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Review Article

Grb2-associated binding (Gab) proteins in hematopoietic and immune cell biology

Tamisha Y. Vaughan, Sheetal Verma, Kevin D. Bunting

Department of Pediatrics, Aflac Cancer Center of Children's Healthcare of Atlanta and Emory University, School of Medicine, Atlanta, GA, USA.

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Abstract: Grb2-associated binding (Gab) scaffolding/adapter proteins are a family of three members including mammalian Gab1, Gab2, and Gab3 that are highly conserved. Since the discovery of these proteins, there has been an extensive amount of work done to better understand Gab functional roles in multiple signaling pathways, typically acting as a downstream effectors of receptor-tyrosine kinase (RTK)-triggered signal transduction. In addition to their participation in hematopoiesis, Gabs play important roles in regulation of immune response and in also in cancer cell signaling. Gabs may play complex roles and thus a complete understanding of their interactions and how they modulate hematopoietic and immune cell biology remains to be determined. This review will cover the most recent findings including the involvement of Gabs in disease development and signaling which will be important for design of future therapeutic interventions.

Keywords: Adapter protein, cytokine signaling, Grb2-associated binding protein, Gab, receptor tyrosine kinase, cancer signaling

Introduction

The first Gab molecule was originally identified as the mammalian homolog of the *Daughter of Sevenless* (DOS) *Drosophila* adapter protein while also displaying sequence similarity to *Suppressor of Clear* 1 (*Soc*1), which was identified by genetic screens in *C. elegans* [1, 2]. In mammals, there are three family members with high sequence identity, Gab1, Gab2, and Gab3. Since the discovery of these Gabs, it has been a priority to understand their similarities, differences and functions [3-12]. To date, they have been shown to be expressed in a variety of cells such as T cells, B cells, macrophages and mast cells [6, 13-16]. However, Gab3 has been found at only low levels in the hematopoietic system and lymphocytes during steady state [7]. Gab proteins are located in the cytosol until modifications occur such as phosphorylation which then causes migration to the plasma membrane where they function as adapter proteins. Although, all Gab proteins become tyrosine phosphorylated and contain PH domains with a recognition sequence PXXXR for Grb2, the protein-protein interaction is distinct [6,15,17-21]. Importantly, consensus binding sites for SH2 domains of SHP2, p85 regulatory subunit of PI3-K, PLCγ and Crk were identified in all 3 Gabs. The exact role of Gabs remains to be determined.

Gab1, the most widely studied Gab member, was originally isolated as a Grb2-binding protein from a human glial tumor expressing library and found to be tyrosine phosphorylated [22]. Gab1 has a vital role in signal transduction of multiple receptors controlling cell growth, differentiation and function. Interestingly, a major limitation in studying the function of Gab1 is embryonic lethality when knocked out [21, 23], occurring between E13.5 and E18.5 as a result of organ development failure and possibly cell differentiation. Conditional Gab1 KO mice have been generated [11]; however until there is a complete grasp on the redundancies of other Gab members it has not been determined whether Gab1 has in vivo functions in hematopoiesis. Gab2 on the other hand is involved in MAPK/PI3K pathways and is a highly phosphorylated protein with 10 tyrosine, 18 serine and 5

Table 1. Description of Gabs phenotype and health relevance

Condition / Protein	KO phenotypes	Disease model
Gab1	Organ development failure Cell differentiation Fetal lethality	colorectal cancer
Gab2	Impaired fertility allergic responsiveness Altered mast cell development	gastric carcinoma melanoma breast cancer Leukemia
Gab3	Involvement in macrophage differentiation	Unknown

threonine phosphorylation sites. Deletion of Gab2 in mice results in impaired fertility, lack of allergic responsiveness, and altered mast cell development [4]. Gab2-null bone marrow mast cells have severely impaired IgE-induced mast cell degranulation and largely absent activation of downstream PI3K/AKT signaling [4]. Gab3 was identified for its homology with the other members of the Dos/Gab family. There hasn't been a phenotypic defect associated with the deletion of Gab3, although a role in macrophage cell differentiation has been inconsistent [24-26](**Table 1**). In this review, we discuss the role of Gabs in signaling.

