Review

GAB2—a Scaffolding Protein in Cancer
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Abstract
Adaptor or scaffolding proteins mediate protein–protein interactions that drive the formation of protein complexes. Grb2-associated binding protein 2 (GAB2) scaffolding protein is an intermediary molecule that links plasma membrane receptor signaling including receptor tyrosine kinases with the downstream effectors, such as protein tyrosine phosphatase, nonreceptor type 11 (SHP2), p85 subunit of phosphoinositide-3 kinase (PI3-K), phospholipase C-gamma 1 (PLC-γ), v-crk sarcoma virus CT10 (CRK), Src homology 2 domain containing transforming protein 1 (SHC), and SH2 containing inositol phosphatase (SHIP). Although, well described in signal transduction, its role in cancer has recently been emerging especially in leukemia, breast and ovarian cancer, and melanoma. GAB2 is essential for two major signal transduction pathways in cancer, the PI3-K-AKT and extracellular signal-regulated kinase (ERK) signaling pathways, and thus regulates a number of key cellular processes. This review focuses on structure and function of GAB2, its regulatory proteins, emerging role in cancer, and potential as a therapeutic target. Mol Cancer Res; 10(10); 1265–70. ©2012 AACR.

Introduction
Grb2-associated binding protein 2 (GAB2) is a scaffolding protein that contains various structural domains and docking sites that serve as a platform for the assembly of signaling systems. Although, GAB2 itself lacks enzymatic activity, it acts downstream of receptor tyrosine kinases (RTK) and non-RTKs, such as cytokine and G-protein–coupled receptors, to transmit and amplify signals to downstream effectors. Upon stimulation, GAB2 becomes phosphorylated on critical tyrosine residues creating binding sites for diverse targets involved in signal transduction. As such, GAB2 serves as a mediator of essential cellular processes including proliferation, survival, migration, and differentiation.

GAB2 belongs to a family of evolutionarily conserved proteins consisting of 3 mammalian paralogues, GAB1, GAB2, and GAB3, Drosophila melanogaster homolog DOS (daughter of sevenless), and Caenorhabditis elegans homolog SOC1 (suppressor of clear). Family members show a 40% to 50% sequence homology but are associated with unique cellular functions (1). Both GAB1 and GAB2 are expressed ubiquitously, but they are most highly expressed in brain, kidney, lung, heart, testis, and ovary (2). GAB3 also has a widespread expression pattern, although it is most highly expressed in lymphoid tissue (2). The role of GAB proteins in signal transduction has been extensively reviewed in the literature (2–5). This article will instead focus on GAB2 with an emphasis on its emerging role in human cancer.

Structural Motifs and Binding Partners of GAB2
GAB proteins contain a number of highly conserved structural motifs that mediate their interaction with binding partners including an N-terminal Pleckstrin homology (PH) domain, a central proline-rich domain, and multiple phospho-tyrosine residues (Fig. 1; refs. 3, 6). The PH domain binds to cell membrane phospholipids, preferentially phosphatidylinositol phosphates (7, 8) and plays a role in the membrane localization of GAB2.

The proline-rich domain contains numerous PXXP motifs, which serve as docking sites for Src homology 3 (SH3) domain–containing proteins. GAB2 constitutively associates with the Grb2 adaptor protein through a “canonical” (PXPPPXR) and “atypical” (PXXXRXXKP) SH3 binding motif (9). GRB2 is the main upstream regulator of GAB2 and indirectly recruits it to the activated plasma membrane receptors. These include RTKs (EGFR, KIT), cytokine receptors (IL-1, IL-3, IL-15, TPO, EPO, KITL, M-CSF, Flt310, gp130), Fc receptors (FceR1, FcγR1), T- and B-cell antigen receptors, and G-protein–coupled receptors (2). GRB2 binds GAB2 via its C-terminal SH3 domain, and the complex associates with membrane receptors by binding to phosphorylated tyrosine residues on their intracellular domain (10). Certain receptors do not have GRB2 binding sites, such as the β-chain of IL-2 and IL-3 receptors. These signaling cascades require a SHC protein to serve as an additional bridging adaptor between tyrosine-phosphorylated receptors and the GRB2–GAB2 complex (11, 12).

GAB2 harbors multiple tyrosine residues that are phosphorylated upon activation of signal transduction. These
critical phospho-tyrosine moieties are capable of interacting with Src homology 2 (SH2) domain–containing proteins and mediate the interaction of GAB2 with downstream effectors, such as SHP2, p85, PLC-γ, CRK, SHC, and SHIP (6). GAB2 plays a central role in the propagation of mitogen-activated protein kinase (MAPK) and PI3-K-AKT signaling pathways. Binding of GAB2 to SHP2 activates extracellular signal-regulated kinase (ERK; GAB2–SHP2–ERK) and MAPK signaling. This well-defined relationship is dependent on tyrosine residues Y614 and Y643 in GAB2, which engage the SH2 domain of SHP2 (6). Conversely, docking of the P85 subunit of phosphoinositide-3 kinase (PI3-K) onto Y452, Y476, and Y584 of GAB2 leads to the activation of AKT (GAB2–p85–AKT; ref. 6).

