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Review

The role of the oncofetal IGF2 mRNA-binding protein 3 (IGF2BP3) in cancer

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ABSTRACT

The post-transcriptional control of gene expression mediated by RNA-binding proteins (RBPs), long non-coding RNAs (lncRNAs) as well as miRNAs is essential to determine tumor cell fate and thus is a major determinant in cancerogenesis. The IGF2 mRNA binding protein family (IGF2BPs) comprises three RBPs. Two members of the family, IGF2BP1 and IGF2BP3, are *bona fide* oncofetal proteins, which are *de novo* synthesized in various human cancers. *In vitro* studies revealed that IGF2BPs serve as post-transcriptional fine-tuners modulating the expression of genes implicated in the control of tumor cell proliferation, survival, chemo-resistance and metastasis. Consistently, the expression of both IGF2BP family members was reported to correlate with an overall poor prognosis and metastasis in various human cancers. Due to the fact that most reports used a pan-IGF2BP antibody for studying IGF2BP expression in cancer, paralogue-specific functions can barely be evaluated at present. Nonetheless, the accordance of IGF2BPs' role in promoting an aggressive phenotype of tumor-derived cells *in vitro* and their upregulated expression in aggressive malignancies provides strong evidence that IGF2BPs are powerful post-transcriptional oncogenes enhancing tumor growth, drug-resistance and metastasis. This suggests IGF2BPs as powerful biomarkers and candidate targets for cancer therapy.

1. Introduction

The mammalian IGF2 mRNA-binding protein family (Gene symbol: IGF2BP) comprises three RNA-binding proteins with a conserved domain structure including two N-terminal RNA recognition motifs (RRM) and four C-terminal hnRNP K homology (KH) domains (Fig. 1a; reviewed in: [1]). Diverse biological roles and distinct target mRNAs identified for the individual IGF2BP family members account for the numerous synonyms and aliases assigned to protein family (CRD-BP, KOC, ZBP, VICKZ or Vg1RBP/Vera in *Xenopus*).

The first family member described was IGF2BP1, which was initially identified as a protein involved in the stabilization of the MYC mRNA [2]. The protein prevents MYC mRNA degradation by binding to the coding region instability determinant (CRD) and thereby promotes tumor cell proliferation and survival in various cancer contexts (reviewed in: [1]). Later on, IGF2BP1 was found to control the subcellular sorting of the ACTB mRNA in primary fibroblasts and neurons by binding to the cis-acting zipcode in the ACTB mRNA's 3′UTR [3]. By controlling the spatially restricted translation of the ACTB mRNA, IGF2BP1 was proposed to enhance neurite outgrowth and axonal guidance ([4]; reviewed in: [1]). The human IGF2BP2 was first described in 1999 due to its association with the IGF2 mRNA [5]. Later on the protein, also termed p62, was proposed as an auto-antigen in hepatocellular carcinoma [6]. Most notably, however, single nucleotide polymorphisms (SNPs) have been identified in the second intron of the human *IGF2BP2* gene. These were correlated with an elevated risk of type two diabetes by various studies (reviewed in: [7]). Consistently, IGF2BP was recently identified as a modulator of mTOR signaling and IGF2 mRNA translation [8]. The human IGF2BP3, which of all human family members shows the highest similarity to *Xenopus* Vg1/RBP, was initially termed KOC and identified due to its high abundance in pancreatic cancer tissue [9]. Since its first identification a bulk of literature reported IGF2BP3 to be the mainly expressed family member in human cancer (reviewed in: [10]). Despite their high degree of