Involvement of Gabs in signaling activation

Phosphorylation of Gab proteins plays several roles in downstream signal activation. All members of the Gab have binding sites for the SH2 domain of PI3-K p85 regulatory subunit. Phosphatidylinositol 3-kinase (PI3K) is a key component of multiple signaling pathways, where it typically promotes survival, proliferation, and/or adhesion [17]. Gab2 has been proven to be a principle activator of PI3K in response to Fc (epsilon)RI activation in vivo [4, 6]. Hepatocyte Growth Factor (HGF), a multipotent protein with several functions which include development functions has been shown to phosphorylate several sites of Gab1 upon activation of its receptor c-Met [27]. In addition, mutations to c-Met receptor show similar defects to that of Gab1 deficiencies [20]. Most recently, studies have been done investigating the protein-protein interaction of Gab1 to Grb2. They were able to eloquently show the cSH3 domain of Grb2 only recognizes two out of four distinct Gab1 RXXK motifs [18]. This signaling information is critical as it leads to better understanding of Gab involvement in health complications such as immune deficiencies and cancer therapeutics.

Gabs involvement in immune response

Cells of the immune system have tyrosine phosphorylated Gab1 and Gab2, occurring by a number of mechanisms including antigen receptors of B and T cells [6,13-15]. In contrast, Gab3 is phosphorylated mostly upon Macrophage-Colony Stimulating Factor (M-CSF) activation [25]. Most recently, Gab1 has been identified to play a role in immune response through macrophage differentiation and toll-like receptor (TLR) signaling [28]. TLRs are key receptors involved in the innate immune processes and play vital roles in host protection against invading pathogens. They function by utilizing downstream signaling molecules and adapter proteins (**Figure 1**). Macrophage, however, have been identified as a key player in innate immune response as well as playing a role in the initiation and progression of diverse inflammatory diseases.

Again, as the first identified family member, Gab1 has been most widely studied in this area. Studies show Gab1 promotes TLR-triggered pro-inflammatory cytokine production by enhancing MAPKs and NFκB activation in macrophage [28]. NFκB activation is not only a key player in inflammation but it plays a novel role in a number of complications including hematological disorders (**Figure 1**). In addition to triggering proinflammatory cytokines, Gab1 has also been linked to the production of type I interferon by directly binding p85 to activate PI3K/Akt PI3K/Akt cascade [28]. Interestingly, it has been shown that SHP-2 deficiency increased TBK1-activated IFN-beta and TNF-alpha expression in which Gab members may be involved [29]. SHP-2 also inhibited poly (I: C)-induced activation of MAP kinase pathway which led to SHP-2 specifically negatively regulating TRIF-mediated gene expression in TLR signaling. Work by An et al., demonstrated that SHP-2 negatively regulated TLR4- and TLR3-activated IFN-beta production [29]. However, Gab involvement hasn't been extensively studied.

Interestingly, an investigation of Gab2 was done on the pharmacological effects of an antisense oligonucleotide targeted at Gab2 on the immune responses of rat basophilic leukemic

