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Essential roles of Gab1 tyrosine phosphorylation in growth factor-mediated signaling and angiogenesis

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Abstract

Growth factors and their downstream receptor tyrosine kinases (RTKs) mediate a number of biological processes controlling cell function. Adaptor (docking) proteins, which consist exclusively of domains and motifs that mediate molecular interactions, link receptor activation to downstream effectors. Recent studies have revealed that Grb2-associated-binders (Gab) family members (including Gab1, Gab2, and Gab3), when phosphorylated on tyrosine residues, provide binding sites for multiple effector proteins, such as Src homology-2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2) and phosphatidylinositol 3-kinase (PI3K) regulatory subunit p85, thereby playing important roles in transducing RTKs-mediated signals into pathways with diversified biological functions. Here, we provide an up-to-date overview on the domain structure and biological functions of Gab1, the most intensively studied Gab family protein, in growth factor signaling and biological functions, with a special focus on angiogenesis.

Keywords

angiogenesis; endothelial cells; Gab1; receptor tyrosine kinase; tyrosine phosphorylation

1. Introduction

Growth factors and their associated receptor tyrosine kinases (RTKs) mediate a number of biological processes controlling cell-cycle progression, motility, survival, migration, metabolism, and differentiation[1-3]. Upon the engagement of the ligand on the cell-surface receptors, their intrinsic protein-tyrosine kinases are activated. Receptor tyrosine-phosphorylation creates docking sites for signal relaying proteins which contain Src-homology 2 (SH2) and phosphotyrosine-binding (PTB) domains[4]. These proteins fall into

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two general categories-enzymes and adaptors. Adaptor proteins, lacking the catalytic domain, can recruit one or more enzymes into signal transduction. The adaptor proteins Grb2-associated binders (Gab) are members of the insulin receptor substrate 1 (IRS1)-like multi-substrate docking adaptor protein family[5, 6], which possess a pleckstrin homology (PH) domain that can bind phosphatidylinositol lipids within biological membranes. These docking adaptor proteins also contain binding sites for SH3 domain-containing proteins and multiple tyrosine phosphorylation sites for recruitment of SH2 and PTB domain-containing proteins, which play important roles in the regulation of signal specificity, signal amplification and assembling multimeric signaling complexes[2, 4]. Gab genes encoding mammalian Gab1, Gab2, and Gab3, the *Drosophila* homolog Daughter of Sevenless (DOS), and the *Caenorhabditis elegans* homolog Suppressor of Clear (Soc1), define a family of docking adaptor proteins. Gab1 was originally identified as a Grb2 SH3-domain binding protein[7, 8]. Gab2 was isolated as a binding partner of the SH2 domain-containing protein tyrosine phosphatase (SHP2)[9]. Gab3 was discovered based on its sequence similarity with Gab1 and Gab2 within a large sequencing database[10]. Gab1 and Gab2 are expressed ubiquitously, while Gab3 is highly expressed in lymphoid tissue in particular. The Gab family proteins contain a PH domain in the amino-terminal region, as well as tyrosine-based motifs and proline-rich sequences (PXXP), which are potential binding sites for SH2 and SH3 domain containing proteins. Although the overall sequence identity among the Gab family is only 40-50%, the N-terminal PH domain, proline-rich motifs, and multiple potential tyrosyl and seryl/threonyl phosphorylation sites are conserved among Gab1, Gab2, and Gab3[5, 6] (Figure 1). However, each Gab protein also has unique structure in individual signal transduction.

Gab proteins can be recruited to activated RTKs through direct and indirect mechanisms. Direct mechanism has been described between Gab1 and c-Met, the receptor for hepatocyte growth factor (HGF)[8, 11-13]. Gab1 interacts with tyrosine-phosphorylated c-Met via the Met-binding domain (MBD, amino acids 450-532), which contains 13 essential amino acids (487-499) and is absent in Gab2 and Gab3[14-16]. Most RTKs recruit Gab1 indirectly via Grb2[5, 6]. Gab proteins harbor several proline-rich motifs which bind to Grb2 SH3 domain, while Grb2 contains an SH2 domain which targets the Grb2-Gab complex to receptors containing Grb2 SH2 domain binding sites[15]. It has been shown that indirect recruitment of Gab1 by c-Met is also physiologically important, since the mutation of Grb2 SH2 domain dramatically decreases the c-Met-Gab1 association[11, 17], thereby, blocking the HGF pathway.