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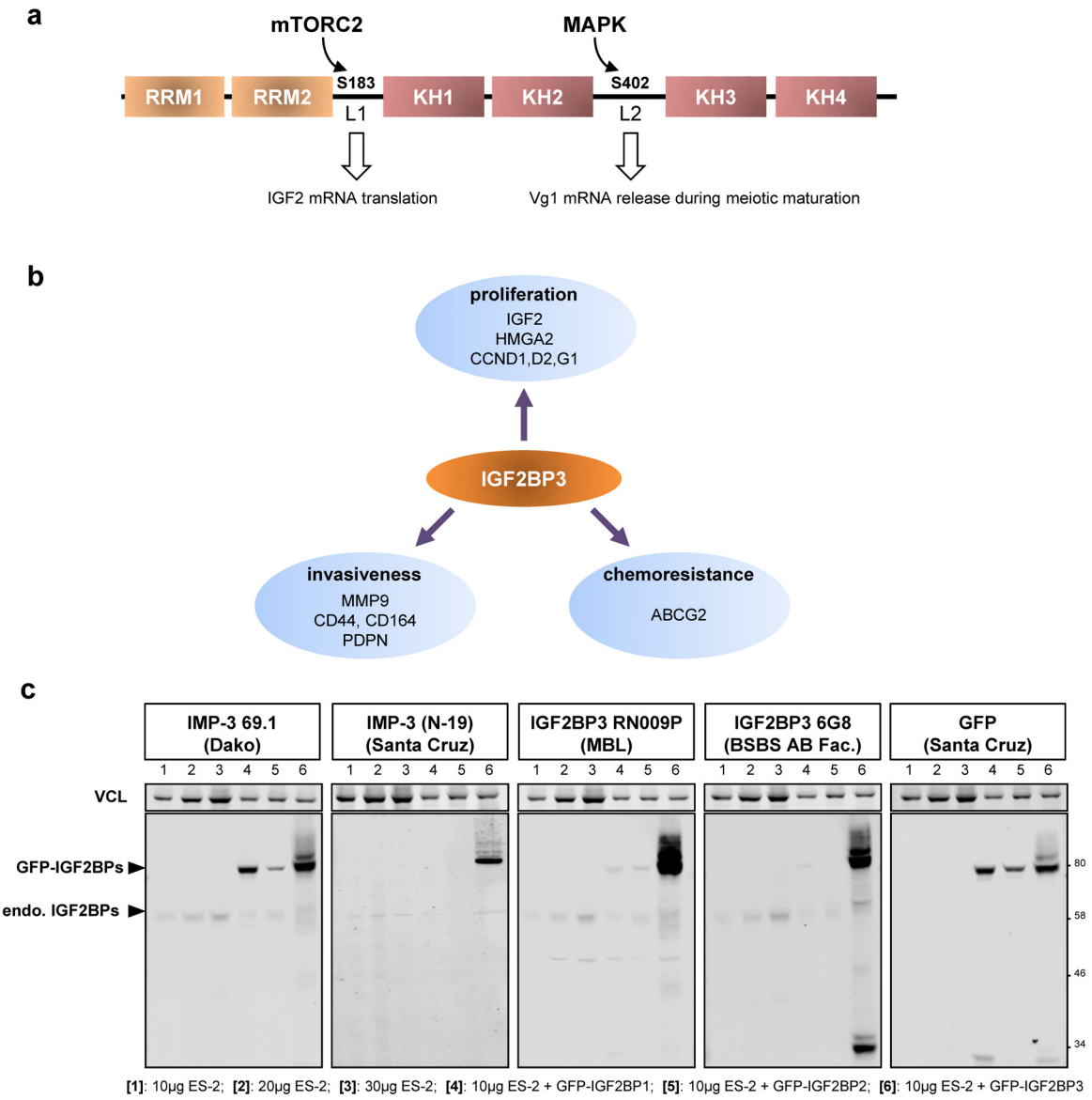

Fig. 1. (a) Domain structure of IGF2BPs with two N-terminal RNA recognition motifs (RRM1 and RRM2), the hnRNP K homology domains (KH1–KH4) and linker regions (L1, L2). S183 in L1 of IGF2BP3 is phosphorylated by the mTOR complex 2 and suggested to promote IGF2 mRNA translation [27]. MAPK-dependent phosphorylation of S402 in L2 of Vg1/RBP, the *Xenopus* ortholog of IGF2BP3, was proposed to release Vg1 mRNA during meiotic maturation of *Xenopus* oocytes [26]. (b) IGF2BP3 enhances indicated tumor cell properties by promoting the expression of indicated target genes by either preventing mRNA decay or stimulating mRNA translation, as depicted in Table 1. (c) The specificity of IGF2BP3-directed antibodies was analyzed by Western blotting of total protein isolated from non-transfected (1–3) or ES-2 cells transfected with GFP-tagged IGF2BP paralogues (4–6), respectively. Increasing amounts of total protein were analyzed in lanes 1–3. VCL (vinculin) served as a loading control. The expression of GFP-tagged proteins was determined by a GFP-directed antibody supplied by Santa Cruz. Note that the DAKO-supplied antibody 69.1 (also termed L523) detects all IGF2BPs. IGF2BP3-selective antibodies were purchased from Santa Cruz (N-19) and MBL (RN009P). The mouse monoclonal antibody 6G8 was generated in collaboration with the antibody facility (BSBS) of the Technical University of Braunschweig, Germany.

similarity the IGF2BP proteins exhibit quite different expression patterns (reviewed in: [1]). Although highly abundant during embryogenesis [5], the only family member ubiquitously expressed in adult mouse tissues is IGF2BP2 [1]. In cancer, however, *de novo* synthesis or a severe upregulation has been described mainly for IGF2BP1 and IGF2BP3 suggesting these two family members as *bona fide* oncofetal proteins ([5]; reviewed in: [1]).

All three IGF2BPs exhibit a high degree of identity (ranging from 66 to 74%) and even higher similarity (79–84%) at the amino acid level. The sequence identity is most prominent in the RRM and KH domains suggesting the distinct biological functions of IGF2BPs to mainly be regulated *via* the highly variable linker regions (Fig. 1a). The C-terminal KH domains of the IGF2BPs are essential for RNA-binding and thereby determine subcellular localization of all three family members, which is typically characterized by a mainly cytoplasmic, granular distribution [11]. Based on crystal structures as well as NMR studies of the C-terminal KH-3,4 di-domain of IGF2BP1, also termed ZBP1, the current view suggests an anti-parallel pseudo-dimer formation of the two KH domains interacting with two appropriately spaced RNA motifs [12,13]. *In vitro* studies revealed that all four KH domains mediate RNA-binding, whereas the RRM domains were proposed to promote the stability of protein–RNA complexes and mediate the association with other RBPs [11,14]. Despite the high degree of sequence identity in the KH domains, all three paralogues associate with the IGF2 mRNA but apparently exhibit distinct RNA-binding properties and presumably associate with variable target transcripts ([11]; reviewed in: [1]). However, all paralogues were described

