The role of the Src Homology-2 domain containing protein B (SHB) in β cells

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Abstract

This review will describe the SH2-domain signaling protein Src Homology-2 domain containing protein B (SHB) and its role in various physiological processes relating in particular to glucose homeostasis and β cell function. SHB operates downstream of several tyrosine kinase receptors and assembles signaling complexes in response to receptor activation by interacting with other signaling proteins via its other domains (proline-rich, phosphotyrosine-binding and tyrosine-phosphorylation sites). The subsequent responses are context-dependent. Absence of *Shb* in mice has been found to exert effects on hematopoiesis, angiogenesis and glucose metabolism. Specifically, first-phase insulin secretion in response to glucose was impaired and this effect was related to altered characteristics of focal adhesion kinase activation modulating signaling through Akt, ERK, β catenin and cAMP. It is believed that SHB plays a role in integrating adaptive responses to various stimuli by simultaneously modulating cellular responses in different cell-types, thus playing a role in maintaining physiological homeostasis.

Key Words

- ▶ signal transduction
- ▶ insulin secretion
- ▶ vascular
- ▶ islet cells
- ▶ immune system

Journal of Molecular Endocrinology
(2016) 56, R21–R31

Introduction

The limited replicative capacity of human pancreatic β cells contributes significantly to the development of diabetes mellitus and thus comprises a major hurdle for possible treatment and/or prevention of this disease. In early attempts to understand β cell function and particularly β cell replication, cDNA library subtraction-screening procedures were performed to identify genes that were selectively expressed in proliferating β cells. Positive hits were considered as genes relating to β cell proliferation and expansion of the β cell mass. One candidate gene identified by such procedures was the *Reg1* (*REG1A*) gene that was induced in β cells regenerating after partial pancreatectomy (Terazono *et al.* 1990). In a similar attempt to identify genes induced in replicating β cells

(β TC1 cells) after serum-stimulation, the Src Homology-2 domain containing adapter protein B (SHB) was identified (Welsh *et al.* 1994). This review will describe the subsequent progress in our understanding of the role of this protein in various processes, with particular focus on β cell function.

The SHB adapter protein

The *SHB* gene is located on chromosome 9 (nt 38069213–37915898 in coding direction, <http://www.ncbi.nlm.nih.gov/gene/6461> for the human *SHB* gene) and ubiquitously expressed (see <http://www.ebi.ac.uk/gxa/genes/ENSG00000107338> and <http://www.gtexportal.org/>

(Welsh *et al.* 1998). Several tyrosine phosphorylation sites have been described (<http://www.phosphosite.org/proteinAction.do?id=5046&showAllSites=true>), of which five have been indicated (boxed). The four C-terminal tyrosine phosphorylation sites exhibit a high degree of identity between each other, showing a tyrosine (Y) followed by two X-X (of which one is D or E)-P-Y/P/W (aromatic)-D/E. In the C-terminus, there is an SH2 domain preferentially recognizing the target sequence pY-T/I/V-X-L that can also be written in a more general form as pY-hydrophobic-X-hydrophobic-D/E-hydrophobic (Welsh *et al.* 1994, Anneren *et al.* 2003, Liu *et al.* 2012). The domains have different interacting partners and the role of SHB is to generate signaling complexes upon activation. These complexes are context-dependent, i.e., dependent on cell type and conditions the cells are exposed to (growth factors, degree of activation, exposure to stress, etc) and consequently have unique effects on the cells in various settings.

Figure 1
SHB schematic structure and amino acid sequence comparison between human, mouse and chick SHB. Proline-rich motifs, tyrosine-phosphorylation sites, SH2 domain and sequence used to define the PTB domain have been indicated.