2. Effector proteins involved in Gab1-mediated signal transduction

Gab1 is tyrosine-phosphorylated in response to many growth factors (including vascular endothelial growth factor (VEGF), HGF, nerve growth factor (NGF), platelet-derived growth factor (PDGF), EGF) and other stimuli [5, 6, 18], thereby propagating signals that are essential for cell proliferation, motility, and erythroblast development. Whereas, hyper-phosphorylation in serine and threonine of Gab1 (by PKC-α and PKC-β1) has been shown to negatively regulate HGF-induced biological responses which is critical for Gab1-induced signaling required for angiogenesis[19]. Gab2 is tyrosine-phosphorylated in response to cytokines IL-2, IL-3, IL-15, TPO, EPO, Kitl, M-CSF, Flt3l, and the stimulation of gp130,

FcεRI, FcγR, and T and B antigen receptors [20]. To date, Gab3 is tyrosine-phosphorylated in response to M-CSF[10]. Our previous study showed that Gab1 was tyrosine-phosphorylated in endothelial cells (ECs) under mechanical stress such as fluid shear stress[21, 22]. These data show that Gab proteins act downstream of receptor tyrosine kinases, cytokine receptors, and possibly other receptor systems.

Gab proteins lack enzymatic activity but become rapidly phosphorylated on tyrosine residues, providing binding sites for multiple SH2 domain-containing proteins such as SHP2, phosphatidylinositol 3-kinase (PI3K) regulatory subunit p85, phospholipase C (PLC), Crk, and GC-GAP. Association of Gab1 with SHP2 and the p85 subunit of PI3K is considered to be essential for activation of extracellular signal-regulated kinase (ERK)1/2 and AKT, respectively. These interactions between Gab protein and effector molecules were found to be critical for transducing Gab-mediated signaling[5, 6, 20, 23].

Among the proteins bind to the Gab proteins, SHP2 has been shown to interact with all mammalian Gab proteins, as well as the *Drosophila* DOS and *C. elegans* Soc1, indicating that recruitment of SHP2 is a conserved feature that Gab family genes retained from *C. elegans* to mammalian systems[6]. Mutants of Gab family proteins incapable of binding SHP2 have been used to study the functional significance of the Gab-SHP2. Met-mediated morphogenesis, EGF-induced and fluid shear stress-induced MAPK signaling transduction, were blocked by overexpressing the Gab1 mutant unable to interact with SHP2. Accumulating evidence indicates that Gab1 Y627 and Y659 phosphorylation recruits and activates SHP2 phosphatase, which in turn activates MAPK signaling[15, 24, 25]. Moreover, DOS or Soc1 with all tyrosines mutated to phenylalanines, except those crucial for SHP2-binding, is sufficient to mediate RTKs signaling and to rescue the developmental lethality resulting from the loss-of-function mutations[26]. These results strongly demonstrated the physiological significance of Gab-SHP2 interaction.

Another well-studied effector protein of Gab family proteins is the PI3K p85-subunit. Mutations at the p85-binding sites of mammalian Gab1 and Gab2 resulted in defective signal transduction in many signaling systems[27-31]. The association between p85 and Gab1 or Gab2 is crucial in mediating the PI3K/Akt signaling pathway induced by a variety of stimuli. Overexpression of Gab1 wild type potentiates FGF-, VEGF-, and HGF-induced Akt activation, whereas overexpression of the p85-binding mutant of Gab1 results in decreased Akt activation[32]. This mutant also failed to convey the anti-apoptotic signaling in NGF stimulation[29]. These results suggest that the Gab–p85 association plays an important role in activating the PI3K/Akt pathway in mammalian cells. In addition, PIP3, the product of activated PI3K, binds to the PH domain of Gab proteins and further potentiates the activation of PI3K, forming a positive feedback loop to amplify the signals through the Gab proteins[33], which are important for signal specificity in certain systems.