to control the turnover, translation and/or transport of their target mRNAs. Among all family members the most functional and mechanistic studies were performed on IGF2BP1 (reviewed in: [1]). Little is known about IGF2BP2, which essentially was reported to control IGF2 mRNA translation, mTOR-signaling and the regulation of PINCH and MURF expression (reviewed in: [1]). Although, IGF2BPs are mainly cytoplasmic [11,15–17], one report suggests that IGF2BP3 in concert with HNRNPM modulates the fate of cyclin D1, D3 and G1 encoding transcripts in the nucleus [18]. Although the latter remains to be validated and might be due to aberrant nuclear protein staining of some commercial antibodies (data not shown), there is a common consensus that all IGF2BPs direct mRNA fate *via* cytoplasmic mRNPs. In these IGF2BPs were proposed to associate with other RBPs, mainly or exclusively in a RNA-dependent manner [11], to regulate the fate of "virgin" mRNAs, which have not undergone the pioneer round of translation and thus remain associated with proteins of the exon-junction complex (EJC) [19,20]. Presumably, IGF2BPs bind their target transcripts already at the site of transcription in the nucleus [21] and protect or "cage" their target mRNAs in cytoplasmic mRNPs (reviewed in: [1]). The release of caged target mRNAs and thus their translation or decay is presumably modulated by cytoplasmic signaling which merges on IGF2BPs and potentially other RBPs associating in virgin mRNPs. Phosphorylation-dependent regulation of the RNA-binding activity of RBPs has been proposed as an essential mechanism modulating the cytoplasmic control of gene expression [22,23]. For IGF2BP1 as well as the *Xenopus* Vg1RBP (IGF2BP3) phosphorylation by the SRC-kinase (IGF2BP1) or MAPKs (IGF2BP3) in the linker region connecting the KH di-domains was reported to modulate growth cone guidance [4,24,25] or the release of the Vg1 mRNA from the vegetal cortex of *Xenopus* oocytes (Fig. 1a; [26]). Strikingly, mTOR-dependent phosphorylation in the linker region connecting the RRM and KH domains of IGF2BP1 and IGF2BP2 was shown to enhance the translation of the IGF2 mRNA (Fig. 1a; [8,27]). Phosphorylation at a homologous residue was also reported for IGF2BP3; however, the functional relevance of this observation remains yet to be evaluated [27].

2. IGF2BP3's role in modulating tumor cell fate

Although little is known about the role of IGF2BP3 in modulating the cytoplasmic fate of mRNAs, IGF2BP3 like IGF2BP1 was proposed to control the translation or turnover of various candidate target transcripts (Table 1). Notably, thousands of target mRNAs, yet a rather small and common binding motif, has been described for all IGF2BPs based on PAR-CLIP [28]. These analyses yet remain to be validated but suggest a significant overlap of target mRNA binding among IGF2BP paralogues. The most prominent and frequently studied target mRNA of IGF2BPs obviously is the IGF2 mRNA, or more precisely one IGF2 transcript variant comprising a highly structured 5′UTR, the leader 3 sequence. While initially reported to repress translation of the respective IGF2 transcript [5], more recent evidence indicates that IGF2BPs promote IGF2 synthesis, presumably in a mTOR-controlled manner [8,27,29,30]. Although, the role of IGF2BP3 in the control of IGF2 mRNA translation remains contradictory, recent studies indicate that an upregulation of the protein in human cancer might enhance tumor growth by promoting the expression of IGF2, as previously suggested by *in vitro* studies [31]. Moreover, recent studies suggest that IGF2BP3 promotes tumor cell proliferation also by synergizing with HNRNPM in the nucleus leading to an enhanced expression of cyclins [18]. Recently, IGF2BP3 was shown to promote the expression of the architectural transcription factor HMGA2 by preventing miRNA attack, predominantly *via* the let-7 family [16]. Consistently, IGF2BP3 was reported as an essential factor in tumor initiating cells in hepatocellular carcinomas (HCCs), was strongly correlated with an enhancement of HMGA2 expression in HCCs and was identified as one of the most severely upregulated RBPs in HCCs [15,32,33]. In lung carcinoma cells, HMGA2 was proposed to enhance tumor cell aggressiveness by acting as a competing endogenous RNA (ceRNA) sequestering members of the let-7 miRNA family [34]. Thus, by protecting and consequently enhancing the abundance of HMGA2 and potentially other let-7 targeting ceRNAs, IGF2BP3 like IGF2BP1 could enhance the expression of let-7 repressed oncogenes and thereby promote tumor cell aggressiveness. In support of an oncogenic role of IGF2BP3, the protein was furthermore proposed to stabilize the ABCG2 encoding mRNA [35]. This was suggested to enhance the chemo-resistance of breast cancer-derived cells *in vitro*.