The proline-rich sequences in the N-terminus have been shown to interact with signaling proteins such as Src-family kinases, phospholipase C-gamma (PLC- γ), the p85 subunit of phosphatidylinositol 3' kinase (PI3K), epidermal growth factor receptor pathway substrate 8 (EPS8), Janus kinase (JAK)-1/3 and c-Abl (Anneren *et al.* 2003, Hagerkvist *et al.* 2007a). The PTB domain interacts with focal adhesion kinase (FAK), Vav1 and linker for activation T cells (LAT) (Anneren *et al.* 2003). The phosphorylation sites interact with CrkII, SH2-domain-containing leukocyte protein of 76 (SLP76), Ras-GTPase activating protein (RasGAP) and c-Abl (Anneren *et al.* 2003, Hagerkvist *et al.* 2007a). Studies using the individually mutated phosphorylation sites failed to reveal any site-specific interaction (Hagerkvist *et al.* 2007a) and this is a likely consequence of the high degree of sequence identity among four of the phosphorylation sites. Finally, the SH2 domain has been shown to interact with the platelet-derived growth factor receptors (PDGFRA and PDGFRB), fibroblast growth factor receptor-1 (FGFR1), vascular endothelial growth factor receptor-2 (VEGFR2), T cell receptor (TCR), interleukin-2R, EphB2 (ephrin receptor) and c-Met (Lindholm 2002, Anneren *et al.* 2003, Holmqvist *et al.* 2004, Jorgensen *et al.* 2009, Leung *et al.* 2014). SHB becomes tyrosine-phosphorylated upon stimulation of the PDGF receptors, FGFR1, T cell receptor, VEGFR2, c-Src-family kinases, EphB2 and the angiogenesis inhibitor endostatin. SHB also binds the growth hormone receptor (Moutoussamy *et al.* 1998) and the Epstein–Barr virus protein LMP2A (Matskova *et al.* 2007), of which the latter causes SHB tyrosine phosphorylation by Syk and Lyn (Dergai *et al.* 2013), thus participating in viral responses (Arbiser 2015). In addition, SHB associates with the tyrosine phosphatase SHP-2 (PTPN11; protein tyrosine phosphatase, non-receptor 11) via unknown mechanism(s) (Cross *et al.* 2002). Known SHB-interacting partners are listed in Table 1. SHB-dependent signaling has been shown to influence the activities of downstream signaling intermediates such as extracellular-signal-regulated kinase (ERK), PI3K, Rap1, FAK, PLC- γ and Rac1, depending on context (Anneren *et al.* 2003).

SHB has three paralogues: SHD, SHE and SHF (Oda *et al.* 1997, Lindholm *et al.* 2000). They all share sequence similarity in the C-terminus and at the phosphorylation sites. In addition, SHD exhibits limited sequence similarity with SHB in the N-terminus. SHD and SHE were identified as c-Abl interacting partners (Oda *et al.* 1997), whereas SHF was characterized based on sequence homology with SHB (Lindholm *et al.* 2000). Whereas little is known about the functions of SHD and SHE, SHF has been reported to negatively regulate anaplastic lymphoma kinase (ALK)

Table 1 Summary of SHB domain interactions. Identified interactions between different domains of SHB and various signaling proteins are shown. GHR has been tentatively assigned as a partner interacting with the SHB SH2-domain. LMP2A is an Epstein–Barr virus product

Proline rich motifs	PTB domain	Tyrosine phosphorylation sites	SH2 domain
c-Src	FAK	CrkII	PDGFRA
p85 PI3K	LAT	SLP76	PDGFRB
PLC- γ	Vav1	RasGAP	FGFR1
EPS8	LMP2A	c-Abl	TCR
GRB2			VEGFR2
JAK1/3			IL-2R
			c-Met
			EphB2
			GHR
			LMP2A

signaling (Takagi *et al.* 2013) and participate in PDGFRA signaling (Lindholm *et al.* 2000).