Gab1 is essential for embryo survival, since $Gab1^{-/-}$ mice are not viable and only reach day 14 to 18 of gestation. Further analysis indicates that these mice have multiple developmental defects in heart, placenta, liver, spleen and muscle development[34]. The phenotype of Gab1 deficient mice showed similarity with those deficient of HGF, c-Met, PDGF, and EGF signaling pathways. In contrast, $Gab2^{-/-}$ and $Gab3^{-/-}$ mice can live to a normal age, with

the exception that Gab2$^{-/-}$ mice have a defect in mast cell signaling[35, 36], while the loss of Gab3 does not result in noticeable developmental defects[36]. To better understand the tissue-specific role of Gab1, several studies have been carried out with cell type-specific (conditional) Gab1 knockout mice. For instance, liver-specific Gab1 knockout mice displayed a phenotype of defective liver regeneration triggered by partial hepatectomy surgery[37]. Very recently, Sun et al reported that cardiomyocyte-specific Gab1 knockout mice exhibited an increase in infarct size and a decrease in cardiac function after ischemia/reperfusion (I/R) injury[38], suggesting that Gab1 is also essential for cardioprotection against I/R oxidative injury. In addition, it has been reported that Gab1 and Gab2 may have the redundant roles for maintenance of cardiac function via neuregulin-1/ErbB signaling when using cardiomyocyte-specific Gab1/Gab2 double knockout mice[39]. Since there have been several excellent reviews on the biological functions of Gab proteins[5, 20, 40], in the next section, we will focus on the specific role of Gab1 in growth factor-mediated signaling and angiogenesis.

3. Gab1 and angiogenesis

In 2011, three independent groups (including our laboratory) simultaneously reported the crucial role of Gab1 in promoting postnatal angiogenesis using endothelial cell-specific Gab1 knockout (Gab1-ecKO) mice and hindlimb ischemia models[41-43] (Table 1). The Gab1-ecKO mice were viable, with no obvious defects on embryonic vasculogenesis and neonatal retinal angiogenesis, which indicate that Gab1 in the endothelium plays no crucial role during developmental vasculogenesis. All three groups consistently showed that Gab1-ecKO mice have severe defects in angiogenesis after hindlimb ischemia. Impaired blood flow recovery, low capillary density and necrotic limb were observed 2 weeks after the femoral artery ligation in Gab1-ecKO mice, while the WT control mice showed a time-dependent recovery of blood flow and increased capillary density in the gastrocnemius muscle[41-43]. Unlike Gab1-ecKO mice, no significant effects on angiogenesis were observed on conventional Gab2 knockout mice[39].

Although increased level of both VEGF and HGF, the potent pro-survival factors were observed in the ischemic hindlimb muscles. Zhao *et al* also reported a significant increase of apoptotic ECs in the gastrocnemius muscle from Gab1-ecKO mice in association with the low capillary density[41]. Furthermore, the viability of Gab1-deficient ECs remained low under the treatment of both growth factors (VEGF and HGF) *in vitro*, whereas wild-type cells are protected from apoptosis. One possible explanation might be that impaired PI3K/Akt signaling and activated caspase-3 in the absence of Gab1[41]. Shioyama *et al* showed that HGF specifically upregulates Krüppel-like factor 2 (KLF2) mRNA and protein expression in ECs overexpressing Gab1[43]. KLF2 functions as a potent anti-apoptotic factor, which acts, in part, through the activation endothelial nitric oxide synthase (eNOS), and mediates the Gab1-dependent cell survival signaling in ECs. Zhao *et al* also demonstrated that Gab1 is essential for HGF-induced ERK$^{1/2}$ phosphorylation through SHP2 activation[41], while Shioyama et al showed that ERK5 is also activated downstream of Gab1-SHP2 after HGF stimulation[43]. In the third report, Lu *et al* revealed an important protein kinase A-dependent pathway for VEGF-induced eNOS activation and angiogenesis[42]. In addition to hindlimb ischemia-induced angiogenesis, Gab1 was also