In addition to growth, survival and chemo-resistance, IGF2BP3 was also reported to enhance the invasive potential of tumor cells *in vitro*. This presumably involves the stabilization of the CD44, CD164, MMP9 and PDPN encoding mRNAs (Fig. 1b; references in Table 1). Moreover, these findings suggest that IGF2BP1 and IGF2BP3 may synergize in promoting tumor cell dissemination. IGF2BP1 was shown to: (1) sustain mesenchymal-like tumor cell properties by enhancing the expression of LEF [36]; (2) promote tumor cell migration and pro-migratory adhesion by modulating actin dynamics in a HSP27-dependent manner [37,38]; (3) enhance the formation of invadopodia by synergizing with IGF2BP3 in promoting the expression of CD44 [39]. In addition to *in vitro* evidence, IGF2BP3 has also been correlated with an aggressive and invasive cancer phenotype in some human malignancies. In breast cancer-derived tumor cells the expression of IGF2BP3 was enhanced by EGFR-signaling but suppressed by estrogen receptor β (ERβ) signaling [40]. This was well correlated with upregulated expression of IGF2BP3 in highly aggressive triple-negative breast carcinomas (TNBC; Table 2) and the IGF2BP3-dependent enhancement of TNBC-derived tumor cell migration *in vitro* [40]. Moreover, IGF2BP3 was reported to promote the chemo-resistance of breast cancer-derived cells suggesting the protein to act as an oncogenic factor in mammary carcinomas [35]. In osteosarcoma, IGF2BP3 was proposed to be upregulated due to epigenetic modifications and enhance anoikis resistance as well as the formation of syngeneic subcutaneous *Xenografts* [17]. In oral squamous cell carcinoma (OSCC), high IGF2BP3 expression was correlated with an overall poor prognosis and a higher incidence of lymph node metastasis (Table 2; [41,42]). This was suggested to partially rely on the IGF2BP3-dependent stabilization of the podoplanin (PDPN) mRNA [43], since elevated PDPN expression was proposed to enhance tumor cell invasiveness and metastasis [44,45].

Consistent with various studies on IGF2BPs' role in cancer, there is strong evidence for a pro-metastatic role of IGF2BP1 *in vivo*, since transgenic expression of the protein in mice induced primary breast cancer lesions as well as metastasis [46]. In contrast, tumor formation was not observed by the transgenic expression of IGF2BP3 [47]. However, the only moderate phenotypic abnormalities in the exocrine pancreas and parotid gland observed in the respective mouse model might be explained by the moderate gastrointestinal expression of the transgene. Thus, although *in vivo* evidence for an oncogenic role of IGF2BP3 remains sparse, the protein clearly promotes tumor cell proliferation, growth, drug-resistance and invasiveness *in vitro*.

3. IGF2BP3's expression in human cancer

The predominant IGF2BP paralogue described in the context of human cancer is IGF2BP3 (reviewed in: [10]). This is largely due to the fact that the vast majority of studies analyzing IGF2BP expression in cancer rely on one antibody, supplied by DAKO,

Table 1
Target mRNAs of IGF2BP3.

Target	cis-Element	Regulation	Refs.
CD44	3′-utr	Control of mRNA stability (mediated by I1 and 3)	[39,81,131]
IGF2	5′-utr	Translational control	[30,131]
H19	ncRNA	Unknown	[30]
ACTB	3′-utr	Unknown (*in vitro* binding)	[11]
MYC	CRD	Unknown (*in vitro* binding)	[11]
CD164	Unknown	Control of mRNA stability	[40]
MMP9	Unknown	Control of mRNA stability	[40,131]
ABCG2	Unknown	Unknown	[35]
PDPN	3′-utr	Control of mRNA stability	[43]
HMGA2	3′-utr	Protection from miR directed degradation	[16]
CCND1	3′-utr	(Presumably) translational control	[18]
CCND3	3′-utr	(Presumably) translational control	[18]
CCNG1	3′-utr	(Presumably) translational control	[18]

which is suitable for immuno-histochemical (IHC) analyses. However, although proposed to be IGF2BP3-specific, the DAKO-supplied antibody, is not paralogue-specific but recognizes all three IGF2BP paralogues (Fig. 1c). In ovarian carcinoma-derived ES-2 cells, the DAKO-supplied antibody identified endogenous IGF2BP expression but also detected the expression of all other transiently expressed GFP-tagged IGF2BP paralogues. Notably, this observation is consistent with a previous, independent report by Natkunam et al. [48]. Only few studies use paralogue-specific antibodies, for instance the N-19 antibody supplied by Santa Cruz or the MBL-supplied polyclonal serum directed against a C-terminal peptide of IGF2BP3. These antibodies are highly IGF2BP3-specific and show a negligible cross-reactivity with the other paralogues in Western blotting (Fig. 1c). This is also observed for a monoclonal antibody (6G8) raised by our lab in collaboration with the BSBS antibody facility (Fig. 1c). Hence, the expression of IGF2BPs in cancer has to be considered with great caution in respect to paralogue-specificity. However, in view of the studies indicating an upregulated expression of IGF2BP1 and IGF2BP3 in various cancers on the basis of RT-PCR or paralogue-specific antibodies and the fact that these both paralogues are barely observed in the adult organism, we propose that upregulated expression determined by the DAKO-supplied antibody strongly indicates expression of IGF2BP1 and/or IGF2BP3. Bearing in mind the above described limitation, we in the following summarize recent findings on the expression of IGF2BPs in human cancer. Where available, we also indicated a correlation of IGF2BP expression with prognosis and/or metastasis (Table 2).