GAB2 forms complexes with a variety of proteins in which functional significance has not been fully characterized. In osteoclasts, PLC-γ2 binds GAB2, is required for GAB2 phosphorylation, and modulates GAB2 recruitment to receptor activator of NF-κB (RANK), thereby regulating osteoclastogenesis (13). 14-3-3 Proteins have an inhibitory effect on GAB2 signaling. They bind to S210 and T391 in a phosphorylation-dependent manner and attenuate GAB2-mediated signal transduction. This may be due to the disassembly of GAB2–GRB2 complexes or shifting of the equilibrium between GAB2–GRB2 and GAB2–14-3-3 complexes favoring the latter (14). A GTP-ase activating protein for the Rho family, named Rho GTPase activating protein 32 (GC-GAP), interacts with both GAB1 and GAB2. GC-GAP is highly expressed in the brain, colocalizes with GAB2 at the cell membrane, and stimulates GTPase activity of the Rho family of small GTPases (15). SHIP-1 and SHIP-2 are SH2 domain–containing inositol 5′-phosphatases that hydrolyze PIP(3,4,5)3 to PIP(3,4)2 and have been shown to associate with GAB2 (16, 17).

Genomic Studies in Cancer

The DNA amplification is a common mechanism leading to oncogenic activation in human cancer. GAB2 is located on chromosomal band 11q14.1. Amplification of 11q13-14.1 is frequently observed in human malignancies (20). CCND1 located on 11q13.2 has long been considered the key gene driving this amplicon (21, 22). However, the large size of 11q13-14.1 (~9–10 Mb), the presence of other potential proto-oncogenes in this region, and the identification of narrow amplicons telomeric and distinct from CCND1 suggest the possibility of multiple drivers underlying these amplifications. Studies have identified several candidates including EMS1, EMSY, PAK1, RSF1, and GAB2 (23).

The identification of GAB2 as a potential oncogene comes from studies in breast (24, 25) and ovarian cancer (23), leukemia (26), and melanoma (27). 11q13-14.1 is amplified in approximately 10% to 15% of breast cancers (28), and GAB2 is overexpressed in human primary breast cancer tumors and cell lines as compared with normal ductal epithelium (29). Eight percent of human breast carcinomas show upregulation of GAB2 by gene expression analysis, and a subset of these have concomitant genomic
amplification (24). Evaluation of DNA copy number changes in 172 primary breast cancers by comparative genomic hybridization shows a proximal amplicon at 11q13.2-13.3 spanning CCND1 in 28% of cases and a distal amplicon at 1q13.5-14.1 with a 15% frequency (25). The distal amplicon occurs without coamplification of CCND1 in 3% of cases, suggesting a distinct driver event. The smallest common region spans ALG8, KCTD21, USP35, and GAB2.

Similar copy number profiles are observed in melanoma. GAB2 was amplified in 11% of 64 human metastatic melanoma samples and 20 cell lines examined by array-comparative genomic hybridization, and a subset occurred without coamplification of CCND1 (27). When taken together, these genomic studies validate the presence of a distinct amplicon in human cancer at 11q13.5-14.1 that is independent of the CCND1 locus. Functional studies implicate GAB2 as a driving event (24, 27).

GAB2 and Breast Cancer

The mechanisms by which GAB2 contributes to breast cancer pathogenesis are beginning to be elucidated (29, 30). GAB2 contributes to an invasive and metastatic phenotype in breast carcinogenesis. Overexpression of GAB2 in MCF10A cells, an immortalized human mammary epithelial cell line, results in increased proliferation and altered dependency on EGF and other growth factors (31). Conversely, silencing of GAB2 in several breast cancer cell lines harboring genomic amplifications leads to a decrease in proliferation, attributing to slowed cell-cycle progression and increased apoptosis, and a reduction in their invasive potential (25).