shown to be important for the tumor angiogenesis. Zhao et al. [41] demonstrated a significant low level of capillary density in tumors engrafted in the Gab1-ecKO mice as well as dramatically decreased tumor weight and volume. A logical follow-up question will be to address the mechanism of how Gab1 regulates the tumor angiogenesis, such as the potential role of Gab1 in matrix metalloproteinases (MMPs) activation and metastasis of tumor cells. Collectively, studies from three independent groups established the critical role of endothelial Gab1 in HGF-and VEGF-induced postnatal angiogenesis[41-43]. Taken together, Gab1 functions as a key molecule that regulates both VEGF- and HGF-mediated downstream signaling pathways involved in EC stabilization, proliferation, migration and survival which are crucial for angiogenic processes (Figure 2).

4. Future perspectives

Since the discovery of Gab docking proteins 18 years ago[7], it has become evident that these family of proteins extend beyond the original definition of docking proteins (as a platform for the assembly of multiple signaling branches) and play critical roles in a variety of pathophysiological processes[40]. Preventing protein-protein interactions involving Gab-family proteins and their associated effectors/adaptors will be a viable therapeutic strategy in diseases involving angiogenesis. One enduring question regarding the Gab1 signaling is: how is the specificity of each RTK achieved while they share the same downstream scaffolding adaptor Gab family proteins? Definitely, further studies on the Gab family members will allow us to understand more the complexity of the receptor-mediated signaling and ensuing biological functions.

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Highlights

- Gab1 relays receptor tyrosine kinase-mediated signal transduction
- Gab1 promotes endothelial cell survival, migration and tube formation
- Gab1 is critical for HGF and VEGF induced postnatal angiogenesis

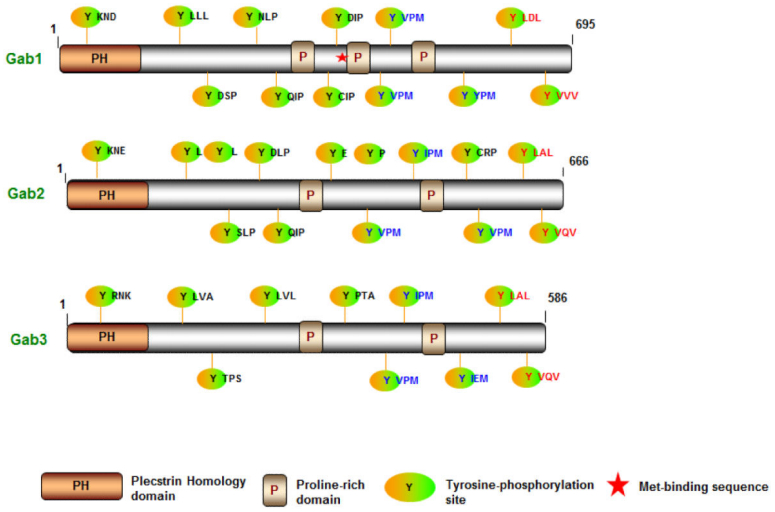

Figure 1. Domain structures of the Gab superfamily of adaptors/scaffolding proteins

The Gab family (Gab1, Gab2, and Gab3) is recruited by a wide variety of receptor tyrosine kinases (RTKs) and has multiple phosphorylation sites. Gab1, the most intensively-studied Gab family member, also contain serine and threonine phosphorylation sites, which negatively regulate HGF/Gab1 signaling. The Met-binding sequence (MBS, amino acid 487-499) within the Met-binding domain (MBD) in Gab1 is indicated with a red star. This specific MBS is absent in Gab2 and Gab3. P, proline-rich domain contains binding site for SH3 domain; motifs in red contain potential tyrosine phosphorylation sites for binding SHP-2 tyrosine phosphatase; motifs in blue contain potential tyrosine phosphorylation sites for binding PI3-K

