was also reduced. IGFBP7 expression increased cell adhesion of DLD-1 cells to laminin-5 and fibronectin [98]. In a separate study, two human CRC cell lines, one with an activating BRAF mutation (HT29) and the second with an activating KRAS mutation (SW-620), when xenografted into nude mice, were significantly growth inhibited upon systemic IGFBP7 treatment [89].

Proteomics was used to identify proteins associated with IGFBP7 in CRC. Six proteins were downregulated upon IGFBP7 reintroduction in colon cancer RKO cells, one of which was heat shock protein (HSP) 60 [99]. The authors focused on HSP60, as a key protein involved in IGFBP7-mediated growth inhibition, since it is overexpressed in CRC tissue and involved in proliferation and inhibition of apoptosis. They argue that one mechanism by which IGFBP7 overexpression inhibits growth of CRC cells, is through downregulation of HSP60.

#### *Prostate cancer*

IGFBP7 expression is found in primary cultures of prostate epithelial cells, and within the conditioned media from these cells. Peripheral nerves and stromal components associated with prostate tissue were strongly positive for IGFBP7 [100]. IGFBP7 protein and mRNA expression was up-regulated by IGF-I, TGF- $\beta$ , and retinoic acid in the nontumorigenic prostate epithelial line, P69, derived by immortalization of human primary prostate epithelial cells with simian virus-40 T antigen. IGFBP7 was undetectable by northern blot from malignant prostate lines such as LNCap, DU145, and PC-3 cells, and M12 cells (the tumorigenic and metastatic subclone of P69) [101,100]. There was a significant loss of detectable IGFBP7 mRNA in metastatic prostate tissue [28]. Re-expression of IGFBP7 in the human prostate cancer cell line, M12, results in an increase in cell doubling time, a decrease in colony formation in soft agar, a marked change in epithelial morphology along with an increased sensitivity to apoptosis, and finally decreased tumor formation and size *in vivo* [102]. In order to identify genes upregulated by IGFBP7 expression in prostate epithelial cells, a cDNA array analysis of IGFBP7-overexpressing M12 was performed, identifying SOX9, a transcription factor associated with differentiation [103]. The overexpression of



SOX9 in M12 cells seemed to recapitulate the effects seen with overexpression of IGFBP7 alone, suggesting that SOX9 is at least partly responsible for the growth inhibitory effect of IGFBP7 on prostate cancer cells. Another group used similar techniques and identified another transcription factor, manganese superoxide dismutase (SOD-2), which they argue was at least in part responsible for the growth inhibitory effects of IGFBP7 in prostate cancer cells [104]. Whether these transcription factors were indeed part of the anti-proliferative mechanism of IGFBP7, or merely a consequence of IGFBP7 overexpression in M12 cells remains to be determined.

#### *Thyroid cancer*

In accordance with prostate, colon and breast cancer, IGFBP7 expression is also significantly downregulated in thyroid cancer tissue samples compared to normal thyroid tissue [105]. IGFBP7 is epigenetically silenced by promoter hypermethylation in PTC-derived NIM1 thyroid tumor cell line. NIM1, along with most other thyroid cancer cell lines, carries the BRAFV600E mutation. Restoration of IGFBP7 in NIM1 cells by cDNA transfection resulted in growth inhibition, reduced colony formation in soft agar, and decreased migration capability in wound healing assay. Furthermore, tumor growth was inhibited upon injection in nude mice [105]. Examination of the mechanism governing IGFBP7 mediated growth inhibition revealed that IGFBP7-expressing NIM1 cells were impaired in cell cycle progression, manifesting cell cycle arrest in G1. The G1 arrest was associated with a strong decline in phospho-ERK levels, and an upregulation of p53 and p21 tumor suppressors. IGFBP7 expression alone resulted in increased apoptosis, as judged by increased cleaved PARP, which was even more pronounced upon exposure to the TRAIL, a proapoptotic agent effective in NIM1 cells [105]. These results suggest that IGFBP7 is a tumor suppressor in thyroid carcinogenesis.

#### *Hepatocellular carcinoma (HCC)*

A strong antitumor activity against HCC has been demonstrated for interferon (IFN)-based combination therapy (IFN- $\alpha$ / 5-FU therapy) [106-116]. However continuous exposure to IFN- $\alpha$  can result in IFN-resistant HCC cells. IGFBP7 was identified by microarray analysis as one of the most significantly downregulated genes in IFN resistant clones. Parental PLC/PRF/5 cells transfected with short hairpin RNA for IGFBP7 showed IFN- $\alpha$  resistance. IGFBP7 transfection into IFN-resistant HCC cells restored IFN sensitivity [106]. These results suggested that IGFBP7 could be a novel marker to predict clinical outcome to IFN- $\alpha$ /5-FU therapy.

A recent report studied PLC/PRF/5 cells treated with shRNA directed towards IGFBP7. They found that in the absence of IGFBP7 expression, the cells grew more rapidly, phospho-ERK was significantly increased, and apoptosis was decreased, as compared to the parental IGFBP7 expressing cells [117]. They found that apoptosis was decreased as a result of decreased expression of proapoptotic proteins, *SMARCB1* and *BNIP3L* by qRT-PCR. Furthermore, upon suppression of IGFBP7 expression, cell cycle progression was increased, concomitantly with increased cyclin D1 and cyclin E, and decreased p27. IGFBP7 reexpression in an HCC line that had very low IGFBP7 levels resulted in growth inhibition and decreased invasive ability. IGFBP7 downregulation was also significantly associated with tumor progression and postoperative poor prognosis in resected human HCC samples [117]. These studies identify IGFBP7 as a tumor suppressor and also an independent significant prognostic factor in HCC.



*Lung cancer*

Expression of IGFBP7 in lung cancer cell lines using RT-PCR revealed decreased expression of IGFBP7 compared to controls, and 42 out of 90 patients with primary lung tumors exhibited negative staining of IGFBP7 by immunohistochemical analysis [118]. There was a significant correlation between DNA methylation of exon/intron 1 region and IGFBP7 downregulation. When a p53 expression vector was transfected into lung cancer cell lines, it could only induce expression of IGFBP7 in the unmethylated cell line, but not in the methylated cell lines, suggesting that IGFBP7 might be regulated by p53 in lung cancer cell lines.

*Squamous cell carcinoma of the head and neck (SCCHN)*

A study found that a single nucleotide polymorphism (G to A) in the IGFBP7 promoter region was significantly associated with a reduced risk of SCCHN, when analyzed in a hospital-based case-control study of 1065 SCCHN patients and 1112 cancer-free control subjects. Upon analyzing reporter gene constructs, the G to A allelic change at -418 of the IGFBP7 promoter had increased promoter and DNA binding activity, suggesting increased IGFBP7 protein expression [119].

Although IGFBP7 has been shown to function as a tumor suppressor in a wide variety of cancers, a few studies suggest that IGFBP7 has an opposite effect, ie. promoting cancer growth. These cancers include the blood cancer, leukemia, and the brain cancer, glioblastoma.

