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RESEARCH ARTICLE

Multifaceted roles of ASB proteins and its pathological significance

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BACKGROUND: Post-translational (PT) modification in cells regulates many intracellular events like signal transduction, transcription, cell cycle, protein quality control, apoptosis and cellular development. Ubiquitination is one of the PT modifications which functions as a marker for degradation of target proteins by the proteasome and as a regulatory mechanism for several signalling pathways. The ubiquitination mechanism requires multiple enzymes, including E1, E2, and E3 ligases. Among them, E3 ligases play a major role in recognizing target proteins and an essential feature of protein homeostatic mechanisms within the cell. Most of the ASB (ankyrin repeat SOCS box) proteins function as RING family of E3 ubiquitin ligases characterized by the presence of two conserved domains N-terminal ankyrin repeat and C-terminal SOCS box domain
METHODS and RESULTS: Current studies have shown that some ASBs function as important regulators of several signalling pathways. This review gives an overview of ASB proteins on numerous cellular processes such as insulin signalling, spermatogenesis, myogenesis and in cellular development. Including various pathological situations, such as cancer, primary open-angle glaucoma, and inflammation, indicating that ASBs has important functions in both normal and pathological development
CONCLUSIONS: This article provides a precise comprehensive focus on ASBs protein structure, its biological functions, and their pathological significance.

Keywords ankyrin repeat, SOCS box, E3 ligase, cancer, spermatogenesis, cellular development

Introduction

ASB genes were discovered as expressed sequence tags (EST) by exploring several DNA databases with SOCS box as consensus sequence (Hilton et al., 1998). ASB gene family refers to the ankyrin repeat and SOCS box protein gene family. There are 18 members of this family which are composed of two motifs first being variable numbers of ankyrin repeats and the second being the SOCS box protein. These combinations result in 18 different biologically active proteins. The ankyrin repeat is a 33-residue motif in proteins consisting of two alpha helices separated by loops and are involved in numerous processes such as cell cycle regulation (Lux et al., 1990), transcription initiation (Pahl,1999), ion transportation, signal transduction and cytoskeletal integrity (Tee and Peppelenbosch, 2010). SOCS stands for suppressor of cytokine signaling proteins a negative regulator of JAK-STAT signaling. Table 1 summarizes the proposed function (where known), protein length and E3 ligase activity of ASB family members and also shows their gene location and potential substrates identified till now.

Ubiquitination and ASB gene family: an overview

One of the most abundant proteins in eukaryotic tissues is a small 8.5 kDa regulatory protein called ubiquitin. The process of regulation occurs through substrate binding and is called ubiquitination. Ubiquitination can mark protein targets for degradation via proteasome or alter cellular localization and also impact on activity by preventing/promoting protein interaction and for some targets of degradation members of ASB gene family members play an enabling role. Ubiquitination is an essential mechanism that regulates numerous

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Table 1 Tabular column of chromosomal location and properties of human ASB genes with their respective substrates identified, cancers involved from NCBI (https://www.ncbi.nlm.nih.gov/pubmed/) and Entrez (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = gene) databases.

Gene	No of ankyrin repeats	Amino acids length	Cellular function	Substrates identified	E3 ligase activity	Human gene locus	Splice variants	Cancers involved
ASB1	6	335	Spermato genesis	-	ND	2q37	1	Nasopharyngeal carcinoma
ASB2	12	587	Muscle differentiation	FLNA-B, JAK	YES	14q31-q32	2	Acute promyelo-cytic cancer
ASB3	11	518	Degradation of TNFR2	TNFR2	YES	2p16-p14	2	
ASB4	6	426	Vascular differentiation, Insulin signaling	GPS1,IRS4, ID2, HIF1α	YES	7q21-q22	2	Hepatocellular carcinoma
ASB5	6	329	Arteriogenesis	-	ND	4q34.2	1	
ASB6	6	421	Insulin signaling	APS	YES	9q34.13	3	Oral carcinoma
ASB7	7	318	Chromosomal stability	DDA3	YES	15q26.31	2	
ASB8	4	288	Spermato genesis	-	YES	12q13.11	1	Lung carcinoma
ASB9	6	294	Inhibition of cell growth	CKB	YES	**Xp21.3**	3	Breast and color-ectal carcinoma
ASB10	7	467	Open-angle glaucoma	-	YES	7q36.1	3	
ASB11	6	323	Neurogenesis & notch signaling	Ribophorin1, Delta A	YES	**Xp.22.31**	3	
ASB12	**5**	**309**	**Not studied**	**-**	**ND**	**Xq.11.2**	**1**	
ASB13	**6**	**278**	**Not studied**	**-**	**ND**	**10p15.1**	**1**	Non-Hodgkin's lymphomas
ASB14	**11**	**587**	**Not studied**	**-**	**ND**	**3p21.1**	**2**	
ASB15	9	588	Myogenesis	-	YES	7q31.31	1	
ASB16	**7**	**453**	**Not studied**	**-**	**ND**	**17q21.31**	**1**	
ASB17	1	295	Spermatogenesis	-	ND	1p31.1	1	
ASB18	**6**	**466**	**Not studied**	**-**	**ND**	**2q37.2**	**1**	

Highlighted genes function is still unexplored. ND-Not defined.

cellular processes in eukaryotes (Groothuis et al., 2006) makes this interaction with ASB significant for study.

ASB genes constitute a conserved chordate-unique gene family, which is defined as the substrate-recognition subunits of elongin-cullin-SOCS (ECS) complexes that specifically transfer ubiquitin to cellular proteins targeting them for degradation by the proteasome (Kohroki et al., 2005, Okumura et al., 2012). The ECS complex is known for its active role in the degradation of hypoxia-inducible factor HIF-1, involved in oxygen homeostasis (Maxwell et al., 1999). Further, it displays a striking overall similarity to the SCF complex (Kile et al., 2002). The ASB subclass of humans consists of 18 members as ASB1-18.

