

Review

## Transforming Growth Factor-Beta-Induced Protein (TGFBI)/( $\beta$ ig-H3): A Matrix Protein with Dual Functions in Ovarian Cancer

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**Abstract:** Transforming growth factor-beta-induced protein (TGFBI, also known as  $\beta$ ig-H3 and keratoepithelin) is an extracellular matrix protein that plays a role in a wide range of physiological and pathological conditions including diabetes, corneal dystrophy and tumorigenesis. Many reports indicate that  $\beta$ ig-H3 functions as a tumor suppressor. Loss of  $\beta$ ig-H3 expression has been described in several cancers including ovarian cancer and promoter hypermethylation has been identified as an important mechanism for the silencing of the *TGFBI* gene. Our recent findings that  $\beta$ ig-H3 is down-regulated in ovarian cancer and that high concentrations of  $\beta$ ig-H3 can induce ovarian cancer cell death support a tumor suppressor role. However, there is also convincing data in the literature reporting a tumor-promoting role for  $\beta$ ig-H3. We have shown  $\beta$ ig-H3 to be abundantly expressed by peritoneal cells and increase the metastatic potential of ovarian cancer cells by promoting cell motility, invasion, and adhesion to peritoneal cells. Our findings suggest that  $\beta$ ig-H3 has dual functions and can act both as a tumor suppressor or tumor promoter depending on

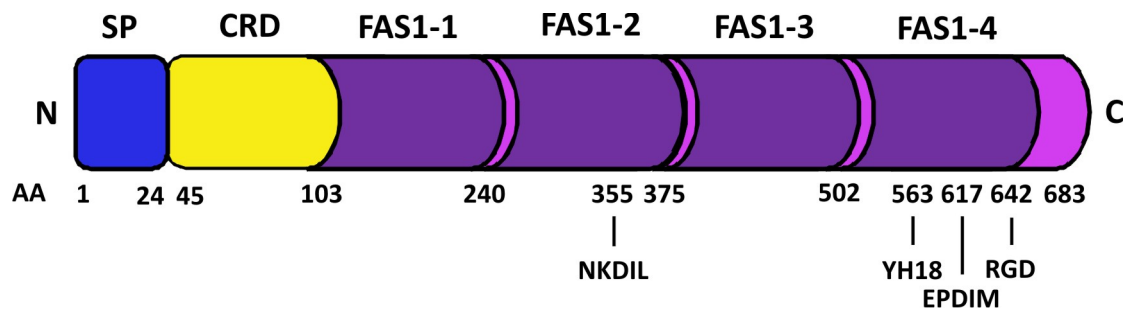
the tumor microenvironment. This article reviews the current understanding of  $\beta$ ig-H3 function in cancer cells with particular focus on ovarian cancer.

**Keywords:** ovarian cancer; extracellular matrix; TGFBI; tumor suppressor; invasion; adhesion; metastasis

## 1. Introduction

$\beta$ ig-H3 (also known as TGFBI for protein and *TGFBI* for gene) is a transforming growth factor beta (TGF $\beta$ ) inducible secreted extracellular matrix (ECM) protein. The name  $\beta$ ig-H3 was derived from its cloning as a major TGF $\beta$  responsive gene in lung adenocarcinoma cell line A549: TGF $\beta$  induced gene human clone 3 [1]. In the literature it has also been referred to as keratoepithelin [2], collagen fibre associated protein (RGD-CAP) [3], P78/70 [4], Big-h3 [5],  $\beta$ -igH3 [6], and  $\beta$ -ig [7].  $\beta$ ig-H3 is comprised of 683 amino acids and its secreted form has a predicted molecular mass of 68 kDa. Two isoforms of  $\beta$ ig-H3 at 78 and 68 kDa have been reported to date [8], both of which are encoded by a single gene, *TGFBI* [9].  $\beta$ ig-H3 contains an *N*-terminal secretory signal (1–24 amino acids), a cysteine rich domain, four internal repetitive fasciclin-1 domains (FAS1 1–4), integrin binding motifs in the *C*-terminus known as Arg-Gly-Asp (RGD), YH18, and EPDIM and an internal NKDIL motif [10,11] (Figure 1).