3.1. Breast cancer

In breast carcinomas, IGF2BP3 expression determined by the DAKO-supplied antibody was observed in the majority of invasive triple-negative mammary carcinomas [49,50]. However, in basal-like breast cancer, a significantly upregulated expression was only found in adenoid cystic carcinomas [51,52].

3.2. Gynecologic cancers

IGF2BP3 expression has been reported in all to date analyzed gynecologic cancers including cervical cancer [53–55], endometrial cancer [56–61] and ovarian cancer [62,63]. Consistent with other cancers, IGF2BP3 expression was proposed to be increased in high-grade malignancies, for instance 90% of endometrial clear cell carcinomas [58] and where investigated was associated with an overall poor prognosis, for instance in ovarian carcinomas [62]. However, in the here reviewed studies the expression of IGF2BP3 was exclusively analyzed by the antibody supplied by DAKO. Thus, the paralogue-specific expression of the oncofetal IGF2BPs, IGF2BP1 or IGF2BP3, remains largely elusive. For ovarian cancer, our own analyses indicate that the expression of IGF2BP1, determined by a highly paralogue-selective polyclonal antibody, was correlated with an overall poor prognosis [64]. In agreement, IGF2BP1 depletion severely impaired the proliferation, survival and migratory capacity of ovarian cancer-derived tumor cells *in vitro* suggesting that both, IGF2BP1 and IGF2BP3, are important oncogenic factors in ovarian cancer [38,64]. Notably, recent studies indicate that IGF2BP2, termed VICKZ2 in the respective study, next to IGF2BP1 is the most severely upregulated IGF2BP paralogue in serous ovarian carcinoma and confers increased invasiveness of ovarian cancer-derived tumor cells *in vitro* [65].

3.3. Gastrointestinal cancer

In the vast majority of studies addressing IGF2BP3 expression in colorectal cancer, the DAKO-supplied antibody was used. All studies reported indicate a significantly elevated expression of IGF2BP3 in the vast majority of analyzed aggressive colorectal carcinomas (CRCs) compared to typically negative mucosa and suggest IGF2BP3 expression to correlate with an unfavorable prognosis [66–69]. Consistently, a strong correlation of IGF2BP3 expression was observed with the pro-proliferative marker Ki67 [67] and lymph node metastasis [69]. Likewise, in esophageal cancer IGF2BP3 expression, analyzed by the pan-IGF2BP antibody supplied by DAKO, was reported to correlate with an overall poor prognosis, higher tumor grading and was identified as a good predictor of regional lymph node metastasis [70,71]. In gastric adenocarcinomas (GAC), upregulated IGF2BP3 expression, determined again by IHC-analyses using the DAKO-supplied antibody, was reported in up to 87% of analyzed samples [72–74]. None or only faint staining was observed in up to 10% of adjacent 'normal' mucosa. The latter could indicate moderate expression of IGF2BP2. As observed for other gastrointestinal cancers, IGF2BP3 expression was correlated with an unfavorable prognosis and metastasis in GAC [72–74].

3.4. Liver cancer

IGF2BP3 expression was reported in carcinomas of the bile duct [75–77], gallbladder carcinomas [78], intrahepatic cholangiocarcinoma [79] as well as hepatocellular carcinomas [32,80,81]. As in other cancers, IGF2BP3 mainly determined by the DAKO-supplied antibody, was correlated with an overall poor prognosis and increased invasiveness [32]. Recent studies also provide functional evidence for an oncogenic role of IGF2BP3 in HCCs. In tumor initiating $CD133^{+}/CD49f^{+}$ stem cells (TICs) derived from HCC mouse models as well as human patients, IGF2BP3 was found to be severely upregulated [15]. *In vitro*, IGF2BP3 was reported to enhance chemoresistance and sustain pluripotency in HCC-derived TICs. Consistently, in a study reporting upregulated expression of IGF2BP3 in 68% of analyzed primary HCCs, the protein was proposed to enhance the invasiveness and motility of HCC-derived

Table 2
IGF2BP expression in human cancer.