The primary mechanism of ASB protein functions via recruitment of the target protein in the SOCS box domain using the ankyrin repeats (ANK) and further processing them for ubiquitination. Majorly all SOCS-box containing proteins are involved in ubiquitination of target proteins for proteasomal-mediated degradation (Linossi and Nicholson, 2012). All representatives of the ASB family have two functional domains (shown in Fig. 1): an ANK repeat region at its N-terminal which is involved in specific protein–protein interactions (PPI), and an SOCS box domain at its carboxyl-terminal, which functions as an adapter providing a link for degradation of targeted proteins (Hilton et al., 1998).

Targeted degradation of proteins controls various cellular processes and variations (mono or polyubiquitination) in the ubiquitin pathway linked with many genetic disorders, neurodegenerative diseases and cancers (Crosetto et al., 2006; Morris et al., 2010). Research has been carried out on the function and mode of action of some of the ASBs in pathological conditions such as cancer (Au et al., 2014). Current research suggests the role of ASB genes associated with the regulation of both normal processes and diseased conditions.

Expression of ASB genes and their Molecular functions

Most of the members of ASB proteins are ubiquitously expressed in mammalian tissues. The tissue distribution of

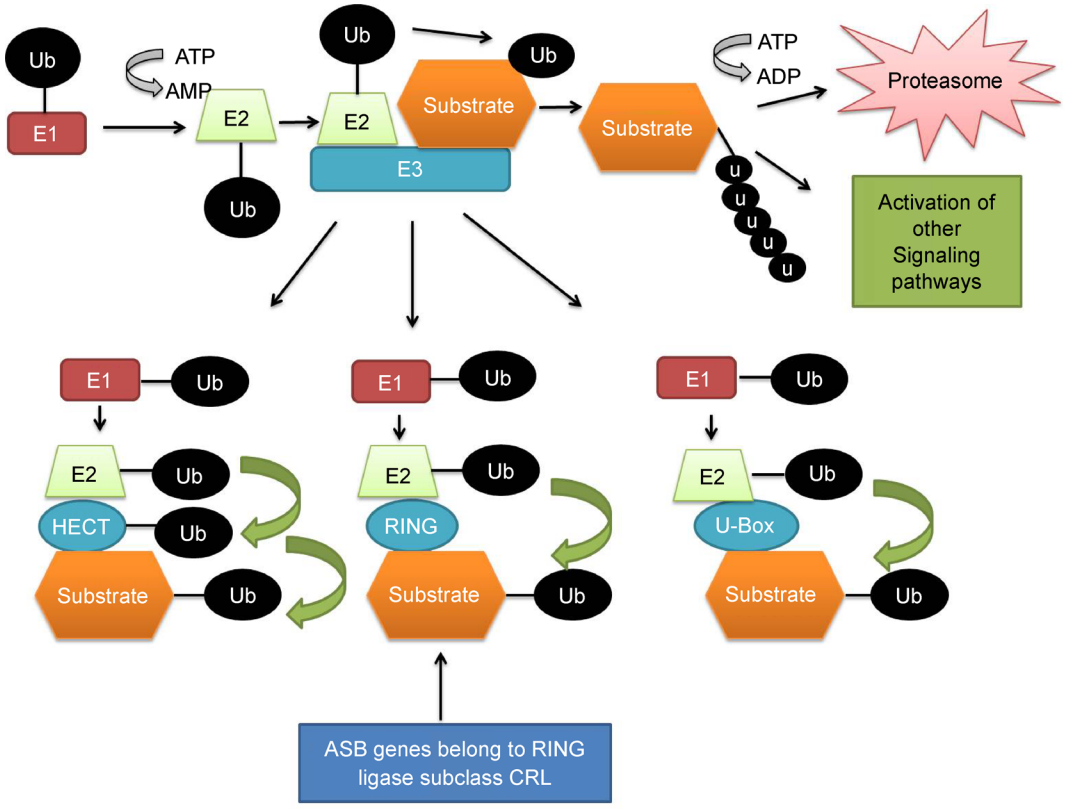

Figure 1 Ubiquitination process and types of E3s. Ubiquitination is an ATP dependent cellular process. Ubiquitin is activated by E1, transferred via transthiolation from E1 to E2. Further, E2~Ub interacts with an E3.E3s then facilitate ubiquitination of the substrate protein by proteasome formation or activation of other signaling Pathways. Multiple ubiquitination of substrate is required for recognition by the 26S proteasome. This may require the involvement of additional E2s and E3. There are 3 types of E3s HECT, RING and U-box. HECT E3s interacts with E2~Ub and transfers ubiquitin directly to the Substrate. In case of RING E3s do not directly catalyse the ubiquitination of the target protein and require the presence of the E2 and often additional components (e.g. in the case of SCF Complexes) for ubiquitination to proceed. U-box E3s have a similar mode of action to RING E3s.

mRNAs and proteins that are encoded by some ASB genes is extremely heterogeneous and tissue-specific (Table. 2). The sub-cellular localization of them varies from the plasma membrane, endoplasmic reticulum, Golgi, nucleus to focal adhesions. Nearly half the genes are ubiquitously expressed in adult tissues whereas the expression patterns of the other genes can vary from a single tissue to multiple organs. ASB17 is the only gene that is known to have restricted expression and is only expressed in the testis. Some ASB genes have differentially spliced isoforms, which can exhibit different subcellular locations PPI.

ASB6, 9, 10, 11 have three isoforms that localize to either the nucleus, Golgi, endoplasmic reticulum or the vesicles and this differential localization may be responsible for achieving different functions. Individual isoforms are also variously susceptible to other post-translational modifications, including SUMOylation, phosphorylation, and ubiquitination, which can markedly affect protein expression levels and its subcellular localization (Von Heijne et al., 1998).