*Glioblastoma*

IGFBP7 is a selective biomarker of glioblastoma (GBM) vessels, strongly expressed in tumor endothelial cells and vascular basement membrane [120]. IGFBP7 was strongly expressed in GBM specimens but not nontumor brain tissue. Moreover, statistical analysis showed that expression of IGFBP7 correlated inversely with overall GBM survival rates. Inhibition of IGFBP7 expression using siRNA transfection in a glioma cell line inhibited cell growth [121]. Addition of IGFBP7 to cell culture medium stimulated cell proliferation. IGFBP7 also promoted glioma cell migration, through downregulation of AKT phosphorylation and enhanced ERK1/2 activation [121]. IGFBP7 expression in brain endothelial cells was found to be upregulated by secreted factors from GBM cells through TGF- $\beta$ 1/ALK5/Smad2 signaling pathway, which has been implicated in angiogenesis [122].

*Acute leukemia*

Overexpression of the human gene BAALC (brain and acute leukemia, cytoplasmic), was shown to be associated with inferior outcome and chemotherapy resistance in adult patients with cytogenetically-normal acute myeloid leukemia (CN-AML), T cell-acute lymphoblastic leukemia (T-ALL) and B-precursor acute lymphoblastic leukemia (B-ALL)[123,124,125,126,127]. IGFBP7 was strongly correlated with BAALC-expression, implicating IGFBP7 in acute leukemia [128]. Aberrant expression of IGFBP7 in adult leukemia was correlated with chemotherapy resistance and inferior survival. Addition of IGFBP7 to leukemic cell lines inhibited cell growth without induction of apoptosis or senescence, suggesting a role of IGFBP7 in contributing to drug resistance through reduced sensitivity to cytostatic drugs [128]. Aberrantly increased levels of IGFBP7 were found in

CSF from children with acute lymphoblastic leukemia, implicating IGFBP7 with a more aggressive subtype of ALL [129]. IGFBP7 was also aberrantly overexpressed in the majority of AML at diagnosis and upon relapse, but not at remission stage [130]. Thus, IGFBP7 was shown to play a positive contributing role in the interaction between leukemia cells and the microenvironment, which may promote the leukemic cells' adhesion, invasion, and migration.

While the data observed in studies of leukemia and glioblastoma portray IGFBP7 in a negative role with respect to cancer, the vast majority of data from studies of solid tumors are in disagreement with these conclusions. It is possible that cell signaling pathways that result in senescence or apoptosis due to IGFBP7 are not present or functional in hematopoietic or glioma cells.

#### 4. Conclusions and perspectives

IGFBP7 has been shown to have tumor suppressive function in breast and other cancers. When examining the summarized data in Table 1, a common thread appears. Overexpression of IGFBP7 leads to inhibition of growth both *in vitro* and *in vivo*, increased expression of apoptotic markers (caspases, cleaved PARP), senescence associated proteins (*i.e.* p21, p27, p53), and decreased expression of proteins associated with proliferation (p-ERK). IGFBP7 appears to affect signaling through the MAP kinase pathway in many tumor models, including breast cancer. OIS may be a mechanism of tumor suppression by IGFBP7. The breast cancer cell lines used in our study, MDA-MB-468 cells, have a mutated PTEN, deregulating the PI3K pathway [131]. OIS can be triggered not only by the activation of oncogenes but also by the loss of tumor suppressor genes, such as PTEN. By upregulating proteins that counteract proliferation, such as cyclin dependent kinase inhibitors, *ie.* p21, which we have shown to occur upon IGFBP7 addition to breast cancer cells, the combined effect can lead to OIS [132]. Our model for the role of IGFBP7 in breast cancer inhibition depicts the entrance of IGFBP7 full length or cleaved IGFBP7 (through matriptase) into the cell, where signals are propagated to the nucleus, leading to the upregulation of expression of cyclin dependent kinase inhibitors, such as p21 and p27 (fig 7). This together with an already hyperstimulated MAP kinase pathway due to oncogenic mutations such as RAS, leads to MAP kinase pathway inhibition, growth arrest, and senescence, as suggested by the conflicting signal model of senescence [132].

The strong link to breast cancer outcome suggests that IGFBP7 may not only be a good prognostic indicator for malignant disease progression, but also a useful surrogate marker for monitoring therapeutic responses in the treatment of breast cancers. Senescence has been shown to be a method of halting tumor growth by many standard chemotherapeutic drugs [133]. Preliminary results indicate that senescence may be one mechanism by which IGFBP7 inhibits breast cancer cell growth in our system. Inhibition of breast cancer growth *in vivo* and *in vitro* together with induction of senescence indicates that IGFBP7 could be further developed as a potential drug to treat breast cancers. The fact that IGFBP7 has growth inhibitory effects when expressed in triple negative breast cancer cells, *i.e.* MDA-MB-468, provides an exciting opportunity to bring to the clinic a potential drug for hard to treat breast tumors.