Cancer/organ	Incidence (%)	Correlation		Refs.
		Poor prognosis	Metastasis/invasion	
Breast				
Mammary carcinoma	8–33	N/A	N/A	[10]
Basal-like breast cancer	21	N/A	N/A	[51]
Triple-negative breast carcinoma	33–56	+	N/A	[49,50]
Adenoid cystic breast carcinoma	81	N/A	N/A	[52]
Apocrine breast carcinoma	25	N/A	N/A	[52]
Gynecologic				
Cervical carcinoma	50–100	N/A	N/A	[53–55]
Endometrial carcinomas	15–100	+	N/A	[56–61]
Ovarian cancer (clear cell; serous papillary)	95; 91	+	N/A	[62,63]
Gastrointestinal				
Colorectal carcinoma	35–91	+	+	[66–69]
Esophageal adenocarcinoma	60–70	+	+	[70,71]
Gastric adenocarcinoma	74–87	+	+	[72–74]
Hepatic				
Bile duct carcinomas	58–78	N/A	N/A	[75–77]
Gallbladder carcinoma	82	N/A	N/A	[78]
Intrahepatic cholangiocarcinoma	41	+	N/A	[79]
Hepatocellular carcinomas (TIC)	61–85 (100)	+	+	[15,32,80,81]
Pancreatic				
Pancreatic ductal adenocarcinoma	62–97	+	+	[82–86]
Genitourinary				
Prostate cancer	18–83	+	+	[87–89]
Renal cell carcinoma	4–62	+	+	[90–93]
Testicular cancer and teratoma	63–100	N/A	N/A	[94,95]
Urothelial carcinoma	20–93	+	+	[96–100]
Lung and esophageal				
Esophageal	38–97	N/A	N/A	[103,106]
Laryngeal squamous cell carcinoma	97	N/A	N/A	[103]
Lung	55–100	N/A	N/A	[101,102,104–106]
Non-small cell lung cancer	55	N/A	N/A	[104]
Large-cell neuroendocrine carcinoma	100	N/A	N/A	[101]
Small cell lung cancer	100	N/A	N/A	[101]
Head–Neck				
Oral squamous cell carcinoma	56–100	+	+	[43,107–110,42,41]
Cutaneous cancer				
Keratocanthomas	26	N/A	N/A	[111]
Squamous cell carcinoma	57	N/A	N/A	[111]
Melanoma	8–100	+	+	[112–115]
Merkel cell carcinoma	90	N/A	N/A	[116]
Thyroid cancer				
Benign (FA, SNC, *etc.*)	0	N/A	N/A	[119]
Follicular thyroid carcinoma	69	N/A	N/A	[119]
Papillary thyroid carcinomas (PTC)	11	N/A	N/A	[119]
Follicular variant of PTC	38	N/A	N/A	[119]
Poorly differentiated	59	N/A	N/A	[117]
CNS				
Sacral chordoma	63	N/A	N/A	[120]
Astrocytoma	31	+	N/A	[121]
Glioblastoma	88	N/A	N/A	[31]
Meningioma	7	N/A	N/A	[122]
Neuroblastoma	58	+	N/A	[123]
Lymphoid				
Hodgkin (classical) lymphoma	94–100	N/A	N/A	[48,124–126]
Hodgkin (lymphocyte predominant)	92–100	N/A	N/A	[48,124–126]
Diffuse large B-cell lymphoma	78–85	N/A	N/A	[48,125]
Anaplastic large cell lymphoma	75–100	N/A	N/A	[48,125]
ALL/AML	+/(100)	N/A	N/A	[48,127]
Sarcoma				
Leiomyoma	0	N/A	N/A	[129]
Leiomyosarcoma	52	+	N/A	[129]
Osteosarcoma	17–90	N/A	N/A	[17,128]

cells *in vitro* by promoting the expression of the pro-stemness factor HMGA2 [32]. These findings were supported by recent studies demonstrating that IGF2BP3 prevents miRNA-dependent inhibition of HMGA2-expression [16]. Also in HCC, we recently demonstrated that IGF2BP1 and IGF2BP3 are among the ten most upregulated RBPs, as revealed by microarray and RT-PCR analyses [33]. Like proposed for IGF2BP3, IGF2BP1 was found to act as an oncogenic factor promoting the survival and proliferation of HCC-derived cells *in vitro* and *in vivo* by enhancing the expression of MYC and Ki67 [33].

3.5. Pancreatic cancer

In pancreatic cancer, IGF2BP3 expression was exclusively assessed by IHC-analyses with three studies using the DAKO-supplied antibody [82–86]. In agreement with other analyses, IGF2BP expression was determined in the vast majority of pancreatic ductal adenocarcinomas (PDAC) and was proposed for 97% of invasive PDACs [83]. In contrast, the vast majority (>74%) of inflamed pancreatic tissue was negative supporting the view that IGF2BPs, in particular IGF2BP1 and IGF2BP3, are potent biomarkers of aggressive and invasive pancreatic carcinomas [83].