SHB in cellular events in non- β cells

Early studies utilized SHB overexpression or expression of a SHB SH2-domain mutant (R522K-SHB) to elucidate SHB's function in cell lines. These revealed pleiotropic effects of SHB such as regulation of apoptosis, differentiation, proliferation and cytoskeletal alterations in various cell types such as fibroblasts, pheochromocytoma PC12 cells, endothelial cells, mouse embryonic stem cells and prostate cancer PC-3 cells (Anneren *et al.* 2003, Davoodpour *et al.* 2007, Funa *et al.* 2008a). The pleiotropicity of these effects is in line with SHB's ability to generate diverse signaling complexes in relation to the cellular context in which SHB is stimulated.

In NIH3T3 fibroblasts, SHB overexpression led to increased apoptosis upon serum deprivation in fibroblasts (Karlsson & Welsh 1996). Serum-withdrawal (Lu *et al.* 2002) or the addition of the angiogenesis inhibitors endostatin or angiostatin (Claesson-Welsh *et al.* 1998, Dixelius *et al.* 2000) to endothelial cells were associated with increased apoptosis upon SHB overexpression, as was exposure to the anti-tumor agent 2-methoxyestradiol in PC-3 cells (Davoodpour *et al.* 2007).

Cell adhesion and the cytoskeletal organization were influenced by SHB in fibroblasts (Hooshmand-Rad *et al.* 2000) and endothelial cells (Funa *et al.* 2008b). In endothelial cells, this resulted in increased cell spreading (Holmqvist *et al.* 2003) and tubular morphogenesis

(Lu *et al.* 2002). SHB overexpression enhanced cell differentiation of PC12 cells (Karlsson *et al.* 1998, Lu *et al.* 2000) and endothelial cells (Lu *et al.* 2002), whereas absence of SHB or expression of the SH2-domain-inactive mutant R522K-SHB impaired various aspects of mouse embryonic stem cell differentiation (Kriz *et al.* 2003, Rolny *et al.* 2005, Kriz *et al.* 2006, Saldeen *et al.* 2006, Funa *et al.* 2008a). SHB expression was required for FGF1-stimulated endothelial cell proliferation (Cross *et al.* 2002).

The *Shb* knockout mouse

The above findings were obtained from studies in various *in vitro* systems. To assess a role of SHB *in vivo*, a global *Shb*-gene knockout mouse was generated. As suspected, this mouse displays various abnormalities in line with SHB's pleiotropic effects *in vitro*. Most notably, there are reproductive disorders in the *Shb* knockout mouse (Kriz *et al.* 2007). The *Shb* knockout mouse is embryonically lethal on the C57Bl6 mouse background, whereas it is viable on a mixed genetic background and in BALB/c mice. Reduced implantation efficacy, increased numbers of resorptions and more malformations (neural tube closure defects, rotation defects, petechiae, etc.) were all noted in the absence of *Shb*. Thus, fetal malformations are common in *Shb* mutant embryos (Kriz *et al.* 2007). In addition, the mutant allele is inherited through a transmission ratio distortion with selective ovulation of oocytes carrying the mutant allele. A more extensive investigation revealed effects of *Shb* deficiency on oocyte maturation, progression of meiosis, ovulated oocyte integrity and early embryo development (Calounova *et al.* 2010). *Shb* knockout oocytes displayed elevated ERK signaling (Calounova *et al.* 2010).

Hematopoietic abnormalities

The *Shb* knockout mouse also displays hematopoietic abnormalities (Gustafsson *et al.* 2013, 2014a; b). These include reduced peripheral blood lymphocytes and monocytes and a Th2 skewing of the T cell receptor response upon activation due to elevated basal T cell receptor signaling in the absence of *Shb* (Gustafsson *et al.* 2011). The consequence of these aberrations was an increased propensity for atopic dermatitis upon stimulation with an allergy-inducing agent (Gustafsson *et al.* 2014b).