Studies show that GAB2 alone is insufficient to transform primary mammary epithelial cells. However, it can cooperate with other proto-oncogenes, such as ErbB2 (Neu or HER2) and Src, to potentiate tumorigenic signaling. GAB2 acts downstream of ErbB2 and is tyrosyl-phosphorylated upon activation of signal transduction (24). GAB2 and ErbB2 are coamplified in a subset of breast cancers, and coexpression of GAB2 with ErbB2 leads to an invasive multiaclinar phenotype in a 3-dimensional culture system. This effect is mediated via downstream GAB2–SHP2–ERK signaling and is independent of PI3-K–AKT activation (24). Transgenic mice with mammary-specific overexpression of GAB2 do not show a significant tumor phenotype. However, when crossed with mice overexpressing ErbB2, an accelerated rate of tumor onset is observed (24). Notably, in ErbB2-driven transgenic mouse models, the deletion of GAB2 has only a minor effect on the initiation and growth of mammary tumors but strongly suppresses the development of pulmonary metastases. In this model, GAB2-deficient tumor cells proliferate normally but display impaired motility in vitro, which can be rescued by the introduction of wild-type GAB2. These effects are mediated by impaired activation of ERK/MAPK signaling and occur independently of the AKT pathway (32).

GAB2 not only cooperates with RTK but also with receptor-associated kinases of the SRC family. This relationship was first defined in primary hepatocytes in which GAB2 was shown to be tyrosine-phosphorylated by c-SRC in response to EGF treatment, leading to downstream activation of PI3-K (33). Both GAB2 and c-SRC are overexpressed in breast cancer, and their cotransfection in MCF10A cells promotes a growth advantage and EGF-independence (34). In addition, expression of GAB2 together with the viral oncoprotein v-SRC (35) or the activated c-SRC-Y527F mutant (36) brings about the dissociation of 3-dimensional spheroids in culture, creating a highly dispersed and disrupted acinar morphology. This is thought to be mediated via localized GAB2–SRC–PI3-K signaling at the cell–cell junction, which disrupts the integrity and binding strength of E-cadherin with resultant loss of cellular adhesion (34). Furthermore, these cells show increased migration and invasion, but these effects are independent of PI3-K activation (34). Finally, overexpression of GAB2 in MCF10A cells promotes anchorage-independent growth, leading to larger and more numerous colonies on soft agar. These effects are dependent on SRC signaling and the downstream activation of the oncogenic transcription factor, STAT3 (37).

Additional insight into the role of GAB2 on cell motility, invasiveness, and thus metastatic spread is obtained through studies examining its effects on Rho family of GTPases. RhoA promotes the formation of contractile actomyosin stress fibers and focal adhesions. GAB2, when overexpressed in MCF10A mammary epithelial cells, was found to decrease activation of RhoA, lead to delayed cell spreading, a decrease in stress fibers and mature focal adhesions, and enhanced cell migration (38). Modulation of focal adhesions and stress fibers by GAB2 was dependent on the SHP2 binding sites. These studies shed light onto mechanisms of GAB2-mediated cancer progression.

GAB2 signaling in breast cancer results in the activation of numerous downstream effectors that contributes to cancer development. A genomic "signature" of 205 transcripts was obtained from GAB2 overexpressing, anchorage-independent MCF10A cells and was found to correlate with metastatic relapse in patients, independent of existing clinical and genomic classifiers (37). This suggests a novel role for GAB2 and its transcriptional targets as a biomarker for the prediction of metastatic disease.

miRNAs posttranscriptionally repress gene expression mostly by recognizing complementary target sites in the 3'-untranslated region of target mRNAs. The human let-7 miRNA family consists of 13 members located in 8 genomic regions frequently deleted in human cancers. Among the let-7 family of miRNAs, let-7g was found to have reduced expression that correlated significantly with lymph node metastasis and poor survival in patients with breast cancer (39). Repression of let-7g expression led to increased GAB2 and fibronectin 1 expression, which, in turn, cooperatively increased the activity of ERK, matrix metalloproteinase (MMP-2), and MMP-4. This exemplifies yet another mechanism of GAB2 regulation in breast carcinogenesis.

GAB2 and Leukemia

GAB2 regulates hematopoietic cell survival and proliferation. Although, GAB2 knockout mice are viable and grossly
normal in appearance, they harbor certain select defects as a result. These mice lack a proper allergic response, which is the result of impaired FcεRI stimulation in mast cells and is PI3-K dependent (40). Their bone marrow is osteoprotic due to the decreased RANK-mediated osteoclast differentiation (41) and, while their bone marrow cellularity and peripheral blood counts are normal, there is defective hematopoiesis as a result of decreased proliferation and attenuation of PI3-K- Akt and ERK/MAPK signaling in response to early-acting cytokines (42). In addition, GAB2 ablation results in defective expansion of colony stimulating factor 1 receptor–dependent mononuclear phagocyte progenitors in the bone marrow through decreased proliferation and survival (43).