3.6. Genitourinary cancer

In prostate cancer (PC), exclusively analyzed by the pan-IGF2BP antibody supplied by DAKO, the expression of IGF2BP3 was observed in 18–83% of analyzed samples [87–89] but considered to be of low prognostic value [88]. However, a significant upregulation of IGF2BP expression was observed in only 15% of localized but strikingly 65% of palliatively treated metastatic PCs supporting the pro-invasive/-metastatic role of IGF2BP1/3 [87]. Notably, upregulated IGF2BP3 expression was not correlated with elevated IGF2 mRNA or protein abundance. However, increased IGF2BP serum levels were independently correlated with poor survival in patients treated with radical prostatectomy [87,89]. In studies focusing on IGF2BP3 expression in renal cell carcinoma (RCC), all using the DAKO-supplied antibody, the expression of IGF2BPs was correlated with an overall poor prognosis and metastasis [90–93]. The vast majority (86%) of IGF2BP-positive patients developed metastases whereas this was only observed for 14% of negative tumors [92]. In agreement, IGF2BP expression was reported for about 50% of metastasizing RCCs but approximately only 4% of non-disseminating RCCs [91]. Notably, IGF2BP expression was also observed in approximately 62% of analyzed metastases of RCC-origin [91]. In testicular cancer and male teratoma, IGF2BP3 expression was observed in the vast majority of analyzed samples [94,95]. The study by Hammer et al. used a highly paralogue-specific set of peptide-directed polyclonal antibodies targeting the C-terminus of IGF2BPs [94], similar to the MBL-supplied antibody (Fig. 1c). These tools revealed that IGF2BPs are expressed to different extent in all cancer samples analyzed but only IGF2BP1 was expressed in all testicular cancers. In agreement, another study using the DAKO-supplied antibody revealed expression in a 100% of analyzed metastatic testis teratoma [95]. Puzzling, however, remains that none of the analyzed female mature teratomas was found to express IGF2BPs [95]. In urothelial carcinomas, IGF2BP3 expression, again exclusively assessed by the DAKO-supplied antibody, was correlated with an overall poor prognosis, increased metastasis and was elevated with increased tumor grade/stage [96–100]. Notably, IGF2BP3 expression again was not associated with upregulated IGF2 or CD44 abundance, as also observed in other cancers [96].

3.7. Lung and esophageal cancer

Consistent with other carcinomas, upregulated expression of IGF2BP3, once again exclusively analyzed by the DAKO-supplied antibody, was observed in lung and esophageal cancer. Expression was associated with higher tumor grading and reached a 100% in small cell and metastatic lung cancer [101–106].

3.8. Head–neck cancer

A bulk of studies indicates IGF2BP expression to be upregulated in oral squamous cell carcinoma (OSCC; [41–43,107–110]). All studies relied on the DAKO-supplied antibody and thus paralogue-specific expression signatures remain yet to be addressed. However, as observed in other carcinomas, the expression of IGF2BP3 was correlated with an overall poor prognosis [41,42,110] and confirmed as a predictor of lymph node status [108] and metastasis [41,43,107]. In agreement, *in vitro* studies suggested IGF2BP3-dependent enhancement of podoplanin (PDPN) expression, which was proposed to promote tumor cell invasiveness [43]. Notably, PDPN and IGF2BP3 expression significantly correlated with lymph node metastasis in OSCC patients.

3.9. Cutaneous cancer

Various studies reported upregulated expression of IGF2BP3 in keratoacanthomas, squamous cell carcinomas (SCC) of the skin [111], melanoma [112–115] and merkel cell carcinoma [116]. All these studies relied on the DAKO-supplied antibody and thus paralogue-specific expression remains yet to be investigated. As observed for various other solid cancers, higher incidence of IGF2BP3 expression was observed in invasive SCC of the skin [111] and metastatic melanoma [113,114]. Notably, one study revealed that the expression of IGF2BP1 (Chr.17q) and/or IGF2BP3 (Chr.7p) in metastatic melanoma could be increased due to chromosomal gain [115]. In agreement, we recently reported that IGF2BP1 enhances the migratory potential and a mesenchymal-like cell phenotype in melanoma-derived tumor cells [36].

3.10. Thyroid cancer

In thyroid cancers of follicular origin, IGF2BP expression, mainly assessed by immunostaining using the DAKO-supplied antibody, was proposed to be of diagnostic value [117–119]. IGF2BP3 expression was exclusively observed in malignant cancers and was proposed to show high specificity in follicular thyroid carcinomas and the follicular variant of papillary thyroid carcinomas [119]. In poorly differentiated variants of thyroid carcinomas, IGF2BP3 expression was observed in 59% of cases [117].

3.11. CNS cancer

Mainly analyzed by the DAKO-supplied antibody, IGF2BP expression was reported in various CNS-derived cancers including sacral chordoma [120], astrocytoma [121], meningioma [122], glioblastoma [31] and neuroblastoma [123]. As observed in carcinomas, the expression of IGF2BPs was proposed to correlate with an overall poor prognosis.

3.12. Lymphoid cancer

The expression of IGF2BPs has extensively been studied in lymphomas. IHC-based analyses revealed a high incidence of IGF2BP expression, as determined by the DAKO-supplied antibody, with up to a 100% of positive classical or lymphocyte-predominant Hodgkin lymphomas [48,124–126]. RT-PCR based analyses in a small cohort

of lymphomas suggested that IGF2BP3 is the predominant paralogue expressed in primary lymphomas [48]. Strong expression of IGF2BP3 was found in lymphocytes within the germinal center (GC), lymph nodes, the spleen and megakaryocytes, myeloid precursors as well as plasma cells of the bone marrow. Consistent with this expression signature, IGF2BP3 expression was also observed in ten acute myeloid leukemia (AML) samples, as determined by staining of immature blasts [48]. One study also suggests that distinct acute lymphoblastic leukemia (ALL) entities are characterized by altered IGF2BP expression, as revealed by RT-PCR analyses [127]. However, the expression signatures of IGF2BPs in leukemia and their potential correlation with clinical parameters or diseases progression remain yet to be analyzed in detail.