**Figure 1.** Schematic diagram of Transforming growth factor-beta-induced protein ( $\beta$ ig-H3) protein structure. Secretory signal (SP) in the *N*-terminal cysteine rich domain (CRD), and four fasciclin-1 domains (FAS1 1–4). Position of several known integrin binding motifs, including NKDIL, YH18, EPDIM and Arg-Gly-Asp (RGD), are indicated.



## 2. $\beta$ ig-H3 Regulation and Function

$\beta$ ig-H3 participates in many physiological processes including morphogenesis, adhesion/migration, angiogenesis, and inflammation [12]. It also has a role in reproduction [13,14] and wound healing [15,16]. A wide range of cells have been shown to induce expression of  $\beta$ ig-H3 following treatment with TGF $\beta$  including, fibroblasts, chondrocytes, smooth muscle cells, corneal epithelial cells, and various types of cancer cells [12].  $\beta$ ig-H3 is regulated not only by TGF $\beta$ , but also by retinoid [17], IL-4 [15], IL-1 [18], and TNF- $\alpha$  [18] in various cell types. TNF-like ligand 1A can regulate the inflammatory processes in a human acute monocytic leukemia cell line (THP-1) through

modulation of the  $\beta$ ig-H3 expression via both protein kinase C and extracellular signal-regulated kinase pathways [19].  $\beta$ ig-H3 could also be induced in human mesenchymal stem cells by treatment with the phospholipid, lysophosphatidic acid that is enriched in the serum of cancer patients [20]. Recent evidence suggests that  $\beta$ ig-H3 expression can also be regulated by the microRNA, miR-21 [5].  $\beta$ ig-H3 has been shown to trigger phosphorylation and to activate several intracellular pathways including AKT, extracellular signal-regulated kinase, focal adhesion kinase (FAK), and paxillin, thus mediating adhesion and migration of vascular smooth muscle cells through interactions with  $\alpha$ v $\beta$ 5 integrins [21].

Immunohistochemical studies show that  $\beta$ ig-H3 is distributed in the ECM of a wide range of developing and mature tissues, including endothelial cells of human vascular tissues [22], papillary dermis [10], primary spongiosa, periosteum, and perichondrium [23]. It has also been associated with bone formation [24,25].  $\beta$ ig-H3 expression is induced in endothelium and stroma-derived cells in the healing cornea [7] and reactive astrocytes in rat cerebral cortex at wound sites [16].

In many cell types,  $\beta$ ig-H3 functions as a linker protein which connects various matrix molecules to each other as well as facilitating cell-collagen interactions [4,26–28].  $\beta$ ig-H3 can bind to type I, II, and IV collagens as well as proteoglycans such as biglycan and decorin [28]. It has been shown that  $\beta$ ig-H3 binds covalently to collagen VI microfibrils [27] and interacts with fibronectin [26] and various integrins [29], which are the only  $\beta$ ig-H3 cell surface receptors identified to date (reviewed in [12]).

$\beta$ ig-H3 plays a role in the adhesion and migration of a wide range of cells including keratinocytes, fibroblasts, chondrocytes, osteoblasts, and endothelial cells (reviewed by [25]). Effects on adhesion are mediated through interactions with various integrins including  $\alpha$ 1 $\beta$ 1,  $\alpha$ 3 $\beta$ 1,  $\alpha$ v $\beta$ 3, and  $\alpha$ v $\beta$ 5 [10,30–33] via integrin binding motifs in the  $\beta$ ig-H3 protein. These include the well characterized RGD motif in the C-terminus [34] as well as the NKDIL motif (amino acids 354–358) [11] and the EPDIM motif (amino acids 617–621) [11] in the second and fourth FAS-1 domains, respectively (Figure 1). The structural analysis of the NKDIL and EPDIM sequence motifs show that they can adopt a  $\beta$ -turn structure similar to the RGD motif to interact with integrins during adhesion [34]. Another adhesion motif shown to support  $\alpha$ v $\beta$ 5 integrin mediated adhesion of lung fibroblast MRC-5 cells [29], vascular smooth muscle cells [21], and endothelial cells [35], is the highly conserved tyrosine and histidine residues YH18 motif (amino acids 563–580) in the fourth FAS-1 domain, which is flanked by several leucine/isoleucine residues (Figure 1).