GAB2 mediates a critical role in leukemogenic signaling driven by oncogenic fusion tyrosine kinases. This has been best studied in chronic myelogenous leukemia (CML), which is caused by a translocation between chromosomes 9 and 22; t(9;22)(q34;q11), creating BCR-ABL, a constitutively active fusion tyrosine kinase capable of transforming hematopoietic stem cells (44). The autophosphorylated Tyr177 residue on BCR-ABL recruits the GRB2–GAB2 complex, which can then activate several divergent pathways involved in promoting leukemogenesis, including RAS–RAF–MEK–ERK, PI3-K–AKT, and JAK-STAT (45). GAB2 is required for myeloid cell, but not lymphoid cell, transformation by BCR-ABL. Cells expressing mutant BCR–ABL–T177F show impaired ERK and AKT activation with diminished proliferation and spontaneous migration (45). In addition, silencing of GAB2 or its downstream effectors, SHP2 and STAT5, leads to a decrease in cell proliferation and colony formation in BCR-ABL–mediated CML (46). Notably, GAB2 has a similar function in propagating oncogenic signaling derived from other fusion oncoproteins including TEL-ABL (47), TEL-JAK2 (48), NPM-ALK (49), and BCR-FGFR1 (50) as well as in SF-STK activation by Friend virus in murine erythroleukemia (51).

Treatment of CML with imatinib, a small-molecule inhibitor of BCR-ABL, has led to significant clinical success. However, resistance mechanisms have evolved including continuous activation of v-yes-1 Yamaguchi sarcoma viral related oncogene homolog (LYN) kinase, which complexes with GAB2 and results in persistent phosphorylation of GAB2 and BCR–ABL. Downstream leukemogenic signaling persists in the presence or absence of imatinib, and it is only in silencing of LYN that drug sensitivity is restored (52).

Persistent activation of the transcription factor STAT5 is frequently found in hematologic malignancies. GAB2 signals upstream of JAK-STAT and is an essential mediator of hematopoietic stem cell homeostasis (6). STAT5 and GAB2 double mutant mice show a significant reduction in stem cell number, reduced cell survival, and a dramatic loss of self-renewing potential (53). On the other hand, GAB2-mutated PI3-K–Akt activation potentiates cell growth and survival in STAT5–induced leukemia (54).

GAB2 is a key mediator of leukemogenic signaling driven by SHP2 mutations. Somatic gain of function mutations in SHP2 causes juvenile myelomonocytic leukemia (55). GAB2 is a direct binding partner of SHP2 and is required for the transformation of primary murine myeloid cells secondary to its ability to activate ERK, AKT, and STAT5 signaling (56). A knockin mouse model with a SHP2D61G–activating mutation leads to hyperactive stem cell functioning with accelerated stem cycling, expansion of the stem cell pool, and improved repopulating capacities, which can bring about the myeloproliferative disease. This aberrant hematopoietic signaling is rescued by the concomitant deletion of GAB2, which highlights this adaptors centrality in promoting oncogenesis (57).

GAB2 and Melanoma

The development of melanoma is inextricably linked to oncogenic activation of ERK and PI3-K–Akt signaling (58, 59). GAB2 scaffolding protein is central to the propagation of these signaling cascades and has been implicated as a driver of melanomagenesis. GAB2 is expressed at significantly higher levels in metastatic melanomas as compared with primary melanomas and melanocytic nevi, and can thus be seen as a marker of neoplastic progression. Silencing of GAB2 in metastatic melanoma cell lines leads to a decrease in their invasive potential, whereas overexpression in primary melanomas promotes migration and invasion. In addition, in vivo studies show that overexpression of GAB2 leads to enhanced tumor growth and development of metastases (27).

Although GAB2 cannot independently transform melanocytes, it can cooperate with other oncogenes to promote tumor development. GAB2 is coexpressed with mutant NRAS in a subset of melanomas, and these proteins act in concert to promote a more aggressive phenotype. GAB2 overexpression leads to increased metastatic potential with anchorage independence in soft agar, and xenograft studies show angiogenic switch as a result of upregulation of HIF-1α and VEGF (60).

GAB2 and Ovarian Cancer

Genomic amplifications of GAB2 have been described in approximately 15% of ovarian cancers (23). GAB2 overexpression is also seen at the mRNA level in serous cystadenocarcinoma tumors and at the protein level in several ovarian cancer cell lines (61). Exogenous overexpression of GAB2 in cell lines with low baseline expression promotes characteristics of the epithelial-to-mesenchymal transition. These cells have increased migratory and invasive potential, assessed by wound healing and transwell migration assays, and show downregulation of E-cadherin. Conversely, silencing of GAB2 in cell lines with high expression inhibits migration and invasion and causes upregulation of E-cadherin. GAB2 mediates these effects by activating the PI3-K pathway, which results in increased expression of Zeb1, a transcription factor involved in epithelial-to-mesenchymal transition (61). This novel GAB2–PI3-K–Zeb1 pathway can be targeted by PI3-K and mTOR inhibitors and can perhaps serve as a therapeutic target in the treatment of GAB2-driven ovarian cancer.
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No potential conflicts of interest were disclosed.

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