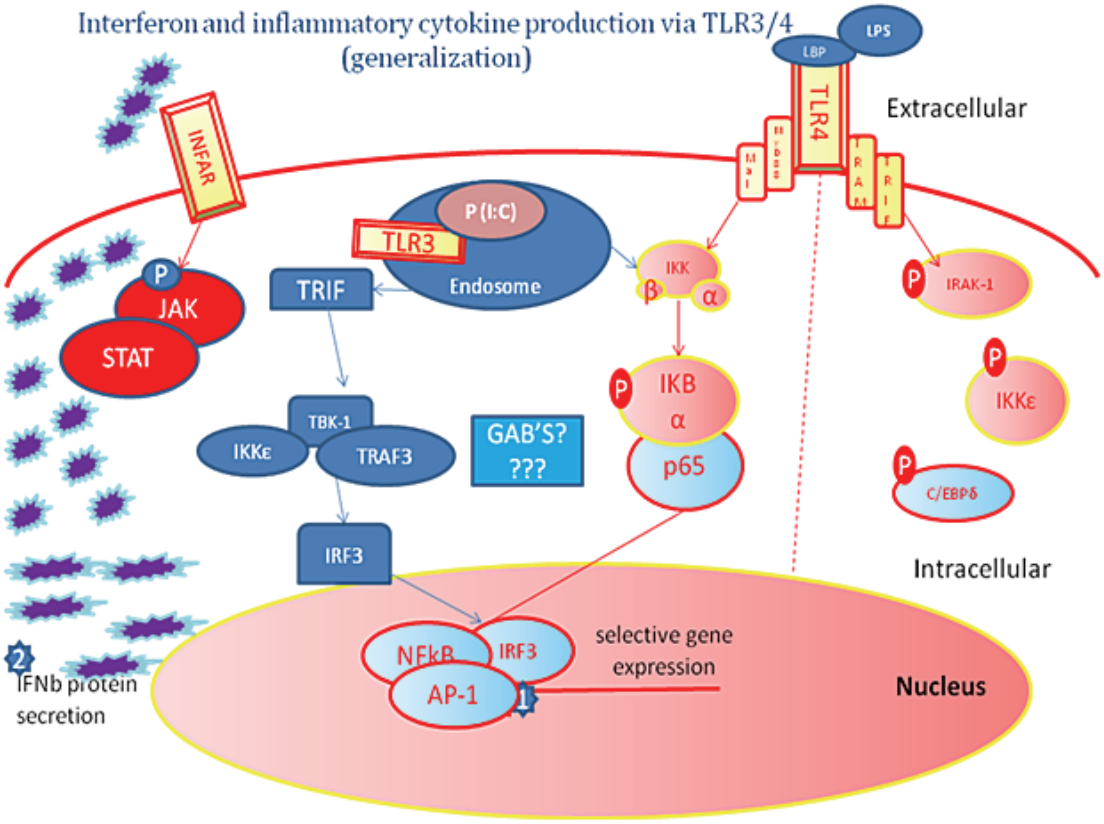

Figure 1. Gabs involvement in immunity. Activation of TLR3/4 initiates a signaling cascade responsible for the production of proinflammatory cytokines and IFNs. In turn, Gab proteins have the potential to interact with several molecules and modulate the interferon response. Demonstration of a role for Gab1 in TLR mediated interferon production in murine macrophages raises questions about the role of Gab2 and Gab3 in this response.

(RBL)-2H3 cells. Not only did they find that Gab2 knockdown inhibits phosphorylation of Akt, p38 mitogen-activated protein kinase and PKCdelta, but knocking down Gab2 under these conditions causes suppression of mast cell functions [30]. These findings give further insight to the Gab family's involvement in immune response and their role as adapter proteins during signaling processes. However, there still remains a gap of knowledge about whether these proteins overlap or have distinct functions.

Gabs in leukemogenesis

Gab1 and Gab2 have both been proven to play critical roles in colon, gastric, and breast cancer [10,19,31-33](**Table 1**). The Bcr-Abl oncoprotein promotes leukemia development by activating a number of signaling pathways regulating cell proliferation, transformation, and survival. Signaling pathways activated by Bcr-Abl include the PI3K-mTOR pathway, the RAS/RAF/MEK/ERK pathway, and the JAK-STAT pathway. However, Gab2 has been most widely characterized for its role in leukemia via its interaction with the BCR-ABL complex. Chronic myelogenous leukemia (CML) is a myeloproliferative disease (MPD) caused by hematopoietic stem cells that possess the Philadelphia chromosome, which encodes the Bcr-Abl oncoprotein. The Bcr-Abl complex has become a target for therapeutic interventions in cancer treatments [8, 34]. A commonly used inhibitor, Imatinib, inhibits kinase activity in Bcr-Abl and has been shown to prolong survival in CML patients [34-36]. However, Gab proteins may contribute to better therapeutics. Currently, Bcr-Abl negative MPD results from mutations in other receptors, most commonly JAK2 [29,30]. New findings show that Bcr-Abl signaling can be suppressed in CML by inhibiting or knocking down Jak2 [34]. Jak2 inhibition subsequently led to the decrease of Grb2-BCR-Abl binding while under normal conditions

would lead to phosphorylation of Gab2 involved in binding the regulatory subunit of PI3 kinase [8, 35]. In addition, they also show in three CML lines such modifications of Jak2 not only compromise the signaling complex of Grb2 but reduce phosphorylation of Gab2 after just 30 minutes of inhibitor treatment [34].

In addition to CML, Gab2 plays important roles in Juvenile Myelomonocytic Leukemia (JMML) [34,35,37]. JMML is a myeloproliferative disease (MPD) of early childhood and studies have shown involvement of Gab2 by the Ras pathway. Interestingly, thirty-five percent of patients diagnosed with JMML have activating mutations in phosphatase *PTPN11* (Shp2), a ubiquitous tyrosine phosphatase. Gab2 plays an important role in *PTPN11* mutations which are associated with this condition [37]. Prior studies have shown how the disruption of Gab2 binding sites for SHP2 [8,19,38] and p85 effect cell growth, migration and activation of downstream cascades. But the *Ptpn11* aberrant hematopoietic stem cell activities caused by the mutation were corrected by deleting Gab2 [37]. Although mechanisms aren't clearly understood, advances in understanding how Gabs promote such diseases will be needed for development of new therapeutic interventions.

Address correspondence to: Kevin D. Bunting, Ph.D. Department of Pediatrics, Division of Hem/Onc/BMT & Aflac Cancer Center and Blood Disorders Service, Emory University School of Medicine, 2015 Uppergate Dr. NE, ECC #444, Atlanta, GA 30322, Tel: 404-778-4039, E-mail: Kevin.bunting@emory.edu

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