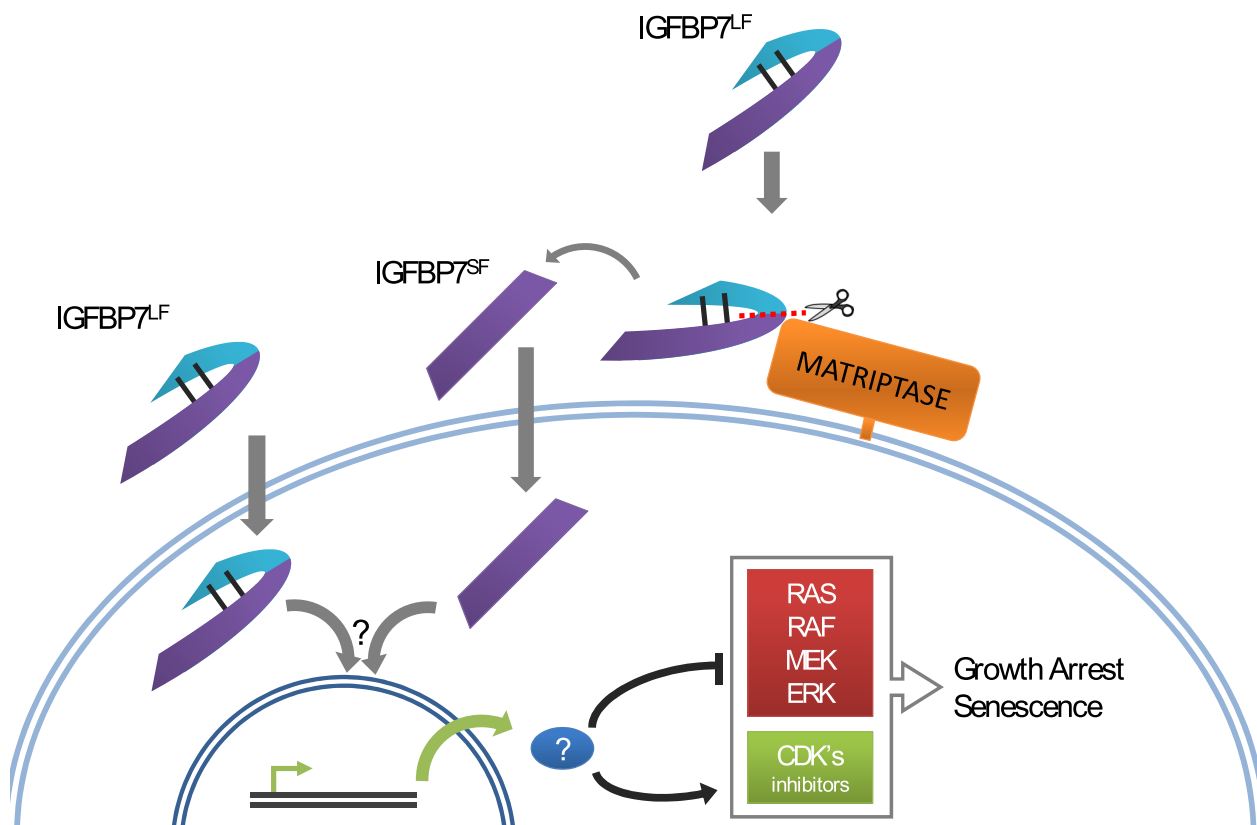


Fig. 7. Model for IGFBP7-mediated inhibition of breast cancer cell growth. IGFBP7 full length (FL) is cleaved by cell surface matriptase to short form (SF). Both forms enter breast cancer cells through an as yet unknown receptor, followed by signal propagation to the nucleus, which leads to upregulation of expression of cyclin dependent kinase (CDK) inhibitors, such as p21 and p27. This ultimately leads to growth arrest and senescence.

## 5. Acknowledgements

This work was supported by a CIHR grant, #MOP-97996 to Arun Seth. We thank Pearl Lam for her contribution to some of the microarray work and Stephanie Bacopulos for help with graphics.

## 6. References

- [1] Chaves, J. and Saif, M. W. (2011) IGF system in cancer: from bench to clinic. *Anticancer Drugs* 22:206-212.
- [2] Stewart, C. E. and Rotwein, P. (1996) Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. *Physiol Rev* 76:1005-1026.
- [3] Roberts, C. T., Jr., Brown, A. L., Graham, D. E., Seelig, S., Berry, S., Gabbay, K. H., and Rechler, M. M. (1986) Growth hormone regulates the abundance of insulin-like growth factor I RNA in adult rat liver. *J Biol Chem* 261:10025-10028.
- [4] Steele-Perkins, G., Turner, J., Edman, J. C., Hari, J., Pierce, S. B., Stover, C., Rutter, W. J., and Roth, R. A. (1988) Expression and characterization of a functional human insulin-like growth factor I receptor. *J Biol Chem* 263:11486-11492.

- [5] Soos, M. A., Whittaker, J., Lammers, R., Ullrich, A., and Siddle, K. (1990) Receptors for insulin and insulin-like growth factor-I can form hybrid dimers. Characterisation of hybrid receptors in transfected cells. *Biochem J* 270:383-390.
- [6] Siddle, K., Urso, B., Niesler, C. A., Cope, D. L., Molina, L., Surinya, K. H., and Soos, M. A. (2001) Specificity in ligand binding and intracellular signalling by insulin and insulin-like growth factor receptors. *Biochem Soc Trans* 29:513-525.
- [7] Braulke, T. (1999) Type-2 IGF receptor: a multi-ligand binding protein. *Horm Metab Res* 31:242-246.
- [8] Mosthaf, L., Grako, K., Dull, T. J., Coussens, L., Ullrich, A., and McClain, D. A. (1990) Functionally distinct insulin receptors generated by tissue-specific alternative splicing. *EMBO J* 9:2409-2413.
- [9] Yamaguchi, Y., Flier, J. S., Benecke, H., Ransil, B. J., and Moller, D. E. (1993) Ligand-binding properties of the two isoforms of the human insulin receptor. *Endocrinology* 132:1132-1138.
- [10] Yamaguchi, Y., Flier, J. S., Yokota, A., Benecke, H., Backer, J. M., and Moller, D. E. (1991) Functional properties of two naturally occurring isoforms of the human insulin receptor in Chinese hamster ovary cells. *Endocrinology* 129:2058-2066.
- [11] Denley, A., Wallace, J. C., Cosgrove, L. J., and Forbes, B. E. (2003) The insulin receptor isoform exon 11- (IR-A) in cancer and other diseases: a review. *Horm Metab Res* 35:778-785.
- [12] Sciacca, L., Prisco, M., Wu, A., Belfiore, A., Vigneri, R., and Baserga, R. (2003) Signaling differences from the A and B isoforms of the insulin receptor (IR) in 32D cells in the presence or absence of IR substrate-1. *Endocrinology* 144:2650-2658.
- [13] Frasca, F., Pandini, G., Scalia, P., Sciacca, L., Mineo, R., Costantino, A., Goldfine, I. D., Belfiore, A., and Vigneri, R. (1999) Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol Cell Biol* 19:3278-3288.
- [14] Blanquart, C., Achi, J., and Issad, T. (2008) Characterization of IRA/IRB hybrid insulin receptors using bioluminescence resonance energy transfer. *Biochem Pharmacol* 76:873-883.
- [15] Soos, M. A., Field, C. E., and Siddle, K. (1993) Purified hybrid insulin/insulin-like growth factor-I receptors bind insulin-like growth factor-I, but not insulin, with high affinity. *Biochem J* 290 ( Pt 2):419-426.
- [16] Kim, H. S., Nagalla, S. R., Oh, Y., Wilson, E., Roberts, C. T., Jr., and Rosenfeld, R. G. (1997) Identification of a family of low-affinity insulin-like growth factor binding proteins (IGFBPs): characterization of connective tissue growth factor as a member of the IGFBP superfamily. *Proc Natl Acad Sci U S A* 94:12981-12986.
- [17] Grimberg, A. and Cohen, P. (2000) Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis. *J Cell Physiol* 183:1-9.
- [18] Jones, J. I. and Clemmons, D. R. (1995) Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev* 16:3-34.
- [19] Hwa, V., Oh, Y., and Rosenfeld, R. G. (1999) The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr Rev* 20:761-787.
- [20] Zhu, W., Shiojima, I., Ito, Y., Li, Z., Ikeda, H., Yoshida, M., Naito, A. T., Nishi, J., Ueno, H., Umezawa, A., Minamino, T., Nagai, T., Kikuchi, A., Asashima, M., and Komuro, I. (2008) IGFBP-4 is an inhibitor of canonical Wnt signalling required for cardiogenesis. *Nature* 454:345-349.