The bone marrow of *Shb* knockout mice displayed a reduced cell number and proliferation of hematopoietic stem cells (HSC) (Gustafsson *et al.* 2013). When such *Shb* knockout cells were subject to competitive transplantation

experiments, they initially performed well in repopulating bone-marrow-depleted recipients but eventually lagged in efficacy, causing relatively less contribution to the myeloid lineages than wild type (WT) cells at 16 weeks after transplantation. These cells also exhibited elevated basal FAK/Rac1/PAK signaling in the absence of *Shb*, and the signaling intermediate that appeared mainly responsible for the effects of SHB was FAK, which showed elevated activity in the absence of *Shb* and which upon its inhibition using a chemical inhibitor increased HSC proliferation. Genotoxic stress-induced HSC proliferation was unaffected by the absence of *Shb*.

When *Shb* knockout bone marrow was infected with the Philadelphia chromosome *BCR-ABL1* oncogene and transplanted to WT recipient mice (Gustafsson *et al.* 2014a), the mice developed leukemia more rapidly with shorter latency. Again, this effect was attributed to elevated FAK activity that promoted the proliferation of leukemic cells in the bone marrow. In addition, the *Shb* knockout leukemic bone marrow promoted the differentiation and proliferation of non-transformed bone marrow cells to mature neutrophils.

Vascular phenotype

Absence of *Shb* confers a vascular phenotype as reviewed (Claesson-Welsh & Welsh 2013). The properties of this includes: reduced tumor and Matrigel angiogenesis (Funa *et al.* 2009, Akerblom *et al.* 2012, Christoffersson *et al.* 2012), reduced vascular permeability (Funa *et al.* 2009), an altered vascular architecture (Christoffersson *et al.* 2012), abnormal endothelial and pericyte morphology and ultrastructure (Akerblom *et al.* 2009, Funa *et al.* 2009, Akerblom *et al.* 2012, Christoffersson *et al.* 2012), reduced blood flow upon ischemia (Christoffersson *et al.* 2012, Nikpour *et al.* 2015) and abnormal properties of leukocyte extravasation (Nikpour *et al.* 2015). Isolated *Shb* knockout endothelial cells displayed *in vitro* altered signaling properties with elevated basal FAK, Rac1, Akt, ERK and myosin light chain kinase signaling and reduced ligand (VEGF) responsiveness (Funa *et al.* 2009, Zang *et al.* 2013). Cellular morphology was different explaining some of the structural and ultrastructural aberrations of the *Shb* knockout vasculature (Funa *et al.* 2009, Zang *et al.* 2013).

With respect to both tumor angiogenesis and reproduction, *Shb* $\pm$ mice displayed a phenotype. This indicates that *Shb* gene expression is under tight control in that a small (50% or less) change in expression will have an impact on the response.

SHB and β cells

Recent mapping of the β cell transcriptome indicates that the SHB gene is under significant transcriptional control in β cells (Pasquali *et al.* 2014). Four key β cell transcription factors (PDX1, NKX2.2, FOXA2 and NKX6.1) have binding sites in the SHB gene (Pasquali *et al.* 2014). The first indications of a functional role of SHB in β cells were obtained using a transgenic mouse expressing SHB under the control of the rat insulin promoter in β cells. In those experiments, SHB overexpression increased the β cell mass (Welsh *et al.* 1999). Increased β cell proliferation could not be detected under basal conditions but upon partial pancreatectomy (Anneren 2002). Furthermore, SHB overexpressing β cells exhibited increased susceptibility to addition of the cytotoxic cytokines IL1 β and IFN- γ (Welsh *et al.* 1999). Thus, a dual response exists to SHB overexpression consisting of increased proliferation but also an increased cytotoxic response to cytokine-induced stress. Whereas the increased proliferation may relate to the increased activation of FAK/IRS/PI3K/ERK (as described below), the augmented stress response could relate to SHB's association with c-Abl (see below). Besides increased proliferation, SHB overexpressing β cells have enhanced insulin secretion and the transgenic mouse shows improved glucose tolerance.

SHB overexpression was found to influence the signaling signature of β cells. Increased association of FAK with IRS1 and elevated basal IRS1 and IRS2 activities were observed in insulin-producing cells overexpressing SHB (Welsh *et al.* 2002). These effects translated to basal PI3K and ERK activation with reduced responsiveness to insulin stimulation.