ASBs have a common domain architecture characterized by a conserved SOCS box domain that imparts E3 ligase activity to ASB family proteins while a variable number of ANK repeats at its N-terminal region can mediate recognition of substrate proteins (Fig. 2). Currently available structural information on these proteins is still largely restricted to sub-domains and its ECS complex structure. Presently, X-ray crystal structure of truncated ASB9-2 isoform (PDB ID:3D9H) which lacks SOCS box, E3 ligase activity, ternary ASB9–Elongin B/C complex (PDB ID:3ZKJ) and ANK repeat domain of ASB11(PDB ID:4UUC) has been identified (Fei et al., 2012; Thomas et al., 2013). ASB complexes are involved in numerous cellular processes and pathways; however, their interactions, assembly, and biological roles remain poorly understood. Recently, crystallographic and biophysical studies conducted with one component of the ASB subclass described how ASB E3 ligase complexes function and assemble in a similar manner to that of other cullin-ring ligases (CRL) systems which provides a stage for a further molecular study on ASB family (Thomas et al., 2013).

Some ASB proteins have the ability to form self homo-oligomers and to form hetero-oligomers with other members of ASB family. Various ASBs are described and predicted in

Table 2 Expression pattern and subcellular localization of ASB genes from human protein atlas database (https://www.proteinatlas.org/) and NCBI (https://www.ncbi.nlm.nih.gov/)

Gene	Tissue expression	Sub-Cellular location (Transmembrane domain)	ASB proteins as binding partners
ASB1	Expressed in all tissues	Nucleoplasm	ASB5, ASB4, ASB6,ASB12
ASB2	Expressed in all, skeletal tissues	ND	ASB2
ASB3	Expressed in all tissues	ND	ASB6
ASB4	Adrenal glands and skeletal tissues	ND	ASB7
ASB5	Skeletal tissues	Plasma membrane (1)	ASB1
ASB6	Expressed in all tissues	ND	ASB15,ASB11,
ASB7	Expressed in all tissues	Golgi, Vesicles, Nucleus	ASB4,ASB6,ASB12
ASB8	Expressed in all tissues	Cytoplasm	-
ASB9	Testis, Pancreas and kidney	Nucleus, Cytosol	-
ASB10	Skeletal and heart muscles	Nucleus, cytoplasm	-
ASB11	Skeletal and heart muscles	Endoplasmic reticulum (1)	ASB6,ASB17
ASB12	Skeletal Tissues	ND	-
ASB13	Expressed in all tissues	Nucleus, Golgi	-
ASB14	Heart and skeletal muscles	ND	-
ASB15	Heart and skeletal muscles	ND	ASB6
ASB16	Skeletal muscles and Cerebellum	Focal adhesion sites	-
ASB17	Testis	ND	ASB1,ASB9,ASB11
ASB18	Heart muscles	ND	-

Predicted and curated interactions among ASB-ASB proteins from various protein interaction databases as IntAct, HIT predict and STRING. ND: Not defined

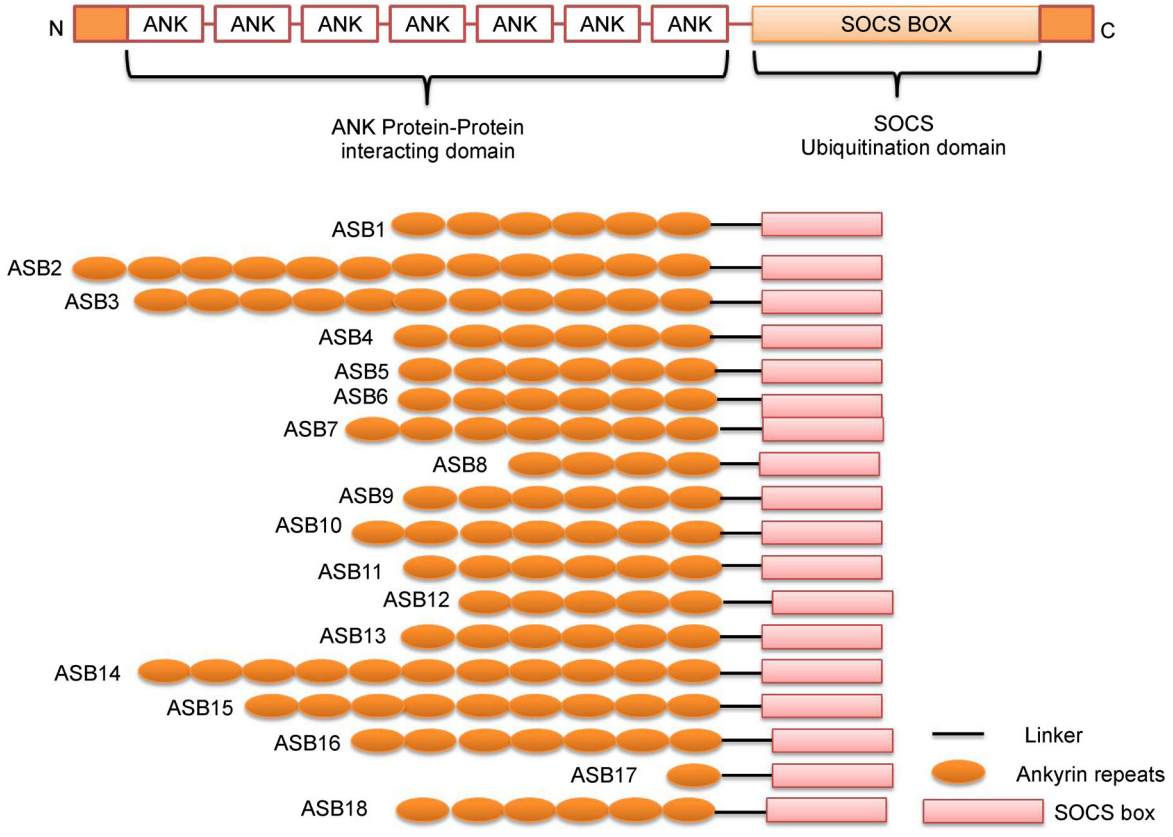

Figure 2 Modular protein structure of ASB and it family members.

Intact (http://www.ebi.ac.uk/intact/) HIT (http://hintdb.hgc.jp/htp/) and STRING (http://string-db.org/) databases (Table 2). Currently, ASB family dimers of ASB3/ASB6 and ASB4/ASB7 proteins have been reported, with no conclusive information for target induced dimerization (Andresen et al., 2014). The implication that despite ASB proteins binding to the same target proteins their affinities will be different and both of them thus serve more as a scaffolding protein instead of direct catalytic function. To further understand and contextualize the information structural studies are essential for describing the precise spatial arrangement of domains and their known or proposed functions. It is crucial to gain insight to understand how ASB genes a dual domain protein operates in the cellular context, and how its dysfunction leads to disease. Further research can shed light on complex interactions among ASB proteins and provide insight required to design new drugs that exploit the pathway. The distinct function of each of the domains present will be described in detail below.

N-terminal ankyrin repeat domain

ANK repeats are common structural domains present at the N-terminal of most proteins (Mosavi et al., 2002, Mosavi et al., 2004). Recently proteins with repeating amino acid sequences have been considered due to their unique protein interactions. They are found in all three kingdoms, including bacteria, archaea, eukaryotes and in few viral genomes (Bork, 1993). They were first discovered in yeast cell cycle as a signaling protein named yeast cell cycle regulator Cdc10/Swi6 having around 24 ANK repeats (Foord et al., 1999). These proteins in consist of 4 to 6 copies of repeated stacks onto each other to make an elongated structure with a continuous hydrophobic core and a significant solvent-accessible surface. Each repeat is made up of 30–34 amino acid residues consisting of two antiparallel α-helices and a long loop ending in a β-hairpin forming a scaffold for specific, high-affinity molecular interactions.

ANK repeats are recognized by their structure rather than function as they are universally recognized and thus do not have a specific sequence either. Among the repeat motifs in humans, ankyrin repeat, leucine-rich repeat, armadillo repeat and tetratricopeptide repeats are the most common (Marcotte et al., 1999).

ANK repeats are flexible and do not recognize any particular amino acid sequence or structure with respect to other PPI domains such as SH2 or SH3. As an alternative depending on the number of repeats they form an elongated surface of varying size. ANK repeat motifs are better described by its tertiary structure than by a specific function, because of the pronounced sequence variation in the individual repeats and copy number across various protein families. The structure of repeated domains is stabilized by local and short-range interactions like globular proteins which shows a new way of understanding the mechanism of protein stability and protein folding (Li et al., 2006). Besides this fact, some proteins with destabilizing mutations in their repeat domains are implicated in several human diseases such as cell cycle (inhibitor p16), which is connected with cancer, notch protein involved in neurological disorders, etc. (Johnson and Walker, 1999).

ANK repeat domain majorly serves as a PPI module to recruit the substrates. The number of ANK repeats varies widely from one to many in a single protein. Protein with the most number of ANK repeats till now is ORF EAA39756 from *Giardia lamblia*, which actually has around 34 repeats from the PFAM and SMART databases (collections of protein family domains and structures). In ASB members, variable no of ANK repeats and other unexplored novel regions are found within the protein, suggesting that they can bind to different target proteins. ASB2 is the longest protein with 12 repeats and ASB17 is the smallest with only one repeat among the family (shown in Fig. 1). The structure and number of ANK repeat influence the interaction of ASB with their respective substrates (Mosavi et al., 2002). Any mutations in the ANK repeats would not let the substrate bound to the repeats ubiquitinate properly and further entry into the degradation pathways would be limited. For example, among ASB family, tumor necrosis factor-alpha receptor-2 (TNF-α, RII) interacts with the ANK repeats of ASB3, while creatine kinase B (CKB) binds to ASB9 ANK repeats (Chung et al., 2005, Debrincat et al., 2007). ASB11 and ribophorin 1 interaction appear to be dependent on the fourth of six ANK repeats (Andresen et al., 2014).

C-terminal SOCS Box domain and ECS complex

SOCS box is protein domains containing about 40-60 (amino acids) region of homology in the members of suppressors of cytokine signaling family (Kile et al., 2002). They are involved in the negative regulation of cytokine signal transduction, majorly involved in the regulation of JAK-STAT signaling pathway (Croker et al., 2008). In addition, to the canonical SOCS (SOCS 1-7) proteins, many SOCS domains are identified in more than 40 proteins in nine different families.

Rather than containing an SH2 domain upstream of the SOCS box, these proteins contain other domains implicated in PPI. The major class of proteins classified are based on the conserved C-terminal SOCS box and N-terminal structural domain into five distinct protein families: (1) those that contain SH2 domains (SOCS proteins), (2) Ankyrin repeats (ASB proteins), (3) SPRY domains (SSB proteins), (4) GTPase domains (RAR family), and (5) those that contain WD-40 repeats (WSB proteins) (Hilton, 1999). Several other proteins with regular or divergent SOCS boxes are also found such as von Hippel-Lindau (VHL) tumor suppressor protein, MUF-1 and elongin A (Okumura et al., 2012). The SH2 SOCS box is the best-characterized protein. Though the functional role of SOCS box is not clearly elucidated yet, it is

expected to play a broadly similar critical role as indicated by the increased level of conservation between species.

The SOCS box is a flexible motif that folds stably with a wide range of E3 substrate-recognition domains. Among ASBs, ASB1, ASB2, ASB3, ASB4, ASB6, ASB7, ASB8, ASB9, ASB11, and ASB12 have been shown to directly interact with either of the components of the ECS complex through their SOCS box domain (Kohroki et al., 2005). In general, SOCS family members use the SH2 domain to recruit substrates with phospho-tyrosine motifs, but ASB family members are expected to use the ANK repeats to recruit substrates as they lack the SH2 domain. An H1–H2 loop insertion aids in the ANK domain interaction that forms the primary adaptation of the ASB family (Thomas et al., 2013). This positions the crescent shape of the ANK repeat domain roughly coaxial to the long axis of the Elongin B/C–SOCS box complex (Linossi and Nicholson, 2012). A 40-residue motif, in SOCS box, determines PPI with four distinct domains, including the ANK domain, Cul5, Elongin B and Elongin C. Elongin BC and Cul-box are sub-domains common to both SOCS box and the VHLα domain that facilitates binding to the Elongin BC complex for substrate identification (Kamura et al., 1998). Elongin C is bound to the BC box which links SOCS box protein to the Cullin RBX (Ring Box protein) module. ASB proteins were reported to bind to scaffold proteins Cullin and RING (Really Interesting New Gene) finger protein RBX through its SOCS box domain. ASBs form complex with Cul5-Rbx2 but neither Cul2 nor Rbx1 complex in ASB1, ASB2, ASB6, ASB7 and ASB12 in HEK293 cells (Kohroki et al., 2005). BC box located downstream of the SOCS box determines whether the given SOCS box protein binds to Cul2-Rbx1 or a Cul5-Rbx2 module. Almost all the ASBs share the consensus sequence of a Cul5 box but not the Cul2 box. Junya et al.(2005) described SOCS boxes of ASB proteins have amino acid sequences of **LC** in its BC box and **LPLP** in its Cul5 box which is needed for its interaction with Cul and Rbx (Kohroki et al., 2005).

But, few ASBs contain sequences different from LC and LPLP. Even though there were slight sequence divergences in the ASB family ASB1, ASB6, ASB7 and ASB12 showed interaction with endogenously expressed Cul5 and Rbx2, and none showed interaction with Cul2 or Rbx1. This suggests Cul5 and Rbx2 interact with ASBs despite slight divergences from the consensus sequences in the BC box and Cul5 box of SOCS box proteins. In most cases, SOCS domain constitutes the catalytic center of ASBs. Some ASB E3 ligases have been shown to require SOCS box as a prerequisite for ubiquitin ligase activity. For example, overexpression of ASB-4 decreased IRS4 protein levels, and deletion of the SOCS box abolished this effect (Li et al., 2011).

Biological and pathological functions of ASB genes

ASB proteins contain properties which contribute to a wide range of cellular process from normal physiology to pathological conditions mentioned below (Fig. 3).

Role in Insulin signaling

Insulin is a key hormone controlling critical energy functions,

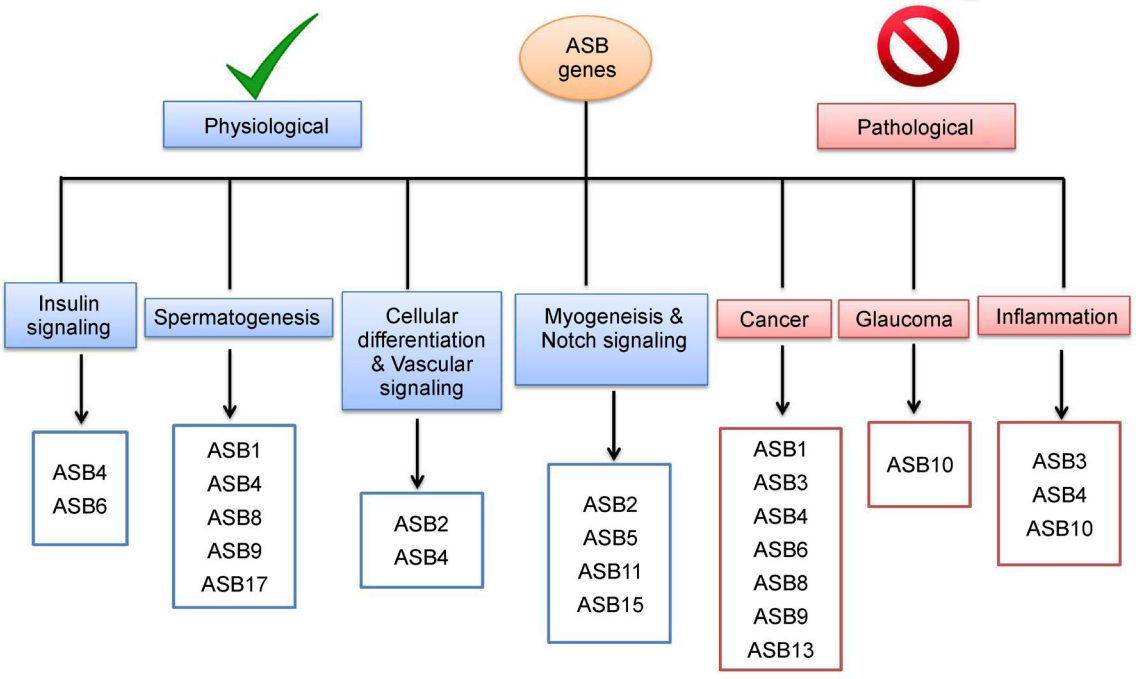

Figure 3 Physiological and pathological significance of ASB proteins.

such as glucose and lipid metabolism (Plum et al., 2006) by activation of insulin receptor tyrosine kinases (IR), which phosphorylate and recruit different substrate adaptors of IRS protein family.Insulin regulates cellular responses such as regulation of blood sugar levels by increased glucose uptake in muscle and fats; increased storage of lipids, energy reserves in fat, liver, and muscle through stimulation of lipogenesis, glycogen synthesis and protein synthesis (Wilcox, 2005). Tyrosine phosphorylated IRS then exhibits binding sites for several signaling partners. Studies show that specific ASB proteins can serve as critical regulators of insulin signaling by binding these receptors. ASB4 was first reported to co-localize and interact with insulin receptor substrate 4 (IRS4) in hypothalamic neurons (Li et al., 2011). IRS4 is an adaptor molecule involved in the signal transduction for both insulin and leptin (Wauman et al., 2008). The interaction of ASB4 with IRS4 mediated the degradation of IRS4 which decreased insulin signaling. JNK is reported as a negative feedback regulator of insulin action. This study illustrates ASB4 inhibits JNK activity by binding with GPS1, providing an additional mechanism through which insulin responses be regulated.

ASB6 is an adipocyte-specific protein reported to regulate components of the insulin signaling pathway in adipocytes (Wilcox et al., 2004). APS (adapter protein with pleckstrin homology and Src homology 2 domains) adapter protein recruits ASB6 which forms a complex with the insulin receptor signaling. Along with APS, recruitment of the Elongin BC complex by ASB6 is essential for the full activation of the insulin receptor. Previous studies described that APS plays a positive role in insulin signaling, but prolonged insulin production resulted in degradation of APS. Hence recruitment of ASB6 and Elongin with APS suggest its negative role in signal transduction of insulin signaling and its link with diabetes. Indicating that ASB4 and ASB6 are important targets in regards therapeutic benefit in diabetes treatments.

Role in cellular differentiation and vascular development

Screening of specific ASB genes expressed in differentiation induced by all-*trans* retinoic acid (ATRA) of human leukemia (HL-60) cells reveals **ASB-2** to be rapidly induced. ASB2 has 2 functional splice variants ASB2α and β in which ASB2β has an ubiquitin binding motif (UIM) in the N-terminal region, but not ASB2α. ASB2α is involved in differentiation of HL-60 cells while ASB2β in myogenic differentiation of C2C12 and primary myoblasts (Bello et al., 2009). ASB2 is found to have typical retinoid receptors (RARs) or retinoid X receptors (RXRs) binding element (RARE/RXRE) in its promoter region (Guibal et al., 2002). These findings show ASB-2 is induced by ATRA and may act as an important regulator, of physiologic processes as cellular differentiation. ASB2 also contributed to hematopoietic differentiation (Hematopoiesis), through MLL(Mixed lineage leukemia 1) degradation and HOX (home box) gene downregulation (Wang et al., 2012). MLL is an essential epigenetic regulator of normal hematopoietic development. Chromosomal translocations involving MLL are one of the most frequent genetic alterations in human leukemia (Ziemin-van der Poel et al., 1991). ASB2α is also associated with the recruitment of one of its substrates, FLNa. By inducing FLNa degradation via proteasome, ASB2α regulates integrin-dependent functions and controls hematopoietic stem cell fate within the niche (Lamsoul et al., 2011).

Vascularization of the placenta is a critical developmental process that ensures fetal viability. Among ASB members apart from their involvement in ubiquitous protein degradation, few are implicated in the vascular development. ASB4 was highly expressed in vascular lineage during early differentiation of endothelial cells. Due to higher levels of ASB4 in the embryonic vasculature, a drastic increase in oxygen tension and placental blood flow takes place, which is likely subjected to be regulated under oxygen tension. However, as vessels mature and oxygen levels stabilize, ASB4 levels downregulate, suggesting ASB4 may function to modulate an endothelium-specific response to increased oxygen tension. ASB4 interacts with factor inhibiting HIF1α (FIH) and acts as a substrate for FIH-mediated hydroxylation via an oxygen-dependent mechanism (Ferguson et al., 2007). Indicating that ASB4 promotes angiogenesis in response to change in oxygen levels. Overexpression of ASB4 in ES cells promoted their differentiation into vascular lineage in an oxygen-dependent manner (Ferguson 3rd and Wu, 2007). Other reports demonstrate **ASB4** expression in early placental development and promote embryonic stem cell differentiation to vascular lineages. ASB4 regulates ID2 (Inhibitor of DNA Binding 2) through its ligase activity in the placenta, and this activity the vascular differentiation (Townley-Tilson et al., 2014). ASB4 ubiquitinated and repressed ID2 expression in a proteasome-dependent fashion in human JAR placental cells. Expression of ASB4 in JAR cells and primary isolated trophoblastic stem cells promoted the expression of differentiation markers. This study shows ASB4 can additionally mediate vascular differentiation in the placenta via degradation of ID2.

Differential genetic screens suggested the **ASB5** function in cardiovascular development (Boengler et al., 2003). Using techniques like northern blot and real-time PCR revealed decreased protein levels of ASB5 in collateral arteries compared to femoral occlusion alone treated with doxorubicin which inhibits angiogenesis showing its involvement in vascular development.

Role in myogenesis and Notch signaling

Data indicates that different ASBs, however, play prominently different roles in muscle development, because of a

various number of ANK repeat these proteins contain. ASBs such as ASB3 and ASB8 proteins are strongly expressed in the skeletal muscle (Liu et al., 2003; Kohroki et al., 2005), while ASB6, ASB7, and ASB9 proteins are weakly expressed in skeletal muscles (Human Protein Atlas). Although some ASB proteins are expressed in skeletal muscle, the important functional role of ASBs in skeletal myogenesis has only recently received recognition. Among them, studies reported **ASB2**β targets Filamin A β (FLNb) for destruction via proteasome in muscle differentiation (Bello et al., 2009). ASB2β regulates the function of FLNb by controlling its degradation. Whereas endogenous knockdown of ASB2β during induced differentiation of C2C12 cells delayed FLNb degradation as well as myoblast fusion and expression of muscle contractile proteins. Studies on hypertrophic cardiomyopathy (HCM) an autosomal-dominant disease, with mutations in genes encoding sarcomeric proteins suggested deregulation of the UPS (Ubiquitination proteasome system) in HCM (Thottakara et al., 2015). They identified desmin as a new target of ASB2β for proteasomal degradation in cardiomyocytes suggesting that an accumulation of desmin could contribute to UPS impairment in HCM. Desmin is a muscle-specific, type III intermediate filament that integrates the sarcolemma, Z-disk, and nuclear membrane in sarcomeres and regulates sarcomere architecture (Costa et al., 2004). In addition, ASB2 plays a major role in ubiquitination of notch targets such as E2A and Janus kinase 2 by the formation of non-canonical E3 ligase complex (Nie et al., 2011). ASB2 is likely bound directly with jak2, but partners with E2A via F-BOX containing protein, Skp2 is also known to associate with Skp1 and Cul1. ASB2 is activated by notch signaling thereby showing its involvement as a regulator of Notch signaling. In multicellular organisms, Notch signaling pathway is a conserved signaling pathway. Despite the need for further studies to understand variations in ASB2-mediated ubiquitylation complexes data clearly demonstrates that ASB2 is involved in degrading key proteins in cell proliferation and differentiation.

ASB5 protein is localized in endothelial cells (ECs) and smooth muscle cells (SMCs) of collateral arteries, and in the satellite cells of myocytes, whereas myocytes itself do not express ASB5 *in vivo* (Boengler et al., 2003). It is involved in the initiation of collateral artery growth via arteriogenesis in rabbits.

ASB11 gets downregulated and regulates neuronal progenitor compartment size by maintaining the neural precursors in the proliferating state through the control of SoxB1 family transcription factors (Diks et al., 2006) (Tee et al., 2012).

ASB15 controls protein turnover and muscle cell development by stimulating protein synthesis and regulating differentiation of muscle cells (McDaneld and Spurlock, 2008). In vivo, overexpression of ASB15 leads to increased protein synthesis and myofibril area in the skeletal muscle (McDaneld et al., 2006).

Role in spermatogenesis

In mammals, spermatogenesis is a vital process in which spermatozoa are produced from spermatogonial stem cells by mitosis and meiosis (Clermont, 1972). Many ASB proteins are expressed and involved in regulating various stages of spermatogenesis (Kim et al., 2008).

Controlled compartment studies on ASB proteins suggested that **ASB8** can play a significant role in spermatogenesis, at the differentiation stage. In humans, ASB8 is abundantly expressed in testis mainly in spermatocytes and spermatids.

ASB9 is expressed in the kidney and testes. Pachytene spermatocytes and spermatids showed mAsb-9 whereas no expression was seen in spermatogonia and the generated spermatozoa (Lee et al., 2008). ASB9 could be a useful target for studies on male germ cell development in spermatogenesis, which makes it a specific marker for spermatogenesis.

ASB17 has the highest expression in testis, with highest in round spermatids but no expression in the Leydig and epididymis region, suggesting its role in testis development and spermatogenesis (Guo et al., 2004; Kim et al., 2004). ASB genes may regulate spermatogenesis at multiple levels though specifics are to be elucidated.

Role in chromosomal abnormality

Chromosomal changes are the markers of gene deregulation in cancer and lead to instability of the genome (Albertson et al., 2003). Chromosomal changes are segregation variable in several types of cancers, and the resultant phenotypic effects are equally variable. Kif2a a microtubule (MT)-associated motor protein interacts with DDA3 (also known as PSRC1), which functions on the mitotic spindles to control chromosome congression by regulating the dynamics of the mitotic spindle (Hirokawa et al., 2009). This interaction increases the efficiency of Kif2a targeting to spindle poles (Jang et al., 2008). The frequency of unaligned chromosomes increased in DDA3 knockdown cells, substantially reduces tension across sister kinetochores at metaphase, and decreases the velocity of chromosome segregation during anaphase (Seki et al., 2008). Recent studies reported that **ASB7** targets DDA3 for proteasomal degradation both *in vivo* and *in vitro* (Uematsu et al., 2016). Knockdown of ASB7 prevented MT polymerization and increased the proportion of cells with unaligned chromosomes. Indeed, depletion of DDA3 in ASB7 knockdown cells reversed these phenotypes. Collectively, these results indicate that ASB7 can modulate spindle dynamics and genome integrity by regulating the level of DDA3, making ASB7 a likely target in numerous cancers.

Role in inflammation

Prolonged or unregulated inflammation can contribute to the pathology of many diseases including immune-mediated

diseases, metabolic disorders, neurodegenerative, and cardiovascular diseases (Coussens and Werb, 2002; Ben-Baruch, 2006; Hotamisligil, 2006). Experiments involving utilization of Antibody array technology against tumor necrosis factor receptor II (TNF-R2) identified it as one of the binding targets of **ASB3** (Chung et al., 2005). In several inflammatory responses, TNF-R2 upregulation is linked with several diseases, including rheumatoid arthritis (Wajant et al., 2003, Mocellin et al., 2005). ASB3 is bound to Elongin BC/Cullin/Rbx1 ligase complex through its SOCS domain, mediating ubiquitination of TNF-R2 followed by its degradation. ASB3 can also affect T cell signaling by degrading TNF-R2, resulting in the inhibition of downstream signaling events in response to TNF-α Studies reveal decreased **ASB4** mRNA in endothelial cells due to hypoxic insult and shear stress whereas it increased in response to TNF-α treatment (Bode et al., 2011). Further experiments establish the role of Nf-κB in TNF-α-induced upregulation of ASB4, exhibiting its role downstream of Nf-κB in the TNF-α signaling pathway. This makes ASB4 a potential regulator of various functions of TNF-α associated with inflammation, angiogenesis, and apoptosis.

Inflammatory cytokines increase the expression levels of **ASB10** (Keller and Wirtz, 2016). It is hypothesized that increase in ASB10 is required to bind a specific target and send it for degradation, similar to the ASB3-TNF-α R2 interaction. **ASB10** mutations appear to contribute a rare familial form of primary open angle glaucoma (POAG); they have the potential to identify important pathways in glaucoma. Mutant ASB10 unlikely to respond appropriately to inflammatory signals in the eye might precipitate the deleterious changes that lead to glaucomatous vision loss.