- [21] Perks, C. M., Newcomb, P. V., Norman, M. R., and Holly, J. M. (1999) Effect of insulin-like growth factor binding protein-1 on integrin signalling and the induction of apoptosis in human breast cancer cells. *J Mol Endocrinol* 22:141-150.
- [22] Fu, P., Thompson, J. A., and Bach, L. A. (2007) Promotion of cancer cell migration: an insulin-like growth factor (IGF)-independent action of IGF-binding protein-6. *J Biol Chem* 282:22298-22306.
- [23] Kiepe, D., Van Der Pas, A., Ciarmatori, S., Standker, L., Schutt, B., Hoefflich, A., Hugel, U., Oh, J., and Tonshoff, B. (2008) Defined carboxy-terminal fragments of insulin-like growth factor (IGF) binding protein-2 exert similar mitogenic activity on cultured rat growth plate chondrocytes as IGF-I. *Endocrinology* 149:4901-4911.
- [24] Mihelic, M. and Turk, D. (2007) Two decades of thyroglobulin type-1 domain research. *Biol Chem* 388:1123-1130.
- [25] Neumann, G. M., Marinaro, J. A., and Bach, L. A. (1998) Identification of O-glycosylation sites and partial characterization of carbohydrate structure and disulfide linkages of human insulin-like growth factor binding protein 6. *Biochemistry* 37:6572-6585.
- [26] Forbes, B. E., Turner, D., Hodge, S. J., McNeil, K. A., Forsberg, G., and Wallace, J. C. (1998) Localization of an insulin-like growth factor (IGF) binding site of bovine IGF binding protein-2 using disulfide mapping and deletion mutation analysis of the C-terminal domain. *J Biol Chem* 273:4647-4652.
- [27] Hwa, V., Oh, Y., and Rosenfeld, R. G. (1999) Insulin-like growth factor binding proteins: a proposed superfamily. *Acta Paediatr Suppl* 88:37-45.
- [28] Hwa, V., Tomasini-Sprenger, C., Bermejo, A. L., Rosenfeld, R. G., and Plymate, S. R. (1998) Characterization of insulin-like growth factor-binding protein-related protein-1 in prostate cells. *J Clin Endocrinol Metab* 83:4355-4362.
- [29] Akaogi, K., Sato, J., Okabe, Y., Sakamoto, Y., Yasumitsu, H., and Miyazaki, K. (1996) Synergistic growth stimulation of mouse fibroblasts by tumor-derived adhesion factor with insulin-like growth factors and insulin. *Cell Growth Differ* 7:1671-1677.
- [30] Oh, Y., Nagalla, S. R., Yamanaka, Y., Kim, H. S., Wilson, E., and Rosenfeld, R. G. (1996) 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* 271:30322-30325.
- [31] Martinerie, C., Viegas-Pequignot, E., Guenard, I., Dutrillaux, B., Nguyen, V. C., Bernheim, A., and Perbal, B. (1992) Physical mapping of human loci homologous to the chicken nov proto-oncogene. *Oncogene* 7:2529-2534.
- [32] O'Brien, T. P., Yang, G. P., Sanders, L., and Lau, L. F. (1990) Expression of *cyr61*, a growth factor-inducible immediate-early gene. *Mol Cell Biol* 10:3569-3577.
- [33] Pennica, D., Swanson, T. A., Welsh, J. W., Roy, M. A., Lawrence, D. A., Lee, J., Brush, J., Taneyhill, L. A., Deuel, B., Lew, M., Watanabe, C., Cohen, R. L., Melhem, M. F., Finley, G. G., Quirke, P., Goddard, A. D., Hillan, K. J., Gurney, A. L., Botstein, D., and Levine, A. J. (1998) WISP genes are members of the connective tissue growth factor family that are up-regulated in *wnt-1*-transformed cells and aberrantly expressed in human colon tumors. *Proc Natl Acad Sci U S A* 95:14717-14722.
- [34] Swisshelm, K., Ryan, K., Tsuchiya, K., and Sager, R. (1995) Enhanced expression of an insulin growth factor-like binding protein (mac25) in senescent human mammary epithelial cells and induced expression with retinoic acid. *Proc Natl Acad Sci U S A* 92:4472-4476.



- [35] Kato, M. V., Sato, H., Tsukada, T., Ikawa, Y., Aizawa, S., and Nagayoshi, M. (1996) A follistatin-like gene, mac25, may act as a growth suppressor of osteosarcoma cells. *Oncogene* 12:1361-1364.
- [36] Murphy, M., Pykett, M. J., Harnish, P., Zang, K. D., and George, D. L. (1993) Identification and characterization of genes differentially expressed in meningiomas. *Cell Growth Differ* 4:715-722.
- [37] Sato, J., Hasegawa, S., Akaogi, K., Yasumitsu, H., Yamada, S., Sugahara, K., and Miyazaki, K. (1999) Identification of cell-binding site of angiomodulin (AGM/TAF/Mac25) that interacts with heparan sulfates on cell surface
- [38] Bartelt, D. C., Shapanka, R., and Greene, L. J. (1977) The primary structure of the human pancreatic secretory trypsin inhibitor. Amino acid sequence of the reduced S-aminoethylated protein. *Arch Biochem Biophys* 179:189-199.
- [39] Ahmed, S., Jin, X., Yagi, M., Yasuda, C., Sato, Y., Higashi, S., Lin, C. Y., Dickson, R. B., and Miyazaki, K. (2006) Identification of membrane-bound serine proteinase matriptase as processing enzyme of insulin-like growth factor binding protein-related protein-1 (IGFBP-rP1/angiomodulin/mac25). *FEBS J* 273:615-627.
- [40] Ahmed, S., Yamamoto, K., Sato, Y., Ogawa, T., Herrmann, A., Higashi, S., and Miyazaki, K. (2003) Proteolytic processing of IGFBP-related protein-1 (TAF/angiomodulin/mac25) modulates its biological activity. *Biochem Biophys Res Commun* 310:612-618.
- [41] Akaogi, K., Okabe, Y., Funahashi, K., Yoshitake, Y., Nishikawa, K., Yasumitsu, H., Umeda, M., and Miyazaki, K. (1994) Cell adhesion activity of a 30-kDa major secreted protein from human bladder carcinoma cells. *Biochem Biophys Res Commun* 198:1046-1053.
- [42] Yamauchi, T., Umeda, F., Masakado, M., Isaji, M., Mizushima, S., and Nawata, H. (1994) Purification and molecular cloning of prostacyclin-stimulating factor from serum-free conditioned medium of human diploid fibroblast cells. *Biochem J* 303 ( Pt 2):591-598.
- [43] Inoguchi, T., Umeda, F., Ono, H., Kunisaki, M., Watanabe, J., and Nawata, H. (1989) Abnormality in prostacyclin-stimulatory activity in sera from diabetics. *Metabolism* 38:837-842.
- [44] Inoguchi, T., Umeda, F., Watanabe, J., and Ibayashi, H. (1986) Reduced serum-stimulatory activity on prostacyclin production by cultured aortic endothelial cells in diabetes mellitus. *Haemostasis* 16:447-452.
- [45] Burger, A. M., Zhang, X., Li, H., Ostrowski, J. L., Beatty, B., Venanzoni, M., Papas, T., and Seth, A. (1998) Down-regulation of T1A12/mac25, a novel insulin-like growth factor binding protein related gene, is associated with disease progression in breast carcinomas. *Oncogene* 16:2459-2467.
- [46] Firth, S. M. and Baxter, R. C. (2002) Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev* 23:824-854.
- [47] Yamanaka, Y., Wilson, E. M., Rosenfeld, R. G., and Oh, Y. (1997) Inhibition of insulin receptor activation by insulin-like growth factor binding proteins. *J Biol Chem* 272:30729-30734.
- [48] Kato, M. V. (2000) A secreted tumor-suppressor, mac25, with activin-binding activity. *Mol Med* 6:126-135.