SHB association with c-Abl was found to be required for an appropriate stress response induced by cisplatin (genotoxicity) and tunicamycin (unfolded protein response) in insulin-producing cells (Hagerkvist *et al.* 2007a). Other studies have implicated the importance of c-Abl for β cell survival via knockdown or use of the inhibitor imatinib (Hagerkvist *et al.* 2005, Hagerkvist *et al.* 2007b).

In the *Shb* knockout mouse, elevated basal blood glucose was observed both in mice fed *ad libitum* and in mice subjected to an over-night starvation period (Akerblom *et al.* 2009). Pancreas perfusion experiments showed a reduction of the first-phase response to glucose occurring at 1–3 min after glucose stimulation suggesting that the elevated blood glucose values were related to impaired insulin secretion. In further support for this, insulin sensitivity was found to be unaffected by the

Shb genotype (Akerblom *et al.* 2009). Other β cell phenotypes observed in the *Shb* knockout were decreased microvascular density in islets of Langerhans (Akerblom *et al.* 2009) and diminished β cell death upon exposure to cytotoxic cytokines (IL1 β + IFN- γ) (Mokhtari *et al.* 2009).

Search for an explanation for the reduced first-phase insulin secretion defect by patch-clamp analysis revealed a reduction in the readily releasable pool of granules as a consequence of *Shb* deficiency, which could be a consequence of a diminished rate of insulin granule supply (Akerblom *et al.* 2009).

In further attempts to obtain a more complete mechanistic understanding of the impaired first-phase insulin secretory response, the islet-signaling signature was investigated in more detail. In *Shb* knockout islets, elevated basal FAK, IRS1/2 and Akt activities were observed (Alenkvis *et al.* 2014). Curiously, this pattern mirrors that observed in SHB-overexpressing β cells in which elevated basal FAK, IRS1/2 and Akt activities were also observed in *Shb*-deficient islets (Welsh *et al.* 2002). Increased expression and nuclear translocation of β catenin was also observed (Alenkvis *et al.* 2014). Akt affects β catenin signaling by phosphorylating and inactivating the GSK3 β kinase that in turn regulates the stability of β catenin. Consequently, the increased nuclear content of β catenin is probably a consequence of elevated Akt activity (Katoh and Katoh 2006). In addition, increased Akt activity is a likely explanation for the reduced β cell death upon exposure to cytotoxic cytokines in the absence of *Shb* (Mokhtari *et al.* 2009).

Determining mRNA levels of several key proteins involved in the exocytotic process showed no effects of *Shb* deficiency, suggesting that altered expression of these proteins is an unlikely explanation for the impaired insulin release occurring in the absence of SHB (Alenkvis *et al.* 2014). There were also no detectable effects on ATP generation, the intracellular Ca²⁺-concentration or Ca²⁺-channel activity (Alenkvis *et al.* 2014). However, the early rise of cAMP in response to glucose was delayed and since cAMP is an important messenger in glucose-induced insulin secretion (Tian *et al.* 2015), this effect may explain the reduction of first-phase insulin secretion (Akerblom *et al.* 2009). The delayed cAMP response may be attributed to FAK-regulation of phosphodiesterase activity (Serrels *et al.* 2010), which upon elevated FAK activity promotes the local breakdown of cAMP in the sub-membrane space (Alenkvis *et al.* 2014).

Both integrins and glucose promote increased β cell FAK activity with enhanced survival, proliferation and insulin secretion as a result (Rondas *et al.* 2011,

Cai *et al.* 2012, Rondas *et al.* 2012, Arous *et al.* 2013). The data of the *Shb* knockout islets suggest negative effects of FAK as well, operating under conditions of chronic over-activity. Altered cAMP signaling is one aspect of this. Another possibility is that elevated basal FAK activity promotes an increased basal insulin secretion in the absence of glucose stimulation, as was observed in *Shb* knockout pancreas (Akerblom *et al.* 2009). Such an effect could help explain the observed depletion of the pool of readily releasable granules.