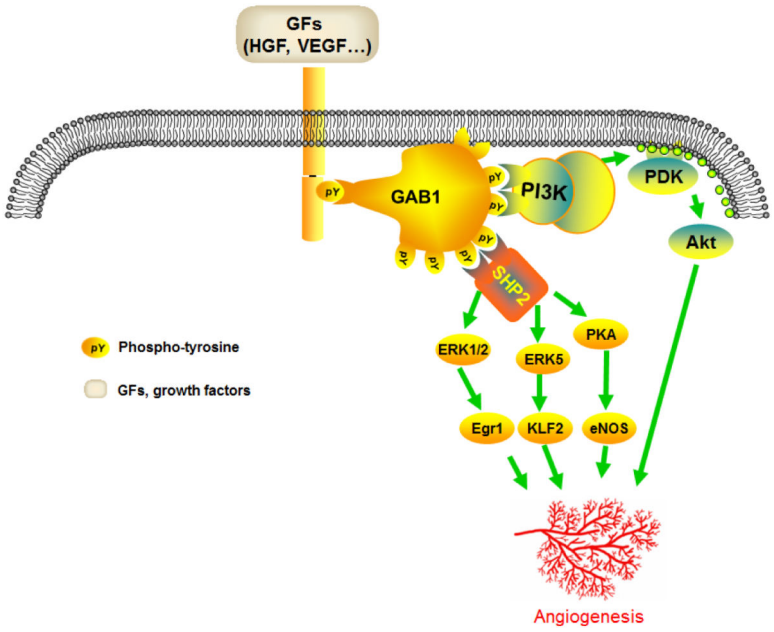

Figure 2. Schematic representation of the role of Gab1 in growth factor signaling and angiogenesis

Growth factors, such as VEGF and HGF stimulate respective receptors on the ECs. Activation of the receptors is associated with phosphorylation of Gab1 and induction of signaling cascades dependent on association of Gab1 with either PI3K (represented by p110/p85 subunits) or the phosphatase SHP2. Downstream targets include Akt (downstream of PI3K), PKA/eNOS, ERK$^{1/2}$/Egr1, and ERK5/KLF2 (all downstream of SHP2). These signaling pathways stimulate EC survival, proliferation, migration, stabilization, and tube formation, thereby contributing to angiogenesis. Collectively, Gab1 serves as the common regulator of ischemia-dependent angiogenesis.

Abbreviations: Egr1, early growth response 1; eNOS, endothelial nitric oxide synthase; FGF2, fibroblast growth factor-2; Gab1-ecKO, endothelial cell-specific Gab1 knockout; HGF; hepatocyte growth factor; HLI, hindlimb ischemia; KLF2, kruppel-like factor 2; VEGF, vascular endothelial growth factor

Table 1

Gab1 as a critical regulator of postnatal angiogenesis.

Studies by	Animal Model	In vitro model	Signaling pathways involved
Zhao et al [41]	Gab1-ecKO+HLI	HGF	SHP2-ERK, PI3K-Akt
Shioyama et al [43]	Gab1-ecKO+HLI; Gab2-KO+HLI; VEGF, HGF gene transfer	HGF; VEGF; FGF2	SHP2-ERK$^{1/2}$-Egr1, SHP2-ERK5-KLF2; PI3K-Akt
Lu et al [42]	Gab1-ecKO+HLI	VEGF	SHP2-PKA-eNOS

Abbreviations: Egr1, early growth response 1; eNOS, endothelial nitric oxide synthase; FGF2, fibroblast growth factor-2; Gab1-ecK0, endothelial cell-specific Gab1 knockout; HLI, hindlimb ischemia; HGF, hepatocyte growth factor; KLF2, kruppel-like factor 2; VEGF, vascular endothelial growth factor.