- [49] Jeruss, J. S., Sturgis, C. D., Rademaker, A. W., and Woodruff, T. K. (2003) Down-regulation of activin, activin receptors, and Smads in high-grade breast cancer. *Cancer Res* 63:3783-3790.
- [50] Nagakubo, D., Murai, T., Tanaka, T., Usui, T., Matsumoto, M., Sekiguchi, K., and Miyasaka, M. (2003) A high endothelial venule secretory protein, mac25/angiomodulin, interacts with multiple high endothelial venule-associated molecules including chemokines. *J Immunol* 171:553-561.
- [51] Wilson, E. M., Oh, Y., and Rosenfeld, R. G. (1997) Generation and characterization of an IGFBP-7 antibody: identification of 31kD IGFBP-7 in human biological fluids and Hs578T human breast cancer conditioned media. *J Clin Endocrinol Metab* 82:1301-1303.
- [52] Degeorges, A., Wang, F., Frierson, H. F., Jr., Seth, A., and Sikes, R. A. (2000) Distribution of IGFBP-rP1 in normal human tissues. *J Histochem Cytochem* 48:747-754.
- [53] Dominguez, F., Avila, S., Cervero, A., Martin, J., Pellicer, A., Castrillo, J. L., and Simon, C. (2003) A combined approach for gene discovery identifies insulin-like growth factor-binding protein-related protein 1 as a new gene implicated in human endometrial receptivity. *J Clin Endocrinol Metab* 88:1849-1857.
- [54] HAYFLICK, L. and MOORHEAD, P. S. (1961) The serial cultivation of human diploid cell strains. *Exp Cell Res* 25:585-621.
- [55] Steinert, S., Shay, J. W., and Wright, W. E. (2000) Transient expression of human telomerase extends the life span of normal human fibroblasts. *Biochem Biophys Res Commun* 273:1095-1098.
- [56] Campisi, J. and d'Adda di, Fagagna F. (2007) Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol* 8:729-740.
- [57] Newbold, R. F. and Overell, R. W. (1983) Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-ras oncogene. *Nature* 304:648-651.
- [58] Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D., and Lowe, S. W. (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88:593-602.
- [59] Wei, S., Wei, S., and Sedivy, J. M. (1999) Expression of catalytically active telomerase does not prevent premature senescence caused by overexpression of oncogenic Ha-Ras in normal human fibroblasts. *Cancer Res* 59:1539-1543.
- [60] Lowe, S. W., Cepero, E., and Evan, G. (2004) Intrinsic tumour suppression. *Nature* 432:307-315.
- [61] Courtois-Cox, S., Genter Williams, S. M., Reczek, E. E., Johnson, B. W., McGillicuddy, L. T., Johannessen, C. M., Hollstein, P. E., MacCollin, M., and Cichowski, K. (2006) A negative feedback signaling network underlies oncogene-induced senescence. *Cancer Cell* 10:459-472.
- [62] Michaloglou, C., Vredeveld, L. C., Soengas, M. S., Denoyelle, C., Kuilman, T., van der Horst, C. M., Majoor, D. M., Shay, J. W., Mooi, W. J., and Peeper, D. S. (2005) BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 436:720-724.
- [63] Chen, Z., Trotman, L. C., Shaffer, D., Lin, H. K., Dotan, Z. A., Niki, M., Koutcher, J. A., Scher, H. I., Ludwig, T., Gerald, W., Cordon-Cardo, C., and Pandolfi, P. P. (2005) Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* 436:725-730.

- [64] Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E. E., Linskens, M., Rubelj, I., Pereira-Smith, O., and . (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 92:9363-9367.
- [65] Campisi, J. (2005) Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 120:513-522.
- [66] Beausejour, C. M., Krtolica, A., Galimi, F., Narita, M., Lowe, S. W., Yaswen, P., and Campisi, J. (2003) Reversal of human cellular senescence: roles of the p53 and p16 pathways. *EMBO J* 22:4212-4222.
- [67] Dirac, A. M. and Bernards, R. (2003) Reversal of senescence in mouse fibroblasts through lentiviral suppression of p53. *J Biol Chem* 278:11731-11734.
- [68] Sage, J., Miller, A. L., Perez-Mancera, P. A., Wysocki, J. M., and Jacks, T. (2003) Acute mutation of retinoblastoma gene function is sufficient for cell cycle re-entry. *Nature* 424:223-228.
- [69] Bennett, D. C. (2003) Human melanocyte senescence and melanoma susceptibility genes. *Oncogene* 22:3063-3069.
- [70] Chin, L., Merlino, G., and DePinho, R. A. (1998) Malignant melanoma: modern black plague and genetic black box. *Genes Dev* 12:3467-3481.
- [71] Sparrow, L. E., Eldon, M. J., English, D. R., and Heenan, P. J. (1998) p16 and p21WAF1 protein expression in melanocytic tumors by immunohistochemistry. *Am J Dermatopathol* 20:255-261.
- [72] Wang, Y. L., Uhara, H., Yamazaki, Y., Nikaido, T., and Saida, T. (1996) Immunohistochemical detection of CDK4 and p16INK4 proteins in cutaneous malignant melanoma. *Br J Dermatol* 134:269-275.
- [73] Pollock, P. M., Harper, U. L., Hansen, K. S., Yudt, L. M., Stark, M., Robbins, C. M., Moses, T. Y., Hostetter, G., Wagner, U., Kakareka, J., Salem, G., Pohida, T., Heenan, P., Duray, P., Kallioniemi, O., Hayward, N. K., Trent, J. M., and Meltzer, P. S. (2003) High frequency of BRAF mutations in nevi. *Nat Genet* 33:19-20.
- [74] Kuilman, T., Michaloglou, C., Vredeveld, L. C., Douma, S., van, Doorn R., Desmet, C. J., Aarden, L. A., Mooi, W. J., and Peeper, D. S. (2008) Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 133:1019-1031.
- [75] Wajapeyee, N., Serra, R. W., Zhu, X., Mahalingam, M., and Green, M. R. (2-8-2008) Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7. *Cell* 132:363-374.
- [76] Acosta, J. C., O'Loghlen, A., Banito, A., Guijarro, M. V., Augert, A., Raguz, S., Fumagalli, M., Da, Costa M., Brown, C., Popov, N., Takatsu, Y., Melamed, J., d'Adda di, Fagagna F., Bernard, D., Hernando, E., and Gil, J. (2008) Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* 133:1006-1018.
- [77] Kortlever, R. M., Higgins, P. J., and Bernards, R. (2006) Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. *Nat Cell Biol* 8:877-884.
- [78] Coppe, J. P., Patil, C. K., Rodier, F., Sun, Y., Munoz, D. P., Goldstein, J., Nelson, P. S., Desprez, P. Y., and Campisi, J. (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 6:2853-2868.
- [79] Kuilman, T. and Peeper, D. S. (2009) Senescence-messaging secretome: SMS-ing cellular stress. *Nat Rev Cancer* 9:81-94.