### 3. $\beta$ ig-H3 Roles in Disease

#### 3.1. Role in Diabetes and Corneal Dystrophies

$\beta$ ig-H3 has been associated with a range of diseases including nephropathy [36], atherosclerosis [22], and rheumatoid arthritis [15,18], as well as corneal disorders. Its role in inflammatory disease processes is not well understood.  $\beta$ ig-H3 expression is prominent in the kidney and increased in the urine of diabetics [37,38]. It has been suggested that combined monitoring of albumin excretion rate and urinary  $\beta$ ig-H3 can predict the severity of diabetic nephropathy [39].  $\beta$ ig-H3 has been shown to induce pericyte apoptosis through its RGD motif, which may constitute an important pathogenic mechanism leading to pericyte loss in diabetes [40]. Recent studies also suggest that  $\beta$ ig-H3 may be

involved in kidney pathology associated with preeclampsia, and was detectable in the urine of these patients but not in non-preeclamptic pregnant women [41].

Mutations in the *TGFBI* gene are well characterized in a number of corneal dystrophies, which lead to the development of corneal deposits and impaired vision [42–45]. Corneal dystrophies represent the only known pathological disease associated with mutations in *TGFBI*. The mechanisms of pathogenesis are unknown but mutations in *TGFBI* may impair protein folding or  $\beta$ ig-H3 secretion and result in the deposition and accumulation of mutant  $\beta$ ig-H3 protein that has increased stability [46].

### 3.2. Roles in Cancer

#### 3.2.1. Role as Tumor Suppressor

Many reports indicate  $\beta$ ig-H3 is an inhibitor of tumorigenesis and suggest that  $\beta$ ig-H3 functions as a tumor suppressor (summarized in Table 1). Furthermore, reduced expression of  $\beta$ ig-H3 has been observed in many tumor types. Down-regulation of  $\beta$ ig-H3 was found to correlate highly with promoter hypermethylation in lung, prostate, and breast cancer cells. Promoter hypermethylation is considered an important mechanism involved in the silencing of the *TGFBI* gene in human cancer cells [47].

**Table 1.** Studies reporting a tumor suppressor role for  $\beta$ ig-H3.

Cell Type	Observation	References
CHO cells	$\beta$ ig-H3 inhibits cell attachment <i>in vitro</i> and suppresses the growth of CHO tumor cells in nude mice	[48]
	RGD peptides released from $\beta$ ig-H3 mediate apoptosis of CHO tumor cells	[49]
HeLa cells	RGD peptides released from $\beta$ ig-H3 mediate apoptosis of HeLa tumor cells	[49]
Bronchial epithelial cells	$\beta$ ig-H3 overexpression suppresses tumorigenicity in radiation-induced tumorigenic human bronchial epithelial cells	[50]
	Loss of $\beta$ ig-H3 expression is associated with the tumorigenic phenotype in asbestos-treated bronchial epithelial cells	[51]
	$\beta$ ig-H3 gene down-regulation is involved in heavy-ion radiation-induced tumorigenesis of human bronchial epithelial cells	[52]
Lung adenocarcinoma	Loss of $\beta$ ig-H3 protein is frequent in primary lung carcinoma and related to tumorigenic phenotype in lung cancer cells	[53]
	Promoter methylation contributes to promoter silencing of the $\beta$ ig-H3 gene in human lung cancer cells	[47]
	$\beta$ ig-H3 is down-regulated in radiation-induced thymic lymphoma model in BALB/c mice	[5]
	$\beta$ ig-H3 overexpression in H522 lung carcinoma cells reduces motility <i>in vitro</i> and metastasis <i>in vivo</i>	[54]
Mesothelioma cell lines	RGD $\beta$ ig-H3 peptides mediate apoptosis of H1299 lung carcinoma cells	[49]
	$\beta$ ig-H3 knockdown increases proliferation and anchorage independent growth of mesothelioma cell lines	[55]

Table 1. Cont.