3.13. Sarcoma

In bone and soft tissue cancer, IGF2BP expression was reported in osteosarcoma [17,128] and leiomyosarcoma [129]. One study explored the expression on the basis of the MBL-supplied antibody (see Fig. 1c) which shows a high specificity for IGF2BP3 in Western blotting suggesting that a vast majority (90%) of analyzed osteosarcomas expresses this paralogue [17]. Most notably, the same study also revealed that the depletion of IGF2BP3 impaired the growth of syngeneic osteosarcoma *Xenografts* and the viability as well as anoikis resistance of tumor cells *in vitro*. In 52% of analyzed leiomyosarcomas but none of the 62 investigated leiomyomas, IGF2BP3 expression was determined using the DAKO-supplied antibody [129].

4. Conclusions and perspectives

The bulk of correlative studies describing elevated expression or *de novo* synthesis of IGF2BPs in human cancer and the various functional *in vitro* studies provide strong evidence that IGF2BPs serve essential roles in modulating tumor cell fate and act in an oncogenic manner in virtually every cancer analyzed to date. With this being said it remains largely elusive *via* which downstream effectors the individual paralogues act, whether or not they synergize in promoting tumor cell aggressiveness and which paralogue is the dominant family member in which cancer.

In view of these and the above outlined limitations, we propose that IGF2BP1 and IGF2BP3 are the main members in driving tumorigenesis, since there is comparatively little evidence for an upregulated expression of IGF2BP2 in cancer or an oncogenic action of this paralogue *in vitro* (also reviewed in: [1]). IGF2BP1/3 have been reported to promote tumor cell survival, proliferation, anchorage-independent growth, chemo-resistance and tumor cell invasiveness *in vitro*. In agreement, an upregulated expression of IGF2BPs has been correlated with an overall poor prognosis and metastasis in various cancers (Table 2). The review of recent literature suggests that IGF2BP3 synergizes with HMGA2 in enhancing tumor cell aggressiveness. By preventing miRNA attack, IGF2BP3 was proposed to promote the expression of HMGA2 [16]. Notably, a similar mechanism was proposed for IGF2BP1, which enhances BTRC1 expression by antagonizing miRNA-dependent degradation of BTRC1 transcripts [130]. These findings support the view, that both proteins serve essential roles in promoting the expression of oncogenic factors by shielding these from being degraded by tumor-suppressive miRNAs. In addition, IGF2BPs promote the expression of other oncogenic transcriptional regulators like MYC and LEF1 [36,64], again two transcripts targeted by tumor-suppressive miRNAs. Thus it is tempting to speculate that IGF2BP1/3 enhance or sustain 'oncogenic' reprogramming of transcription at the post-transcriptional level. Moreover, the target transcripts identified, for instance HMGA2, and the expression signatures of IGF2BP1/3, for instance in the hematopoietic system, suggest that both factors sustain a stemness-like cell phenotype (reviewed in: [1]). This is consistent with reports on a role of IGF2BP1 in modulating stemness-like cell properties during development and the strikingly upregulated expression of IGF2BPs in aggressive and thus frequently de-differentiated cancers (reviewed in: [1]). In addition to promoting a stemness-like tumor cell phenotype, IGF2BP1/3 were also shown to promote the migratory and invasive potential of tumor cells *in vitro*. We propose, that this is mainly facilitated by IGF2BP1, which has been shown to modulate actin dynamics, pro-migratory adhesion, tumor cell invasiveness and was reported to induce metastasis in a transgenic mouse model [36–39,46]. Evidence for a pro-invasive role of IGF2BP3 is mainly based on loss-of-function studies yet downstream effectors remain largely elusive or require further validation. Moreover, the only to date reported mouse model does not support an oncogenic nor pro-metastatic role of the protein [47].

In conclusion, we propose that IGF2BP1 and IGF2BP3 present potent post-transcriptional oncogenes, which enhance tumor cell aggressiveness. To further explore their potential as cancer biomarkers it is essential to characterize their expression by the use of paralogue-specific antibodies, which are available but largely ignored by most studies. Moreover, additional transgenic mouse models are required to test the role of both paralogues as well as their putative synergistic action *in vivo*. These need to be analyzed with respect to the potential of both paralogues in inducing cancer and promoting tumor cell dissemination. In view of promoting our understanding of how IGF2BPs enhance a malignant tumor cell phenotype, future studies should aim at a more holistic view by comparing the role of all three paralogues side-by-side in the cell models of choice. Finally, rescue studies are required to finally prove *via* which downstream effectors IGF2BP paralogues facilitate their multiple roles in promoting tumor cell aggressiveness.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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