- [80] Burger, A. M., Leyland-Jones, B., Banerjee, K., Spyropoulos, D. D., and Seth, A. K. (2005) Essential roles of IGFBP-3 and IGFBP-rP1 in breast cancer. *Eur J Cancer* 41:1515-1527.
- [81] Burger, A. M., Zhang, X., and Seth, A. (1998) Detection of novel genes that are up-regulated (Di12) or down-regulated (T1A12) with disease progression in breast cancer. *Eur J Cancer Prev* 7 Suppl 1:S29-S35.
- [82] Komatsu, S., Okazaki, Y., Tateno, M., Kawai, J., Konno, H., Kusakabe, M., Yoshiki, A., Muramatsu, M., Held, W. A., and Hayashizaki, Y. (2000) Methylation and downregulated expression of mac25/insulin-like growth factor binding protein-7 is associated with liver tumorigenesis in SV40T/t antigen transgenic mice, screened by restriction landmark genomic scanning for methylation (RLGS-M). *Biochem Biophys Res Commun* 267:109-117.
- [83] Smith, P., Nicholson, L. J., Syed, N., Payne, A., Hiller, L., Garrone, O., Occelli, M., Gasco, M., and Crook, T. (2007) Epigenetic inactivation implies independent functions for insulin-like growth factor binding protein (IGFBP)-related protein 1 and the related IGFBPL1 in inhibiting breast cancer phenotypes. *Clin Cancer Res* 13:4061-4068.
- [84] Landberg, G., Ostlund, H., Nielsen, N. H., Roos, G., Emdin, S., Burger, A. M., and Seth, A. (2001) Downregulation of the potential suppressor gene IGFBP-rP1 in human breast cancer is associated with inactivation of the retinoblastoma protein, cyclin E overexpression and increased proliferation in estrogen receptor negative tumors. *Oncogene* 20:3497-3505.
- [85] Amemiya, Y., Yang, W., Benatar, T., Nofech-Mozes, S., Yee, A., Kahn, H., Holloway, C., and Seth, A. (2010) Insulin like growth factor binding protein-7 reduces growth of human breast cancer cells and xenografted tumors. *Breast Cancer Res Treat*
- [86] Wilson, H. M., Birnbaum, R. S., Poot, M., Quinn, L. S., and Swisshelm, K. (2002) Insulin-like growth factor binding protein-related protein 1 inhibits proliferation of MCF-7 breast cancer cells via a senescence-like mechanism. *Cell Growth Differ* 13:205-213.
- [87] Cailleau, R., Olive, M., and Cruciger, Q. V. (1978) Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. *In Vitro* 14:911-915.
- [88] Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M. J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., Menzies, A., Mould, C., Parker, A., Stevens, C., Watt, S., Hooper, S., Wilson, R., Jayatilake, H., Gusterson, B. A., Cooper, C., Shipley, J., Hargrave, D., Pritchard-Jones, K., Maitland, N., Chenevix-Trench, G., Riggins, G. J., Bigner, D. D., Palmieri, G., Cossu, A., Flanagan, A., Nicholson, A., Ho, J. W., Leung, S. Y., Yuen, S. T., Weber, B. L., Seigler, H. F., Darrow, T. L., Paterson, H., Marais, R., Marshall, C. J., Wooster, R., Stratton, M. R., and Futreal, P. A. (2002) Mutations of the BRAF gene in human cancer. *Nature* 417:949-954.
- [89] Wajapeyee, N., Kapoor, V., Mahalingam, M., and Green, M. R. (2009) Efficacy of IGFBP7 for treatment of metastatic melanoma and other cancers in mouse models and human cell lines. *Mol Cancer Ther* 8:3009-3014.
- [90] Chen, R. Y., Chen, H. X., Jian, P., Xu, L., Li, J., Fan, Y. M., and Tu, Y. T. (2010) Intratumoral injection of pEGFC1-IGFBP7 inhibits malignant melanoma growth in C57BL/6J mice by inducing apoptosis and down-regulating VEGF expression. *Oncol Rep* 23:981-988.



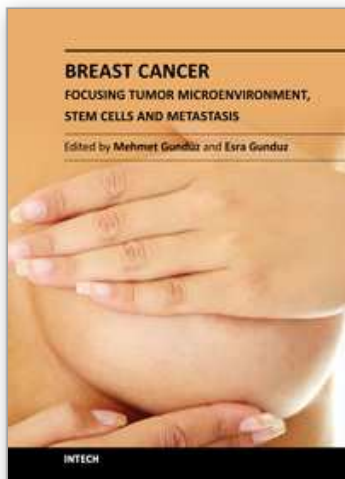
- [91] Ruan, W., Xu, E., Xu, F., Ma, Y., Deng, H., Huang, Q., Lv, B., Hu, H., Lin, J., Cui, J., Di, M., Dong, J., and Lai, M. (2007) IGFBP7 plays a potential tumor suppressor role in colorectal carcinogenesis. *Cancer Biol Ther* 6:354-359.
- [92] Lin, J., Lai, M., Huang, Q., Ma, Y., Cui, J., and Ruan, W. (2007) Methylation patterns of IGFBP7 in colon cancer cell lines are associated with levels of gene expression. *J Pathol* 212:83-90.
- [93] Lin, J., Lai, M., Huang, Q., Ruan, W., Ma, Y., and Cui, J. (2008) Reactivation of IGFBP7 by DNA demethylation inhibits human colon cancer cell growth in vitro. *Cancer Biol Ther* 7:1896-1900.
- [94] Suzuki, H., Igarashi, S., Nojima, M., Maruyama, R., Yamamoto, E., Kai, M., Akashi, H., Watanabe, Y., Yamamoto, H., Sasaki, Y., Itoh, F., Imai, K., Sugai, T., Shen, L., Issa, J. P., Shinomura, Y., Tokino, T., and Toyota, M. (2009) IGFBP7 is a p53 Responsive Gene Specifically Silenced in Colorectal Cancer with CpG Island Methylator Phenotype. *Carcinogenesis*
- [95] Ma, Y., Lu, B., Ruan, W., Wang, H., Lin, J., Hu, H., Deng, H., Huang, Q., and Lai, M. (2008) Tumor suppressor gene insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) induces senescence-like growth arrest in colorectal cancer cells. *Exp Mol Pathol* 85:141-145.
- [96] Braig, M., Lee, S., Loddenkemper, C., Rudolph, C., Peters, A. H., Schlegelberger, B., Stein, H., Dorken, B., Jenuwein, T., and Schmitt, C. A. (2005) Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* 436:660-665.
- [97] Collado, M., Gil, J., Efeyan, A., Guerra, C., Schuhmacher, A. J., Barradas, M., Benguria, A., Zaballos, A., Flores, J. M., Barbacid, M., Beach, D., and Serrano, M. (2005) Tumour biology: senescence in premalignant tumours. *Nature* 436:642-
- [98] Sato, Y., Chen, Z., and Miyazaki, K. (2007) Strong suppression of tumor growth by insulin-like growth factor-binding protein-related protein 1/tumor-derived cell adhesion factor/mac25. *Cancer Sci* 98:1055-1063.
- [99] Ruan, W., Wang, Y., Ma, Y., Xing, X., Lin, J., Cui, J., and Lai, M. (2010) HSP60, a protein downregulated by IGFBP7 in colorectal carcinoma. *J Exp Clin Cancer Res* 29:41-
- [100] Degeorges, A., Wang, F., Frierson, H. F., Jr., Seth, A., Chung, L. W., and Sikes, R. A. (1999) Human prostate cancer expresses the low affinity insulin-like growth factor binding protein IGFBP-rP1. *Cancer Res* 59:2787-2790.
- [101] Lopez-Bermejo, A., Buckway, C. K., Devi, G. R., Hwa, V., Plymate, S. R., Oh, Y., and Rosenfeld, R. G. (2000) Characterization of insulin-like growth factor-binding protein-related proteins (IGFBP-rPs) 1, 2, and 3 in human prostate epithelial cells: potential roles for IGFBP-rP1 and 2 in senescence of the prostatic epithelium. *Endocrinology* 141:4072-4080.
- [102] Sprenger, C. C., Damon, S. E., Hwa, V., Rosenfeld, R. G., and Plymate, S. R. (1999) Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) is a potential tumor suppressor protein for prostate cancer. *Cancer Res* 59:2370-2375.
- [103] Drivdahl, R., Haugk, K. H., Sprenger, C. C., Nelson, P. S., Tennant, M. K., and Plymate, S. R. (2004) Suppression of growth and tumorigenicity in the prostate tumor cell line M12 by overexpression of the transcription factor SOX9. *Oncogene* 23:4584-4593.
- [104] Plymate, S. R., Haugk, K. H., Sprenger, C. C., Nelson, P. S., Tennant, M. K., Zhang, Y., Oberley, L. W., Zhong, W., Drivdahl, R., and Oberley, T. D. (2003) Increased manganese superoxide dismutase (SOD-2) is part of the mechanism for prostate