Cell Type	Observation	References
Breast carcinoma	$\beta$ ig-H3 protein expression is reduced in <i>in situ</i> ductal carcinoma and breast carcinoma tissues, compared to benign tissues	[54]
	$\beta$ ig-H3 overexpression in MCF-7 cells reduces motility <i>in vitro</i> and metastasis <i>in vivo</i>	[54]
Neuroblastoma	$\beta$ ig-H3 significantly reduces proliferation and invasion of neuroblastoma cell <i>in vitro</i> and <i>in vivo</i>	[2,56]
Osteosarcoma	C-terminal fragment of $\beta$ ig-H3 is required for apoptosis in human osteosarcoma cells	[57]
Hepatoma	RGD $\beta$ ig-H3 peptides mediate apoptosis of Hep3B hepatoma cells	[47]
Knockout mice	$\beta$ ig-H3 knockout mice are prone to spontaneous tumors	[58]
	$\beta$ ig-H3 silencing and promoter hypermethylation is a frequent occurrence in ovarian cancer cell lines and ovarian cancer tissues	[59,60]
Ovarian carcinoma	$\beta$ ig-H3 is down-regulated in serous ovarian carcinoma and borderline serous ovarian tumors	[61]
	$\beta$ ig-H3 induces apoptosis in serous ovarian carcinoma cell lines	[61]

$\beta$ ig-H3 overexpression has been shown to markedly reduce tumorigenicity of CHO cells and lung cancer cells *in vivo* [48,50].  $\beta$ ig-H3 expression is markedly suppressed in asbestos- and radiation-induced tumorigenic cells, whilst ectopic expression of  $\beta$ ig-H3 significantly suppresses tumorigenicity and progression in human bronchial epithelial cells [50–52].  $\beta$ ig-H3 has also been reported to mediate apoptosis through the RGD motif in CHO cells [49] and the EPDIM motif in osteosarcoma cells [57]. A recent observation by Becker *et al.* suggested that increased expression of  $\beta$ ig-H3 suppresses neuroblastoma cell adhesion to various ECM proteins, thus inhibiting their proliferation and invasion [2]. More recent studies demonstrating that the loss of  $\beta$ ig-H3 predisposes mice to spontaneous tumor development have provided strong *in vivo* evidence that  $\beta$ ig-H3 functions as a tumor suppressor [58]. Mouse embryonic fibroblasts isolated from *TGFBI*<sup>-/-</sup> mice displayed increased frequencies of chromosomal aberration, abnormal mitoses, and enhanced proliferation [58]. The loss of chromosomal integrity may explain the increased tumor tendency in the *TGFBI* knockout mice.

Recent studies using lung and breast cancer cell lines have also shown that  $\beta$ ig-H3 induced adhesion to ECM proteins, but reduced the motility and invasive ability of these cells both *in vitro* and *in vivo* [54]. These findings indicate that  $\beta$ ig-H3 can restrain the metastatic potential of cancer cells and thus support the tumor suppressor function of  $\beta$ ig-H3. Stable  $\beta$ ig-H3 knockdown mutants established from a mesothelial cell line, Met-5A, exhibited an elevated proliferation rate, enhanced plating efficiency, increased anchorage-independent growth, and a more active PI3K/AKT/mTOR signaling pathway [55]. These findings suggest that  $\beta$ ig-H3 may repress mesothelioma tumorigenesis and progression by inhibiting the PI3K/AKT signaling pathway.

### 3.2.2. Roles as Tumor Promoter

Although there is strong evidence that  $\beta$ ig-H3 has a tumor suppressor function, there is also convincing data in the literature reporting a tumor-promoting role for  $\beta$ ig-H3 (summarized in Table 2).

High  $\beta$ ig-H3 expression has been shown for various tumor tissues and cell lines [6,62–70] and in many cancers elevated expression also relates to more aggressive tumors [6,70,71]. Furthermore, several reports indicate that  $\beta$ ig-H3 can mediate cancer cell invasion and metastasis as well as enhance cancer cell extravasation [71–74].

$\beta$ ig-H3 has been shown to mediate lymphatic endothelial migration and adhesion to ECM under low oxygen conditions [75]. These observations suggest that during hypoxia, which commonly occurs in tumors,  $\beta$ ig-H3 may aid the metastatic process by promoting the adhesion to lymphatic endothelial cells. More recently  $\beta$ ig-H3 has been shown to be highly expressed by mesenchymal stem cells derived from human adipose tissue and to stimulate proliferation and adhesion of the A459 human lung adenocarcinoma cell line [20]. Furthermore,  $\beta$ ig-H3 observed at the invasion front of melanomas co-localized with fibrillar fibronectin/tenascin-C/periostin structures, suggesting an important role for  $\beta$ ig-H3 in ECM deposition and invasive growth of melanoma cells [76]. siRNAs against  $\beta$ ig-H3 transfected into human hepatocellular carcinoma cells showed that  $\beta$ ig-H3 increases the invasive potential of those cells by regulating MMP-2 and -9 secretion [77]. Thus, due to its tumor promoting role  $\beta$ ig-H3 is a promising therapeutic target.