Signaling pathways relevant in β cells, hematopoietic cells and endothelial cells that are modulated by the absence of SHB are summarized in Fig. 2. These include PLC- γ , c-Abl, PI3K, Src-kinase/FAK and Vav1 with consequences for cell survival, proliferation and the cellular cytoskeleton and β cell cAMP-signaling regulating insulin secretion.

SHB and the FAK

The FAK tyrosine kinase is a key element serving a regulatory role at the interface between the extracellular matrix, ligand-stimulated tyrosine kinase/cytokine receptors and intracellular events such as regulation of cell shape, cell motility, the cytoskeleton and signal transduction (Parsons 2003). FAK activity is under

complex control involving numerous elements and/or processes (Tomakidi *et al.* 2014, Lee *et al.* 2015). The SHB/FAK relationship is described above and this interaction will have implications for several downstream signaling systems, such as the Akt, ERK and Rac1 pathways as observed in β cells, endothelial cells and hematopoietic stem cells (Funa *et al.* 2009, Gustafsson *et al.* 2013, Alenkvist *et al.* 2014). Curiously, both SHB overexpression and absence of SHB seem to have similar effects on FAK activity, with increased basal activity and reduced ligand responsiveness. Whereas such effects appear to be plausible consequences of SHB overexpression, it is less apparent why they would also occur in response to SHB deficiency. The response recorded as a consequence of SHB overexpression could result from SHB's ability, via its proline-rich motifs, to interact with Src-family kinases (Karlsson *et al.* 1995). This complex will then more easily be recruited to focal adhesions via SHB's binding through its PTB domain to partially activated FAK, leading to further stimulation of FAK activity (Holmqvist *et al.* 2003). Such chronic over-stimulation will desensitize FAK to further ligand activation.

As noted above, increased FAK activity in the absence of SHB is counterintuitive but could result from several mechanisms. One is that maximal FAK responsiveness requires cycles of stimulation/absence of stimulation and

Figure 2

SHB involvement in cellular signaling of particular relevance for β cells, hematopoietic cells and endothelial cells. SHB may directly interact with PLC- γ , c-Abl, PI3K, Src-family kinases, FAK and Vav1. These interactions will

have pleiotropic effects, including modulating the activities of ERK, Akt, Rac1 and cAMP phosphodiesterase (PDE). Numerous cellular responses will ensue.

that absence of SHB will disrupt this process. Such an oscillatory pattern of FAK activity does indeed exist, with sequential phosphorylation of tyrosine residues present in FAK during activation culminating with the phosphorylation of Y925. When this occurs, GRB2 binds, paxillin displaces from the complex and the focal adhesion complex disintegrates, allowing another cycle to start (Tomakidi *et al.* 2014). If SHB plays an important role in the progression of this cycle, desensitization of FAK activity could take place in the absence of SHB since a block may occur at or subsequent to Y397-FAK phosphorylation, thus disrupting progression of the cycle and normal FAK function. An alternative option could be mis-targeting of intracellular Src-family kinases upon SHB depletion. Alternatively, SHB could interact with a phosphatase that dephosphorylates and inactivates FAK, thus promoting FAK activation. The stimulatory effect of SHB deficiency could additionally involve indirect effects via changes in the expression of any of the numerous components that regulate FAK activity, such as cytokines/growth factors, integrins/extracellular matrix proteins, talin, vinculin, paxillin and FRNK (Tomakidi *et al.* 2014). Finally, SHB may interact with FAK and regulate FAK activity and availability at locations outside focal adhesions and absence of SHB would then facilitate FAK's recruitment to focal adhesions. The latter possibility lends

experimental support from the fact that the SHB protein has not yet been detected as selectively present in focal adhesions (Winograd-Katz *et al.* 2014).