- tumor suppression by Mac25/insulin-like growth factor binding-protein-related protein-1. *Oncogene* 22:1024-1034.
- [105] Vizioli, M. G., Sensi, M., Miranda, C., Cleris, L., Formelli, F., Anania, M. C., Pierotti, M. A., and Greco, A. (2010) IGFBP7: an oncosuppressor gene in thyroid carcinogenesis. *Oncogene* 29:3835-3844.
- [106] Tomimaru, Y., Eguchi, H., Wada, H., Noda, T., Murakami, M., Kobayashi, S., Marubashi, S., Takeda, Y., Tanemura, M., Umeshita, K., Doki, Y., Mori, M., and Nagano, H. (2010) Insulin-like growth factor-binding protein 7 alters the sensitivity to interferon-based anticancer therapy in hepatocellular carcinoma cells. *Br J Cancer* 102:1483-1490.
- [107] Eguchi, H., Nagano, H., Yamamoto, H., Miyamoto, A., Kondo, M., Dono, K., Nakamori, S., Umeshita, K., Sakon, M., and Monden, M. (2000) Augmentation of antitumor activity of 5-fluorouracil by interferon alpha is associated with up-regulation of p27Kip1 in human hepatocellular carcinoma cells. *Clin Cancer Res* 6:2881-2890.
- [108] Kondo, M., Nagano, H., Sakon, M., Yamamoto, H., Morimoto, O., Arai, I., Miyamoto, A., Eguchi, H., Dono, K., Nakamori, S., Umeshita, K., Wakasa, K., Ohmoto, Y., and Monden, M. (2000) Expression of interferon alpha/beta receptor in human hepatocellular carcinoma. *Int J Oncol* 17:83-88.
- [109] Kondo, M., Nagano, H., Wada, H., Damdinsuren, B., Yamamoto, H., Hiraoka, N., Eguchi, H., Miyamoto, A., Yamamoto, T., Ota, H., Nakamura, M., Marubashi, S., Dono, K., Umeshita, K., Nakamori, S., Sakon, M., and Monden, M. (2005) Combination of IFN-alpha and 5-fluorouracil induces apoptosis through IFN-alpha/beta receptor in human hepatocellular carcinoma cells. *Clin Cancer Res* 11:1277-1286.
- [110] Wada, H., Nagano, H., Yamamoto, H., Arai, I., Ota, H., Nakamura, M., Damdinsuren, B., Noda, T., Marubashi, S., Miyamoto, A., Takeda, Y., Umeshita, K., Doki, Y., Dono, K., Nakamori, S., Sakon, M., and Monden, M. (2007) Combination therapy of interferon-alpha and 5-fluorouracil inhibits tumor angiogenesis in human hepatocellular carcinoma cells by regulating vascular endothelial growth factor and angiopoietins. *Oncol Rep* 18:801-809.
- [111] Sakon, M., Nagano, H., Dono, K., Nakamori, S., Umeshita, K., Yamada, A., Kawata, S., Imai, Y., Iijima, S., and Monden, M. (2002) Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer* 94:435-442.
- [112] Yamamoto, T., Nagano, H., Sakon, M., Wada, H., Eguchi, H., Kondo, M., Damdinsuren, B., Ota, H., Nakamura, M., Wada, H., Marubashi, S., Miyamoto, A., Dono, K., Umeshita, K., Nakamori, S., Yagita, H., and Monden, M. (2004) Partial contribution of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/TRAIL receptor pathway to antitumor effects of interferon-alpha/5-fluorouracil against Hepatocellular Carcinoma. *Clin Cancer Res* 10:7884-7895.
- [113] Ota, H., Nagano, H., Sakon, M., Eguchi, H., Kondo, M., Yamamoto, T., Nakamura, M., Damdinsuren, B., Wada, H., Marubashi, S., Miyamoto, A., Dono, K., Umeshita, K., Nakamori, S., Wakasa, K., and Monden, M. (2005) Treatment of hepatocellular carcinoma with major portal vein thrombosis by combined therapy with subcutaneous interferon-alpha and intra-arterial 5-fluorouracil; role of type 1 interferon receptor expression. *Br J Cancer* 93:557-564.