**Table 2.** Studies reporting a tumor-promoting role for  $\beta$ ig-H3.

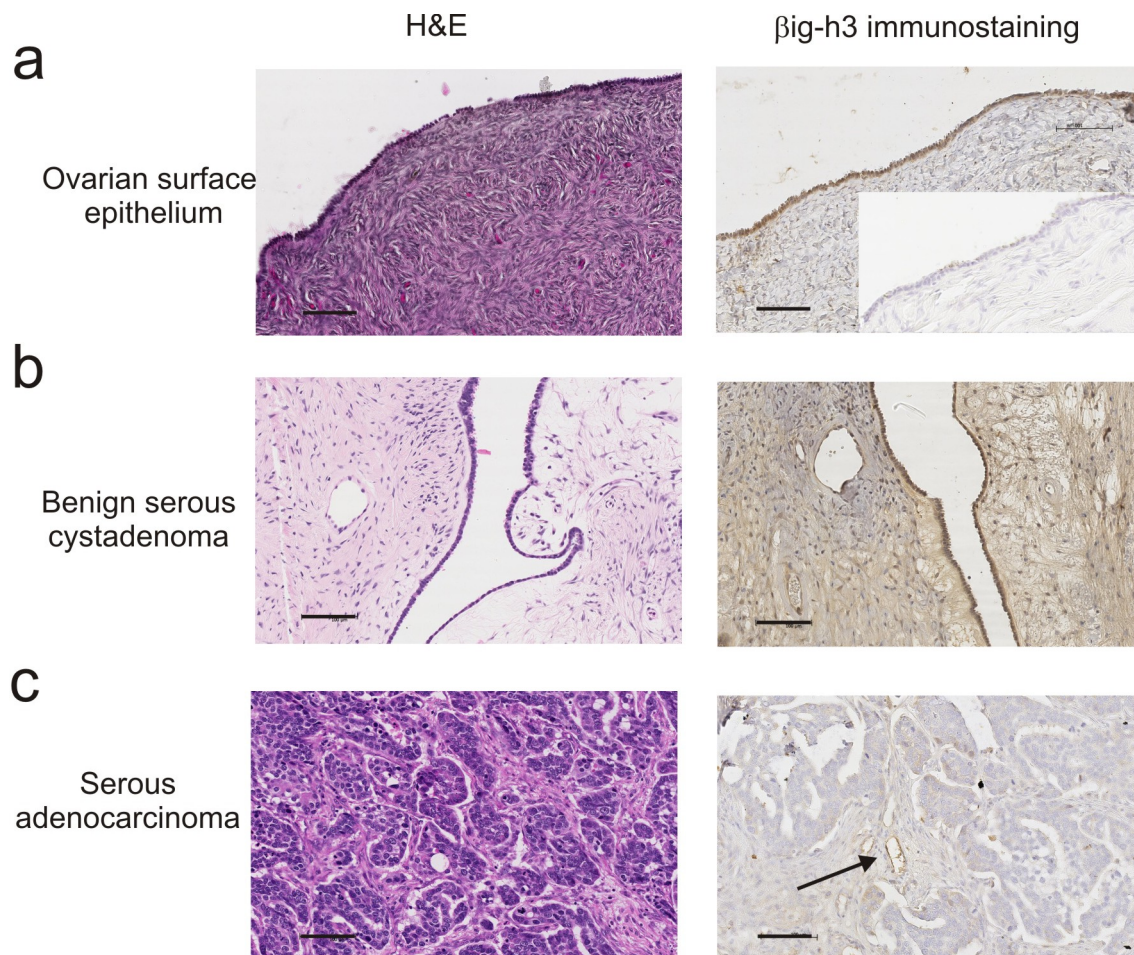
Cell type	Observation	References
Lung adenocarcinoma	$\beta$ ig-H3 is overexpressed in lung cancer	[6]
	Recombinant $\beta$ ig-H3 stimulates proliferation and cell adhesion of A549 cells	[20]
Oesophageal adenocarcinoma	$\beta$ ig-H3 is up-regulated in oesophageal adenocarcinoma and esophageal squamous cell carcinoma tissues and cell lines tissue	[65,78,79]
Pancreatic cancer	$\beta$ ig-H3 expression is increased in pancreatic cancer cell lines and tissues	[68,80]
Oral squamous cell carcinoma	$\beta$ ig-H3 expression is increased in oral squamous cell carcinoma tissues	[81]
Brain tumors	$\beta$ ig-H3 promotes cell adhesion of human astrocytoma cells <i>in vitro</i> via interactions with $\alpha$ 6 $\beta$ 4 integrin	[72]
	$\beta$ ig-H3 expression is elevated in glioblastoma multiforme tissues	[82]
Hepatocellular carcinoma	Knockdown of $\beta$ ig-H3 inhibits glioma cell invasion and MMP secretion	[83]
	$\beta$ ig-H3 knockdown reduced invasion of 7721 cells	[73]
Colon carcinoma	$\beta$ ig-H3 interacts with $\alpha$ 3 $\beta$ 1 integrin to promote adhesion and invasion of 7721 cells	[74]
	$\beta$ ig-H3 expression is elevated in human colon carcinoma tissues	[64,84]
Renal cell carcinoma	Overexpression of $\beta$ ig-H3 promotes extravasation and enhances metastasis of colon cancer cells	[71]
	$\beta$ ig-H3 is up-regulated in clear cell renal carcinoma	[63,64]
Ovarian carcinoma	$\beta$ ig-H3 expression is increased in metastatic renal cell carcinoma	[85]
	$\beta$ ig-H3 suppression leads to a chemoresistant phenotype	[86,87]
	Recombinant $\beta$ ig-H3 promotes motility and invasion of OVCAR-5 and SKOV3 cells	[61]
	Recombinant $\beta$ ig-H3 promotes adhesion of OVCAR-3, OVCAR-5 and SKOV3 cells	[61]