Role in cancer

Current pathological studies indicated a change in the expression of some ASB genes are linked with the malignancy of cancers and prognosis. Recently, circulating E3s have been increasingly considered as cancer biomarkers. Mutated ASB proteins are found in various cancerous tissues such as glioma (ASB4), kidney carcinoma (ASB3, ASB8, and ASB16), breast and skin cancer (ASB11) and lung cancer (ASB15) whose functional role needs to be elucidated. Mutation in ASB11 presented 2 incidences in breast cancer; both placed in the SOCS box region (http://www.sanger.ac.uk/genetics/CGP/cosmic/) (Yang et al., 2005).

A study identified ***ASB3*** mutations in patients with colorectal cancer (CRC) (Du et al., 2017). Wild-type ASB3 inhibits CRC cell proliferation, migration and invasion in vitro with a decrease in the tumorigenicity and hepatic metastasis in vivo; while mutated ASB3 lost this tumor-suppressive role. The non-functional roles of *ASB3*, which result from mutations or its downregulation, are possible events that can lead to the pathogenesis or progression of CRC. ASB3 exerts a tumor-suppressive role in the pathogenesis and progression of CRC.

ASB4 display involvement in HCC (hepatocellular carcinoma cells) (Lee et al., 2008) and recent studies examined the mRNA, and tumorigenic properties of ASB4 in HCC (Au et al., 2014). Suppression of ASB4 alleviates migration and invasion, but not proliferation, of HCC cells. In addition, similar results were exhibited in this study when miR-220a was overexpressed. miRNAs are small non-coding RNA molecule (containing about 22 nucleotides) that functions in RNA silencing and post-transcriptional regulation of gene expression take part in tumorigenesis by modulating the balance between oncogenes and tumor suppressor gene (Van Kouwenhove et al., 2011; Baer et al., 2013). ASB4 had an inhibitory effect on miR-200a expression levels. Indeed, downregulation of miR-200a and its suppression promoted tumor phenotypes of HCC (Yuan et al., 2011). Overexpressing miR-200a in HCC did not alter cell proliferation but could decrease cell migration by increasing E-cadherin levels. This study deciphers the tumorigenic properties of ASB4 and a novel miRNA-based regulatory mechanism of ASB4 in HCC.

Studies in oral squamous cell carcinoma (OSCC) found **ASB6** abundantly expressed with relatively lower induction in adjacent normal tissues (Lai et al., 2006).

Higher tissue expression of **ASB8** transcripts is observed in normal skeletal muscle, heart, brain, liver, placenta, pancreas, and kidney. Whereas, no expression is seen in the normal lung tissues. Interestingly, ASB8 is found to be regulated in all tumor cell lines, exceptionally and upregulated in lung carcinoma cell lines SPC-A1, A549, and NCI-H446. Upregulation of ASB8 in lung cancers shows an important regulatory role of it in the growth of cancer cells. Consistently, inhibition of cancer growth was evident when a mutant ASB8 lacking the SOCS box domain was used. To which the authors explained this effect to be an act of the truncated ASB8 protein which acts as a dominant negative regulator role in cancer cells (da Silva et al., 2003).

ASB9 exhibited overexpression in cancerous tissues compared to the non-cancerous regions in colorectal cancer (CRC) (Tokuoka et al., 2010). It shows the ASB9s general role in the proliferation of cells and as a specific indicator of CRC. In addition, it was also identified in one of the three breast tumor-associated genes from breast cancer cDNA library screening with antibodies in breast cancer serum patients, showing its correlation with breast cancer. Meanwhile, ASB9 is also a strong candidate to drive compartment expansion in distal intestinal structures and cancer.

First known gene expression of **ASB13** was obtained in result data set of diffuse large B cell lymphoma (DLBCL). B cell Non-Hodgkin's lymphomas are one of the most prevalent cancers found in humans (Blenk et al., 2007). ASB13 was overexpressed in activated B cell-like (ABC) group compared to the germinated center B cell-like (GCB) group. GCB

groups have a higher survival rate as compared to the ABC group which is aggressive and have a lower survival rate. Even though the ASB13 expression is seen in many malignant tumor cells, yet no data has been available on the functional role of ASB13.

Conclusion and future perspectives

In conclusion, ASB genes are multifunctional proteins that carry features of both biological and pathological consequences. Further characterization of unknown functions of ASBs, identifying additional substrates and their interaction, will provide further insights into disease mechanisms. Though the functional and structural role of other ASB genes such as ASB12, ASB13, ASB14, ASB16, and ASB18 are not fully characterized. ASBs provide potential targets for cancer treatment as they control entry into the ubiquitination degradation pathways and are suitable targets for therapeutic interventions. ASBs can be treated as biomarkers in the diagnosis, prognosis and monitoring of different diseases, such as diabetes, cancer and neurodegenerative disorders. Further work to explain the expression pattern of ASBs in various forms of cancer may provide additional biomarkers and valuable insight into new therapeutics. In addition, interpretation of the function of all ASBs described above would uncover the pathophysiology of related diseases and lead to new strategies for diagnosis, treatment, and development of drugs.

Abbreviations

PT- Post-translational
ANK- Ankyrin repeats
ASB-Ankyrin repeat SOCS box
SOCS- Suppressor of cytokine signalling
POAG- Primary open-angle glaucoma
ATP- Adenosine triphosphate
HECT- Homologous to the E6-AP carboxyl terminus
RING- Really interesting new gene
EST-Expressed sequence tags
ECS- Elongin-cullin-SOCS
HIF-1- Hypoxia-inducible factor 1
SCF- Skp, Cullin, F-box containing complex
THMMM-Transmembrane hidden Markov model
CRL-Cullin-ring ligases
PPI- protein-protein interaction
TNF-α, RII-tumor necrosis factor-alpha receptor-2
NF-κB- Nuclear factor-κB
SKP2- S-Phase kinase-associated protein 2
CKB-Creatine kinase B
ATRA-All-*trans* retinoic acid

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Compliance with ethical standards

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