SHB and inflammation

A characteristic feature of SHB based on data obtained from the *Shb* knockout mouse is its involvement in inflammatory processes (Arbiser 2015). An example of this involves effects in T cells causing an exaggerated Th2-response as described above (Gustafsson *et al.* 2011, 2014b). Furthermore, peritoneal recruitment of a specific subset of macrophages was also affected by the absence of *Shb* (Nikpour *et al.* 2015). In addition, B16F10 melanoma tumors exhibited less recruitment of CD8+ cytotoxic T cells when grown on an *Shb*-deficient background (Zang *et al.* 2015). However, studies on the RT2 (RIPTag-2; rat insulin promoter-SV40 large T antigen) insulinoma model has provided further knowledge on SHB-related inflammatory responses. In that tumor model, VEGFA-dependent tumor angiogenesis was reduced by the absence of SHB, causing smaller tumors. However, VEGFA-independent tumor expansion was not affected by *Shb* deficiency (Akerblom *et al.* 2012). In order to elucidate the underlying mechanisms, Affymetrix microarray analysis on WT and *Shb*-deficient tumor RNA

Table 2 Summary of Ingenuity analysis of WT and *Shb* deficient RT2 microarrays. Changes among top pathways, molecular and cellular functions, physiological functions and networks identified by the analysis have been listed

Top canonical pathways	Top molecular and cellular functions	Top physiological systems	Top networks
Leucocyte extravasation signaling (+)	Cellular function and maintenance	Hematological system development and function	Infections disease, humoral immune response, protein synthesis
Granulocyte adhesion and diapedesis (+)	Cellular development	Immune cell trafficking	Inflammatory response, cell-to-cell signaling, cell death and survival
Fcg receptor-mediated phagocytosis in macrophages and monocytes (+)	Cellular growth and proliferation	Tissue development and morphology	Humoral immune response, protein synthesis, cellular development
T-helper-cell differentiation (+)	Cellular movement	Humoral immune response	Cellular movement, hematological system development and function, immune cell trafficking
Natural killer cell signaling	Cell-cell signaling and interaction	Hematopoiesis	Cellular development and growth, hematological system development and function
TCR signaling (+)			
iCOS-iCOSL signaling in T helper cells (+)			
T-cell receptor signaling (+)			
Crosstalk between dendritic cells and NK cells (+)			

samples was performed. Principal component analysis (PCA) of seven WT and seven *Shb* +/– tumor microarrays revealed significant heterogeneity in the gene expression patterns between the different tumors, and, in particular, *Shb* +/– tumors displayed the strongest deviation from the mean. The figure showing the results of the PCA can be found at the website <http://www.mcb.uu.se/res/groups/mw/publ/suppl/>. The finding suggests variation between different tumors in their adaptation to the limitations of the microenvironment and that, in particular, absence of SHB will promote diversification in developing escape mechanisms from insufficient VEGFA-dependent angiogenesis. Significant changes in microarray gene expression were subjected to Ingenuity analysis and the results have been listed in Table 2. The top canonical pathways that were significantly altered include those causing myeloid cell extravasation and phagocytosis, T helper cell differentiation, natural killer cell signaling and inducible T-cell co-stimulator (iCOS) signaling (Table 2). The majority of these responses were increased in the absence of *Shb*. These pathways are predicted to have implications for molecular and cellular functions such as cellular maintenance, development, growth, movement and cell–cell signaling. Consequently, the main physiological systems affected are hematological development and function, immune cell trafficking, tissue development and morphology, humoral immune response and hematopoiesis. These inflammatory alterations are not easily

categorized as the belonging to any single well-defined response but rather in concert imply changes in immune/inflammatory reactivity as a key element affected by the absence of SHB. In summary, the data make a strong case for an inflammatory response occurring in *Shb*-deficient RT2 tumors that substitutes the absence of effective VEGFA-dependent angiogenesis in allowing for tumor expansion. The altered inflammatory profile may include both aberrant properties of the vascular component and the composition of the immune cells that infiltrate the tumors. Tumor-associated macrophages, for instance, have been shown to promote tumor expansion in many studies, thus setting precedent for inflammatory responses rescuing tumor growth when angiogenesis is limiting (Allavena *et al.* 2008). For access to the complete RT2 microarray data, Ingenuity analysis and PCA, see the website (<http://www.mcb.uu.se/res/groups/mw/publ/suppl/>).