### 3.2.3. Role of $\beta$ ig-H3 in Ovarian Cancer

#### 3.2.3.1. Tumor Suppressive role of $\beta$ ig-H3 in Ovarian Cancer

One of the crucial steps in ovarian cancer metastasis involves the implantation of ovarian cancer cells onto the peritoneal lining. As the underlying molecular mechanisms have not been well characterized we have studied the interaction between ovarian cancer and peritoneal cells *in vitro*. The ECM protein  $\beta$ ig-H3 was found to be differentially regulated in the secretome of peritoneal-ovarian cancer cell co-culture. We demonstrated that  $\beta$ ig-H3 is abundantly expressed by peritoneal cells and can promote ovarian cancer cell motility, invasion, and adhesion to LP-9 peritoneal cells [61].

**Figure 2.** H & E and  $\beta$ ig-H3 immunostaining of ovarian tissues. (a) Normal ovary surface epithelium; (b) Benign serous cystadenoma; (c) Serous ovarian carcinoma. Scale bar = 100  $\mu$ m for all images. Immunostaining with polyclonal rabbit  $\beta$ ig-H3 antibody (Santa Cruz Biotechnology) as described in [61].



Our recent studies investigating the role of  $\beta$ ig-H3 in ovarian tumorigenesis have demonstrated low expression of  $\beta$ ig-H3 in ovarian cancer cell lines and ovarian cancer tissue [61]. This is consistent with other studies demonstrating a down-regulation of  $\beta$ ig-H3 in cancer cells and more recent studies demonstrating that the *TGFBI* gene is frequently hypermethylated in ovarian tumors [59,60]. Our data, showing high levels of  $\beta$ ig-H3 immunostaining in normal ovarian surface epithelial cells (Figure 2a)



and benign serous ovarian tumors (Figure 2b) but low  $\beta$ ig-H3 immunostaining in human serous ovarian cancer cells (Figure 2c), suggest that  $\beta$ ig-H3 is down-regulated during the process of ovarian cancer tumorigenesis [61]. Our findings, that high concentrations ( $>5 \mu\text{g/mL}$ ) of  $\beta$ ig-H3 can induce ovarian cancer cell death, also support an anti-tumorigenic role for  $\beta$ ig-H3 [61]. The use of *TGFBI* methylation as novel epigenetic biomarker for discriminating ovarian cancer from non-cancer or borderline tumors [59] should be further explored.

### 3.2.3.2. Pro-Tumorigenic Role of $\beta$ ig-H3 in Ovarian Cancer

In our recent study we have demonstrated that  $\beta$ ig-H3 induces both motility and invasion of OVCAR-5 and SKOV-3 cells, but does not affect motility or invasion of OVCAR-3 ovarian cancer cells that are known to be less metastatic [61]. We have also shown that  $\beta$ ig-H3 promotes attachment of OVCAR-5, SKOV-3, and OVCAR-3 to LP-9 peritoneal cells [61]. These findings suggest that  $\beta$ ig-H3 may function in multiple ways to promote ovarian cancer metastasis and that the effects on motility may be independent of those on adhesion.

In our study, the effects of  $\beta$ ig-H3 on OVCAR-5 cells were independent of the  $\beta$ ig-H3 RGD integrin binding motif (amino acids 642–644), since treatment with ERGDEL peptide did not block the ability of  $\beta$ ig-H3 to promote ovarian cancer cell motility, invasion, or adhesion to peritoneal cells. Our data suggests that  $\beta$ ig-H3 activity on OVCAR-5 cells is mediated by other sites in the  $\beta$ ig-H3 molecule other than the RGD motif, which may include the EPDIM and NKDIL motifs as well as the sequence spanning the YH18 motif.

### 3.2.3.3. $\beta$ ig-H3 Processing by Ovarian Cancer Peritoneal Interactions

We have shown that  $\beta$ ig-H3 cleavage in the ovarian cancer-peritoneal cell co-culture occurs between amino acid residues 27–76 in the *N*-terminus and amino acid residues 626–657 in the *C*-terminal domain [61]. Although the functional role of the *N*-terminal  $\beta$ ig-H3 domain has not been well studied, the *C*-terminus has several integrin binding motifs including the RGD, YH18, and EPDIM sequences.  $\beta$ ig-H3 fragments including the EPDIM and the RGD motif, have recently been shown to promote apoptosis of osteosarcoma cells [57]. A truncated  $\beta$ ig-H3 lacking the EPDIM but not the RGD motif failed to induce apoptosis in this cell type [57].

Whilst it is not known whether the *C*-terminal processed  $\beta$ ig-H3 in the secretome of the ovarian cancer-peritoneal co-culture retains its RGD sequence at amino acid 642–644, the EPDIM motif at amino acid 617–621 is maintained in the *C*-terminal processed  $\beta$ ig-H3. Crystal structure of the FAS-1 domains (*Drosophila* TGFBI/ $\beta$ ig-H3 homologue) has identified a novel fold domain consisting of a seven-stranded  $\beta$ -wedge and a number of  $\alpha$ -helices in the 3rd and 4th FAS-1 domains [88]. The EPDIM motif maps to a conserved kink in the  $\beta$ 6 strand of the fourth  $\beta$ ig-H3 FAS-1 domain and is predicted to be buried within the domain protein core [88].  $\beta$ ig-H3 processing by proteases, including plasmin between amino acids 626–655 may expose the EPDIM motif (amino acids 617–621) site for integrin interactions and may promote the integrin binding activity on the surface of the peritoneum [89–91] with ovarian cancer cells [92,93] and increase ovarian cancer metastatic behavior.

Interestingly,  $\beta$ ig-H3 processing was only observed when ovarian cancer cells and peritoneal cells were in direct physical contact in culture, or when the cells shared the same growth media in the



co-culture system [61].  $\beta$ ig-H3 processing did not occur when conditioned media from peritoneal cells was added to cultured ovarian cancer cell lines, or when conditioned media from ovarian cancer cells was added to the cultured peritoneal cells. This indicates that  $\beta$ ig-H3 processing is not mediated by a simple up-regulation of ovarian cancer cell derived proteases but requires multiple levels of cross-talk between both ovarian cancer and peritoneal cells. A similar paracrine effect was previously reported for endometrial cancer epithelium–stroma cell co-cultures, where hepatic growth factor secreted by the stromal cells acted on the endometrial cancer cells by inducing the cleavage of MMPs pro-forms to mature active forms [94]. Our findings suggest, however, that cleavage of  $\beta$ ig-H3 in the ovarian cancer and peritoneal cell co-culture is not MMP mediated as the broad spectrum MMP inhibitor, GM6001, failed to inhibit  $\beta$ ig-H3 processing. Instead, we found that the protease plasmin cleaved  $\beta$ ig-H3 in the same region as observed in the ovarian cancer-peritoneal cell co-culture and that this could be inhibited by a cocktail of protease inhibitors, including serine protease inhibitors. We demonstrated that plasmin activity was increased in the conditioned medium of co-cultured OVCAR-5 and LP-9 cells, whilst no plasmin activity could be detected in the conditioned medium collected from those cells cultured alone [61]. These findings add to our understanding of the interaction between ovarian cancer and peritoneal cells and suggest that increased plasmin production and  $\beta$ ig-H3 cleavage may be early events in the process of ovarian cancer metastasis.