- [114] Nakamura, M., Nagano, H., Sakon, M., Yamamoto, T., Ota, H., Wada, H., Damdinsuren, B., Noda, T., Marubashi, S., Miyamoto, A., Takeda, Y., Umeshita, K., Nakamori, S., Dono, K., and Monden, M. (2007) Role of the Fas/FasL pathway in combination therapy with interferon-alpha and fluorouracil against hepatocellular carcinoma in vitro. *J Hepatol* 46:77-88.
- [115] Damdinsuren, B., Nagano, H., and Monden, M. (2007) Combined intra-arterial 5-fluorouracil and subcutaneous interferon-alpha therapy for highly advanced hepatocellular carcinoma. *Hepatol Res* 37 Suppl 2:S238-S250.
- [116] Nagano, H., Sakon, M., Eguchi, H., Kondo, M., Yamamoto, T., Ota, H., Nakamura, M., Wada, H., Damdinsuren, B., Marubashi, S., Miyamoto, A., Takeda, Y., Dono, K., Umeshita, K., Nakamori, S., and Monden, M. (2007) Hepatic resection followed by IFN-alpha and 5-FU for advanced hepatocellular carcinoma with tumor thrombus in the major portal branch. *Hepatogastroenterology* 54:172-179.
- [117] Tomimaru, Y., Eguchi, H., Wada, H., Kobayashi, S., Marubashi, S., Tanemura, M., Umeshita, K., Kim, T., Wakasa, K., Doki, Y., Mori, M., and Nagano, H. (2011) IGFBP7 downregulation is associated with tumor progression and clinical outcome in hepatocellular carcinoma. *Int J Cancer*
- [118] Chen, Y., Pacyna-Gengelbach, M., Ye, F., Knosel, T., Lund, P., Deutschmann, N., Schluns, K., Kotb, W. F., Sers, C., Yasumoto, H., Usui, T., and Petersen, I. (2007) Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) has potential tumour-suppressive activity in human lung cancer. *J Pathol* 211:431-438.
- [119] Huang, Y. J., Niu, J., Liu, Z., Wang, L. E., Sturgis, E. M., and Wei, Q. (2010) The functional IGFBP7 promoter -418G>A polymorphism and risk of head and neck cancer. *Mutat Res* 702:32-39.
- [120] Pen, A., Moreno, M. J., Martin, J., and Stanimirovic, D. B. (2007) Molecular markers of extracellular matrix remodeling in glioblastoma vessels: microarray study of laser-captured glioblastoma vessels. *Glia* 55:559-572.
- [121] Jiang, W., Xiang, C., Cazacu, S., Brodie, C., and Mikkelsen, T. (2008) Insulin-like growth factor binding protein 7 mediates glioma cell growth and migration. *Neoplasia* 10:1335-1342.
- [122] Pen, A., Moreno, M. J., Durocher, Y., Deb-Rinker, P., and Stanimirovic, D. B. (2008) Glioblastoma-secreted factors induce IGFBP7 and angiogenesis by modulating Smad-2-dependent TGF-beta signaling. *Oncogene* 27:6834-6844.
- [123] Tanner, S. M., Austin, J. L., Leone, G., Rush, L. J., Plass, C., Heinonen, K., Mrozek, K., Sill, H., Knuutila, S., Kolitz, J. E., Archer, K. J., Caligiuri, M. A., Bloomfield, C. D., and de La, Chapelle A. (2001) BAALC, the human member of a novel mammalian neuroectoderm gene lineage, is implicated in hematopoiesis and acute leukemia. *Proc Natl Acad Sci U S A* 98:13901-13906.
- [124] Baldus, C. D., Tanner, S. M., Ruppert, A. S., Whitman, S. P., Archer, K. J., Marcucci, G., Caligiuri, M. A., Carroll, A. J., Vardiman, J. W., Powell, B. L., Allen, S. L., Moore, J. O., Larson, R. A., Kolitz, J. E., de la Chapelle, A., and Bloomfield, C. D. (2003) BAALC expression predicts clinical outcome of de novo acute myeloid leukemia patients with normal cytogenetics: a Cancer and Leukemia Group B Study. *Blood* 102:1613-1618.
- [125] Bienz, M., Ludwig, M., Leibundgut, E. O., Mueller, B. U., Ratschiller, D., Solenthaler, M., Fey, M. F., and Pabst, T. (2005) Risk assessment in patients with acute myeloid leukemia and a normal karyotype. *Clin Cancer Res* 11:1416-1424.

- [126] Baldus, C. D., Martus, P., Burmeister, T., Schwartz, S., Gokbuget, N., Bloomfield, C. D., Hoelzer, D., Thiel, E., and Hofmann, W. K. (2007) Low ERG and BAALC expression identifies a new subgroup of adult acute T-lymphoblastic leukemia with a highly favorable outcome. *J Clin Oncol* 25:3739-3745.
- [127] Langer, C., Radmacher, M. D., Ruppert, A. S., Whitman, S. P., Paschka, P., Mrozek, K., Baldus, C. D., Vukosavljevic, T., Liu, C. G., Ross, M. E., Powell, B. L., de la Chapelle, A., Kolitz, J. E., Larson, R. A., Marcucci, G., and Bloomfield, C. D. (2008) High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: a Cancer and Leukemia Group B (CALGB) study. *Blood* 111:5371-5379.
- [128] Heesch, S., Schlee, C., Neumann, M., Stroux, A., Kuhn, A., Schwartz, S., Haferlach, T., Gokbuget, N., Hoelzer, D., Thiel, E., Hofmann, W. K., and Baldus, C. D. (2010) BAALC-associated gene expression profiles define IGFBP7 as a novel molecular marker in acute leukemia. *Leukemia* 24:1429-1436.
- [129] How, H. K., Yeoh, A., Quah, T. C., Oh, Y., Rosenfeld, R. G., and Lee, K. O. (1999) Insulin-like growth factor binding proteins (IGFBPs) and IGFBP-related protein 1-levels in cerebrospinal fluid of children with acute lymphoblastic leukemia. *J Clin Endocrinol Metab* 84:1283-1287.
- [130] Hu, S., Chen, R., Man, X., Feng, X., Cen, J., Gu, W., He, H., Li, J., Chai, Y., and Chen, Z. (2011) Function and Expression of Insulin-Like Growth Factor-Binding Protein 7 (IGFBP7) Gene in Childhood Acute Myeloid Leukemia. *Pediatr Hematol Oncol*
- [131] Hollestelle, A., Elstrodt, F., Nagel, J. H., Kallemeijn, W. W., and Schutte, M. (2007) Phosphatidylinositol-3-OH kinase or RAS pathway mutations in human breast cancer cell lines. *Mol Cancer Res* 5:195-201.
- [132] Blagosklonny, M. V. (2003) Cell senescence and hypermitogenic arrest. *EMBO Rep* 4:358-362.
- [133] Schmitt, C. A. (2007) Cellular senescence and cancer treatment. *Biochim Biophys Acta* 1775:5-20.
- [134] Clausen, T., Kaiser, M., Huber, R., and Ehrmann, M. (2011) HTRA proteases: regulated proteolysis in protein quality control. *Nat.Rev.Mol.Cell Biol.* 12:152-162.
- [135] Shibata, Y., Tsukazaki, T., Hirata, K., Xin, C., and Yamaguchi, A. (2004) Role of a new member of IGFBP superfamily, IGFBP-rP10, in proliferation and differentiation of osteoblastic cells. *Biochem.Biophys.Res.Comm.* 325:1194-1200.



## **Breast Cancer - Focusing Tumor Microenvironment, Stem cells and Metastasis**

Edited by Prof. Mehmet Gunduz

ISBN 978-953-307-766-6

Hard cover, 584 pages

**Publisher** InTech

**Published online** 14, December, 2011

**Published in print edition** December, 2011

Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed characteristics of breast cancer cell, role of microenvironment, stem cells and metastasis for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Tania Benatar, Yutaka Amemiya, Wenyi Yang and Arun Seth (2011). Insulin-Like-Growth Factor-Binding-Protein 7: An Antagonist to Breast Cancer, Breast Cancer - Focusing Tumor Microenvironment, Stem cells and Metastasis, Prof. Mehmet Gunduz (Ed.), ISBN: 978-953-307-766-6, InTech, Available from: <http://www.intechopen.com/books/breast-cancer-focusing-tumor-microenvironment-stem-cells-and-metastasis/insulin-like-growth-factor-binding-protein-7-an-antagonist-to-breast-cancer>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen