

Expression patterns and physiological roles of SH3BGR protein family as adaptor proteins

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Abstract

Cell signal transduction is a complex system of communication that governs basic cellular activities and coordinates cell actions. Adaptor proteins containing SH2 or/and SH3 domain play critical roles in cell signaling and diseases. Recently, SH3 domain-binding glutamic acid-rich (SH3BGR) protein family members were characterized, except SH3BGR13, they share a consensus SH3-binding motif and a Homer EVH1 binding motif, and function in physiological and pathological events of many diseases, including Down syndrome, diabetes and cancers. In this review, we summarized the recent advances in expression patterns and physiological roles of SH3BGR family members in model systems and cancers, and suggested the further endeavors to unmask their real facets in homeostasis and pathogenicity when dysfunctional to provide potential hints for the diagnosis and therapy of related diseases.

Introduction

Cell signal conduction is a complex system of communication that governs basic cell physiologies and coordinates cellular activities. In this process, protein interactions allow extracellular signals to select the respective cell and plasma membrane receptors and subsequently to conduct information to specific intracellular sites, which is an essential facet of cellular regulation [1]. Protein interactions, especially those involved SH3 domains to recruit enzymes into signaling networks or place enzymes close to their substrates are commonly applied in signal transduction, cytoskeletal rearrangements, membrane trafficking, and other key cellular regulations [2,3]. Recently, a SH3 domain-binding glutamic acid-rich (SH3BGR) protein family composed of SH3BGR [4], SH3BGR1 [5], SH3BGR2 [6] and SH3BGR13 [7] is characterized by the presences of a proline-rich region with SH3-binding motif and an acidic carboxyl terminal region with Homer EVH1 binding motif, respectively [4]. The presence of two domains often involved in protein-protein interactions suggests that SH3BGR protein family may play multiple roles in signaling transduction processes of cells.

Biological characters of SH3BGR family

SH3 domain-binding glutamic acid rich (SH3BGR) was firstly found in the identification of genes involved in the pathogenesis of Down Syndrome patients and named based on two contained motifs, the proline-rich region and the glutamic acid rich region [4]. After characterization of other homologs, including SH3BGR1 [5], SH3BGR2 [6] and SH3BGR13 [7], the shared highly conserved N-terminal region with a proline-rich sequence (PLPPQIF), a SH3 binding (PXXP) [1] and the Homer EVH1 binding (PPXXF) motifs [1,8] were featured, respectively (Figure 1), thus these four genes were assigned as a new SH3BGR gene family [6].

Although SH3BGR family members have very similar structure, their loci in genome are distinct. *sh3bgr* is mapped to chromosome

21q22.3 [4], *sh3bgr1*, *sh3bgr2* and *sh3bgr13* locate in chromosome Xq13.3 [5], chromosome 6q13-15 [6] and chromosome 1p34.3-35 [7], respectively. The member *sh3bgr* encodes a 239 amino acids protein that contains a highly conserved N-terminal region of about 100 amino acids and a variable C-terminal region highly enriched with glutamic acid residues [4]. *Sh3bgr1* encodes a small protein of 114 amino acids, sharing 60% identical and 84% conservative amino acids with the middle portion, proline-rich region of the *sh3bgr* gene [5]. *sh3bgr2* encodes a protein of 107 amino acids and appears to be related to Glutaredoxins and PKC-interacting cousin of thioredoxin (PICOT) homology domain [6]. *sh3bgr13* encodes a small protein of 93 amino acids, showing 39% identity and 76% similarity to SH3BGR protein, but lacks both SH3 and Homer EVH1 binding motifs, indicating that SH3BGR13 may function differently from other members of the family [7]. Whatever, all SH3BGR members have very high similarity to Glutaredoxin1 (GRX1) of *Escherichia coli* at both amino acid sequence and the predicted structural level [7], but they completely lacks the CXXC motif that is essential for glutaredoxin enzymatic function [9]. Given the structural feature, it could be expected that SH3BGR members may participate the redox-dependent processes in cells [7], but there are no enough evidence to support this hypothesis.

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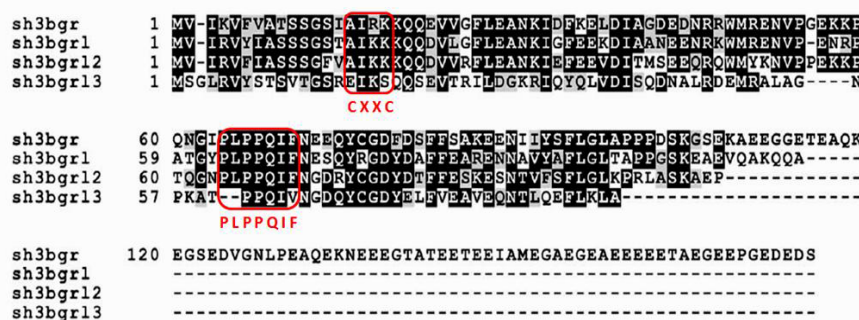


Figure 1. ClustalW alignment of the amino acid sequences of the human SH3BGR family.

Identical and conserved residues are shaded in black and gray, respectively. The conservative proline-rich motif PLPPQIF and the lacking catalytic motif Cys-X-X-Cys in the human SH3BGR family are boxed in red. This figure is cited with modification from Ref.[6].

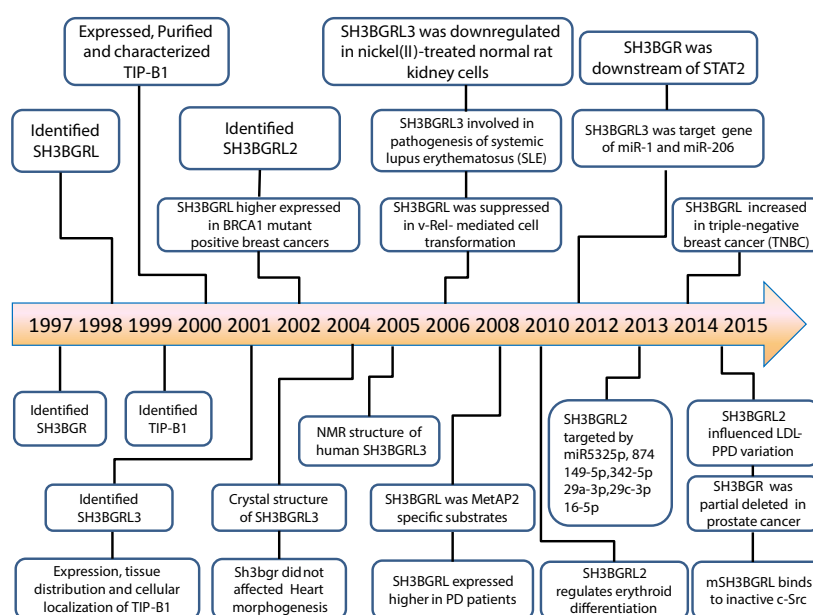


Figure 2. Major achievements in the research of SH3BGR family.

After SH3BGR and its homologs were identified in Down Syndrome (DS), a new family was established as a thioredoxin-like protein superfamily. The family members, except SH3BGR13, share both functional SH3-binding and EVH1-binding motifs, which could be involved in various physiological and pathological processes, including in cancers.

Expression pattern, physiological and pathological functions of SH3BGR family

Since identification of SH3BGR family, their physiological functions *in vivo* are largely unknown. Expression patterns of SH3BGR and SH3BGR1 in *Xenopus* have been reported and indicated that *Sh3bgr* could be critical for sarcomere formation in striated muscles through regulating localization of Enah, and heart development formation by affecting the Enah protein level in *Xenopus* [10]. Our current organ-specific expression analysis of SH3BGR members in zebrafish embryos demonstrated that *sh3bgr13* is uniquely expressed in liver, and *sh3bgr* in sarcomere (unpublished data). Similarly, the mRNA expression of all four members are also expressed in chicken and demonstrated that *sh3bgr* is restricted to muscle tissues, both *sh3bgr1* and *sh3bgr13* are highly expressed in lymphoid tissues (spleen, bursa, and thymus), exhibiting low levels of expression in the liver, skeletal muscle, and kidney, while *sh3bgr12* is expressed highest in the brain and lung, and marginally expressed in the other tissues [11].