SHB and diabetes

It is well established that type 2 diabetes (T2D) exhibits a low-grade chronic inflammatory component (McNelis & Olefsky 2014). This has been shown to promote peripheral insulin resistance in adipose tissue and skeletal muscle. An inflammatory contribution to abnormal β cell function may also exist in T2D (Eguchi & Manabe 2013). The current review suggests that SHB by its regulation of FAK

Figure 3

Schematic diagram showing identified physiological responses affected by *Shb* deficiency. Physiological systems altered are in boxes, below are cell types with aberrant characteristics and, in parenthesis, signaling

components involved possibly explaining the effects listed. EC, endothelial cell; HSC, hematopoietic stem cell.

activity in numerous cell types, such as β cells, endothelial cells and inflammatory hematopoietic cells, will exert an influence on different target organs of relevance to inflammatory homeostasis. Such effects could involve numerous components: an altered signaling signature in β cells with chronically elevated FAK and Akt activities, participation in c-Abl-dependent death signaling upon cytotoxic stress, dysfunctional vasculature in islets of Langerhans and skeletal muscle, disturbed myeloid cell extravasation and skewed T cell responses that cause altered profiles of cytokine production. Although the listed aberrations have been demonstrated in certain systems under specific conditions, it remains unproven whether a systemic low-grade inflammation has any relationship to SHB signaling. This is due to the unfortunate fact that the *Shb* knockout is not viable on the C57Bl6 background and thus no comprehensive data exist on peripheral inflammation of relevance to the metabolic syndrome in the absence of *Shb*, only indications of alterations in vascular/inflammatory cell function, as mentioned above. Searching human-genome-wide association studies (GWAS) for *SHB* gene alterations in relation to the type 2 diabetes parameters (Pruim *et al.* 2010) of fasting glucose levels and HbA1c (<http://locuszoom.sph.umich.edu/locuszoom/>) revealed *SHB* gene single-nucleotide polymorphisms at various positions (rs776023; rs2091415) that correlated with these disease variables at low levels of significance ($0.01 < P < 0.001$). Such low levels of significance do not hold up when multiple comparisons are done, as is the case for GWAS. If indicative of anything, the associations could suggest that *SHB* gene alterations play a role in glucose homeostasis in subpopulations of the disease, or, alternatively, that other genetic markers for the *SHB* gene that hitherto have not been investigated show closer associations with the metabolic variables. More in-depth analysis is required to elucidate these possibilities. An additionally relevant aspect to explore would be the relationship between type 1 diabetes and the *SHB* gene, since the pathogenic mechanisms of that disease involve all or most of the aspects that SHB has been shown to regulate.

Summary and conclusions

SHB is a ubiquitously expressed adapter protein, which participates in diverse signaling pathways, thus affecting signaling efficiency and downstream events. Its expression is under tight control, since a small change in expression has been shown to result in an altered signaling outcome.

Research on SHB has so far focused on its role in β cells/diabetes, reproduction, hematopoiesis, angiogenesis and inflammatory processes as schematically outlined in Fig. 3. Consequently, SHB is implicated as a multifaceted cellular signaling component active in different scenarios, probably by coordinating physiological responses in relevant cell populations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

The work was supported by the Swedish Diabetes Foundation, the Swedish Cancer Foundation and the Family Ernfors Fund. The microarrays were performed by the Uppsala Array Platform, Department of Medical Science, Science for Life Laboratory, Uppsala University, Uppsala.

Acknowledgements

We are grateful to Nils Welsh and Anders Tengholm at the Department of Medical Cell Biology, Uppsala University, for comments.

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Received in final form 15 October 2015

Accepted 20 October 2015

Accepted Preprint published online 20 October 2015