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.

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 (βTC1 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/
home/gene/SHB for recent RNAseq data and http://biogps.org/#goto=genereport&id=6461 for microarray data on SHB gene expression in various human tissues). The human SHB protein has a mass of approximately 55 kDa (509 amino acids) and consists of several identified domains; N-terminal proline-rich motifs followed by a phosphotyrosine-binding (PTB) domain, tyrosine phosphorylation sites and a C-terminal SH2 domain (Anneren et al. 2003). Interspecies SHB sequence identity is very high in the C-terminus (Fig. 1), whereas it is much lower in the N-terminal half except for certain segments, including one of the proline-rich motifs (boxed sequences) and parts of the sequences that potentially comprise the PTB domain. The PTB domain has not been mapped in detail but is contained within a region starting at amino acid 77 and ending at amino acid 389 (human SHB sequence, blue line with arrow over the sequences in figure) and preferentially recognizes the sequence D-D-X-pY on interacting partners (Welsh et al. 1998). Several tyrosine phosphorylation sites have been described (http://www.phosphosite.org/protein-Action.do?id=50468&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-γ (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 Crkl, 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 (PDGFRα and PDGFRβ), 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 (Matksova 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) signaling (Takagi et al. 2013) and participate in PDGFRα 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 pleiotropy 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.
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 RS22K-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).

Vascular phenotype


With respect to both tumor angiogenesis and reproduction, Shb−/+ 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 IL1β 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 (IL1β + 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 (Alenkvist 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 (Alenkvist et al. 2014). Akt affects β catenin signaling by phosphorylating and inactivating the GSK3β 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 (Alenkvist et al. 2014). There were also no detectable effects on ATP generation, the intracellular Ca\(^{2+}\)-concentration or Ca\(^{2+}\)-channel activity (Alenkvist 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 (Alenkvist 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 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

<table>
<thead>
<tr>
<th>Top canonical pathways</th>
<th>Top molecular and cellular functions</th>
<th>Top physiological systems</th>
<th>Top networks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocyte extravasation signaling (+)</td>
<td>Cellular function and maintenance</td>
<td>Hematological system development and function</td>
<td>Infections disease, humoral immune response, protein synthesis</td>
</tr>
<tr>
<td>Granulocyte adhesion and diapedesis (+)</td>
<td>Cellular development</td>
<td>Immune cell trafficking</td>
<td>Inflammatory response, cell-to-cell signaling, cell death and survival</td>
</tr>
<tr>
<td>Fcg receptor-mediated phagocytosis in macrophages and monocytes (+)</td>
<td>Cellular growth and proliferation</td>
<td>Tissue development and morphology</td>
<td>Humoral immune response, protein synthesis, cellular development</td>
</tr>
<tr>
<td>T-helper-cell differentiation (+)</td>
<td>Cellular movement</td>
<td>Humoral immune response</td>
<td>Cellular movement, hematological system development and function, immune cell trafficking</td>
</tr>
<tr>
<td>Natural killer cell signaling</td>
<td>Cell–cell signaling and interaction</td>
<td>Hematopoiesis</td>
<td>Cellular development and growth, hematological system development and function</td>
</tr>
<tr>
<td>TCR signaling (+)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>iCOS-iCOSL signaling in T helper cells (+)</td>
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<tr>
<td>T-cell receptor signaling (+)</td>
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<tr>
<td>Crosstalk between dendritic cells and NK cells (+)</td>
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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 components involved possibly explaining the effects listed. EC, endothelial cell; HSC, hematopoietic stem cell.

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**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 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.

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