So far, to our knowledge, no knockout mice model is reported to dissect the developmental and physiological functions of these genes in mammals. Considering that SH3BGR protein family was screened from the pathogenesis of congenital heart disease (CHD) in Down Syndrome (DS) [4] and *Sh3bgr* is strictly expressed in the earliest stages of mouse heart development [12], it is possible this gene family plays a role in heart morphogenesis and, consequently, in the pathogenesis of CHD in DS. When forcing expression of *Sh3bgr* in transgenic mice with a cardiac- and skeletal-muscle-specific promoter, it seems that SH3BGR has no effect on heart morphogenesis [13]. Moreover, SH3BGR was activated in STAT2 high-expression metabolism coupling postmitotic outgrowth to visual and sound perception network [14].

SH3BGR1 was found to be one of 16 proteins differentially expressed in Parkinson disease (PD) patients comparing with healthy people [15]. For SH3BGR2, although higher level of SH3BGR2 expression was reported to participate in erythropoietin (EPO) stimulated erythrocyte differentiation [16], the expression level of

SH3BGR12 in peripheral blood lymphomononuclear cells (PBMC) showed no difference between Type I diabetes and Type II diabetes [17]. SH3BGR13, previously named TIP-B1 (TNF inhibitory protein-B1) [18] was upregulated under TNF prior simulation to protect cells from TNF induced apoptosis [18,19]. In addition, higher expression of SH3BGR13 in CD4+ T lymphocytes was proved to participate in the pathogenesis of systemic lupus erythematosus (SLE) [20]. We summarized all these milestone events of SH3BGR family from their first identification (Figure 2).

Function of SH3BGR member in tumorigenesis

In cancers, SH3BGR1 was demonstrated to be overexpressed in BRCA1 mutated breast tumors [21], ER positive breast tumors [22] and oral squamous carcinoma, even higher in the invasive oral squamous carcinomas and breast cancers, compared to the non-invasive counterparts or normal breast tissues [23]. Overexpression of murine SH3BGR1 effectively promoted tumor formation and metastasis in xenograft model [23]. Unexpectedly, human SH3BGR1 is a tumor suppressor [23], which is consistent to the observation that SH3BGR1 can repress the viral v-Rel-induced cell transformation [11]. Mechanistically, somatic mutation of human SH3BGR1, such as R76C could trigger tumorigenesis and pulmonary metastasis as murine SH3BGR1 [23], which may partially interpret why human SH3BGR1 is upregulated in various cancers. Moreover, Blocking c-Src and FAK attenuated the downstream AKT and MAPK activities, leading to much less tumor masses and metastatic sites, which provided a possible strategy for the related cancer therapy. However, based on the TCGA dataset, the somatic mutation frequency of human SH3BGR1 is quite low, even less than 5%. Therefore, we suspect the protein modification could be involved in governing SH3BGR1 protein stability and its physiological functions, dependent of the particular cell contexts. Therefore, much effort should be placed on dissecting the detailed mechanisms how SH3BGR members work, what interacting partners are involved in both normal and transformed cells and tissues.

SH3BGR13 was found to be downregulated in nickel (II)-induced carcinogenesis as well in normal rat kidney cells [24]. Further study uncovered that it can be targeted by small noncoding RNAs miR-1 and miR-206 to regulate C2C12 myoblast cell differentiation [25], suggesting the tumor-repressing role of SH3BGR13. In contrast, SH3BGR13 protein was recently observed to be upregulated in urothelial carcinoma samples, and molecularly binds to epidermal growth factor receptor for tumorigenesis, indicating that it could be a potential prognostic biomarker [26]. Similar to SH3BGR1, the real function of these adaptor proteins would be mainly dependent on the specific cell components, and their protein modification would also be a critical cause to their functions as well.

Except the above two players, neither SH3BGR nor SH3BGR12 is yet to be documented in tumorigenesis. Thus the detailed function and the underlying mechanism of SH3BGR members need to be investigated, especially in animal tumor models.

Signaling pathways regulated by SH3BGR family

SH3BGR1 proteins mainly locate in nucleus, only small portion in cytosol and on the inner cell membrane [23]. As a simple protein family with only a SH3 motif, a proline-rich region and a EVH1 domain, it is positively expected that SH3BGR members mainly work as adaptor proteins via the protein-protein interaction in signal transduction processes [27], since SH3 domain in a protein can easily dock to the proline-rich region in another protein. Thus, they

can integrate into signaling networks anywhere in cells, depending on the cellular contexts. Here, we summarized the roles of SH3BGR members in signaling transduction processes (Figure 3). Indeed, in a study of metabolism coupling post-mitotic outgrowth to visual and sound perception network, SH3BGR was manifested as a downstream target of STAT2 [14]. We also presented that murine SH3BGR1 can specifically bind to the inactive c-Src phosphorylated at tyrosine 527 via its SH3 domain to subsequently activate the downstream AKT and MAPK signaling pathways for tumorigenesis and metastasis, and breakdown of the SH3 domain in turn impeded its binding to c-Src and blocked its oncogenic role [23]. Additionally, SH3 domain is critical for SH3BP-1(SH3 binding protein 1) function in small GTPase activation, including Rac1 [28]. In line with this, forced overexpression of murine SH3BGR1 in both CHO and CT-26 cells remarkably increases Rac1-GTP and cdc42-GTP activities [23]. Previously, SH3BGR1 was reported to be suppressed in v-Rel mediated transformation and rescued SH3BGR1 could reversely block v-Rel mediated cell transformation and tumor formation through the inhibition NF- κ B signaling pathway [11], but the molecular mechanism is lacking.

When searching for the MicroRNAs influenced by interferon-beta treatment in the peripheral blood of multiple sclerosis patients, SH3BGR12 was found to be the target of a cluster of MicroRNAs (miR532-5p, miR874, miR149-5p, miR342-5p, miR29a-3p, miR29c-3p and miR16-5p) [29]. SH3BGR13, also named TIP-B1, could protect cell from TNF- α induced apoptosis, which indicates its possible role in apoptosis regulation [18,19]. Additionally, SH3BGR13 was reported to be mediated by miR-1 and/or miR-206, and its sustained expression resulted in increased proliferation and inhibition of C2C12 cell myogenesis [25].

Conclusions and expects

In this review, we summarized the big events on the characterization of SH3BGR family members and their expression patterns. With the accumulated evidence, we just preliminarily understood the SH3BGR family works mainly through their SH3 domain or proline-rich region, rather than the EVH1 domain or their thioredoxin function, in various signaling pathways as adaptor proteins. However, compared to other adaptor protein, such as Grb2, very little information on physiological and pathological roles of SH3BGR family is available. As docking proteins, their working manners should be indispensably dependent

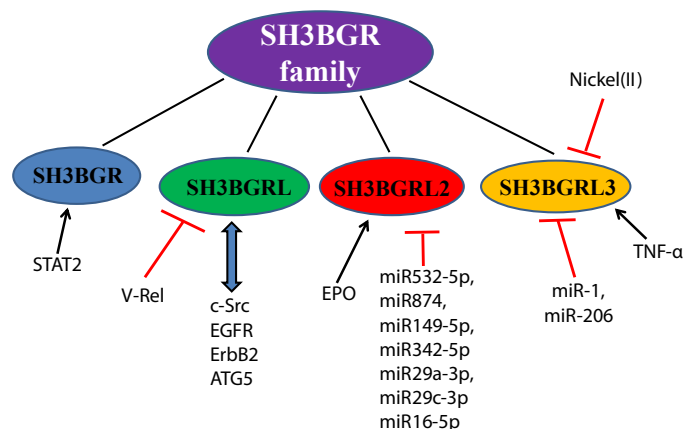


Figure 3. Signaling pathways involved with SH3BGR family members. This figure summarizes the molecules that regulate SH3BGR family members and the signaling pathways involved with SH3BGR members, based on the published data (see Ref.). Red lines with dashes present the suppressive function; Arrows in black indicate the positive regulation, and arrows in blue stand for protein-protein interactions.

on the interacting partners in a specific cell type and the cell contexts, resulting in very complicated events in a particular disease involved with SH3BGR dysfunction, including carcinomas. Therefore, it is urgently to dissect and compare the normal and the aberrant functions of SH3BGR members in both normal and diseased tissues and cells, including their protein modification, location transportation and expression regulation, which would shed lights on the diagnosis and therapeutical reference to a related disease.

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