#### 3.2.3.4. $\beta$ ig-H3 as a Predictor of Therapy Response

The level of  $\beta$ ig-H3 in ovarian cancer tissue has been shown to be a predictive marker of response to treatment with the aromatase inhibitor letrozole [95] and the chemotherapeutic drug paclitaxel [86]. The loss of  $\beta$ ig-H3 induces a specific resistance to paclitaxel and is associated with mitotic spindle abnormalities in ovarian cancer cells [86]. Paclitaxel-resistant cells treated with recombinant  $\beta$ ig-H3 protein show integrin-dependent restoration of paclitaxel sensitivity via FAK- and Rho-dependent stabilization of microtubules [86]. More recent studies have also shown that the suppression of  $\beta$ 3 integrin and  $\beta$ ig-H3 increase the resistance of SKOV3 to paclitaxel [87]. A strong association between elevated  $\beta$ ig-H3 expression and the response to chemotherapy has also been identified in lung cancer patients [96]. Lung cancer cells over-expressing  $\beta$ ig-H3 displayed increased sensitivity to etoposide, paclitaxel, cisplatin, and gemcitabine.  $\beta$ ig-H3-mediated induction of apoptosis occurred through its binding to  $\alpha$  $\beta$ 3 integrin by proteolytic fragments of  $\beta$ ig-H3 and not full length protein [96]. Together these data show that  $\beta$ ig-H3 is also a potential therapeutic to improve response to chemotherapy in ovarian cancer patients.

## 4. Conclusions

Studies over the last 5 years have increased our understanding of the role of  $\beta$ ig-H3 in cancer. However, there is conflicting data in the literature reporting that  $\beta$ ig-H3 can have a tumor suppressive as well as a tumor promoting role in different cancer cells. These opposing effects of  $\beta$ ig-H3 have been identified in several different laboratories and are unlikely to be due to biased observations.  $\beta$ ig-H3 expression and function in cancer cells appears to be cell type specific and is affected by  $\beta$ ig-H3 concentration but also by processing events by protease enzymes which can liberate integrin binding sites. As truncated forms of  $\beta$ ig-H3 have been well documented to have differing functions it is likely

that alterations in  $\beta$ ig-H3 processing in different cell types is an important factor contributing to the disparate findings in literature. Our findings highlight the need for amino acid sequencing to confirm the presence of full length or truncated forms of  $\beta$ ig-H3 [61]. The findings that siRNA *TGFBI* knockdown increased melanoma cell growth and invasion *in vitro* but greatly impaired subcutaneous tumor growth in nude mice highlights the importance of the tumor microenvironment for  $\beta$ ig-H3 function [76]. Whether  $\beta$ ig-H3 functions as a tumor suppressor or tumor promotor may also be dependent on interactions between other ECM proteins and specific integrin receptors present in the tumor microenvironment.

Our research demonstrating that  $\beta$ ig-H3 is down-regulated in ovarian cancer and promotes ovarian cancer cell death supports a tumor suppressor role. However  $\beta$ ig-H3 is abundantly expressed by peritoneal cells and can promote metastatic behavior of ovarian cancer cells. Consequently, in ovarian cancer,  $\beta$ ig-H3 may act as a “double-edged sword”. The loss of  $\beta$ ig-H3 promotes ovarian tumorigenesis, microtubule and chromosome instability and a more chemoresistant phenotype, however in the peritoneal microenvironment;  $\beta$ ig-H3 produced by the peritoneal cells aids the metastatic process. Our ovarian cancer studies to date indicate that  $\beta$ ig-H3 is a potential therapeutic target to inhibit ovarian cancer metastasis to the peritoneum. Further studies investigating therapeutic strategies to block  $\beta$ ig-H3 action in ovarian cancer are therefore warranted.  $\beta$ ig-H3 derived peptides could be used to both block ovarian cancer metastasis and enhance chemotherapy response.

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