The Rho family of small GTPases, including Rho, Rac, and Cdc42, play essential roles in diverse cellular functions. The ability of Rho family GTPases to participate in signaling events is determined by the ratio of inactive (GDP-bound) and active (GTP-bound) forms in the cell. The activation of Rho family proteins requires the exchange of bound GDP for GTP, a process catalyzed by the Dbl family of guanine nucleotide exchange factors (GEFs). The GEFs have high affinity for the guanine nucleotide-free state of the GTPases and are thought to promote GDP release by stabilizing an intermediate transition state. In this study, we have identified and characterized a new Rac/Cdc42-specific Dbl family guanine nucleotide exchange factor, named GEFT. GEFT is highly expressed in the excitable tissues, including brain, heart, and muscle. Low or very little expression was detected in other nonexcitable tissues. GEFT has specific exchange activity for Rac and Cdc42 in our in vitro GTPase exchange assays and glutathione S-transferase-PAK pull-down assays with GTP-bound Rac1 and Cdc42. Overexpression of GEFT leads to changes in cell morphology and actin cytoskeleton re-organization, including the formation of membrane microspikes, filopodia, and lamellipodia. Furthermore, expression of GEFT in NIH3T3 cells promotes foci formation, cell proliferation, and cell migration, possibly through the activation of transcriptional factors involved in cell growth and proliferation. Together, our data suggest that GEFT is a Rac/Cdc42-specific GEF protein that regulates cell morphology, cell proliferation, and transformation.

Received for publication, August 30, 2002, and in revised form, December 11, 2002
Published, JBC Papers in Press, January 23, 2003, DOI 10.1074/jbc.M208896200
PH domains have been found and invariably follow the DH domain in the Dbl family of proteins. PH domain contains ~100 amino acids and has been found in a number of proteins (40, 41). Although DH-associated PH domains promote the translocation of Dbl-related proteins to plasma membranes (42, 43), PH domains have been shown to participate directly in GTPase binding and regulation of GEF activity in the presence or absence of phosphoinositides (28, 34, 44, 45).

Two of the well characterized effectors of Rac/Cdc42 GTPases are the PAK family of serine/threonine kinases and the WASP members of the Dbl family: Tiam-1, Kalirin (Duo), and UNC-73 (64, 65, 67, 76). Bold letters indicate identical amino acids.

**MATERIALS AND METHODS**

**DNA Constructs**—The mouse GEFT fragment was initially identified by enhanced retroviral mutagen (ERM) strategy (53). Briefly, we constructed enhanced retroviral mutagen (ERM) vectors that contained several engineered sequences (e.g. an ERM tag and a splice donor) controlled by a tetracycline-responsive promoter. The ERM vectors were introduced into the NIH3T3 cells. Endogenous genes can thus be randomly activated and tagged in a conditional system. NIH3T3 cells were used to screen for focus-forming genes using the ERM strategy. Full-length cDNAs encoding human GEFTs were obtained by screening human brain library (Clontech). For mammalian expression, cDNAs encoding GEFT were inserted into the HindIII and SalI sites of pCMV-Tag2B (Stratagene), respectively, to produce three GST-fused pEGX vectors. Expression and Purification of Recombinant GEFT and GTPases—Bacterially expressed His6-tagged GEFT protein and GST fusion GTPases were purified according to the standard procedures of the manufacturers. Escherichia coli strain BL21 was transformed by pQE-GEFT and pGEX vectors, respectively, grown to midlog phase at 37 °C, and then induced with 1 mM isopropyl-β-D-galactopyranoside for 3–4 h. Two of the well characterized effectors of Rac/Cdc42 GTPases were the PAK family GTPases, Cdc42, Rac1, and RhoA, which were subcloned into the BamHI and SalI sites of pGEX-4T-1, a GST gene fusion vector (Amersham Biosciences), respectively, to produce three GST-fused pGEX vectors.

**Expression and Purification of Recombinant GEFT and GTPases**—Bacterially expressed His6-tagged GEFT protein and GST fusion GTPases were purified according to the standard procedures of the manufacturers. Escherichia coli strain BL21 was transformed by pQE-GEFT and pGEX vectors, respectively, grown to midlog phase at 37 °C, and then induced with 1 mM isopropyl-β-D-galactopyranoside for 3–4 h. Two of the well characterized effectors of Rac/Cdc42 GTPases were the PAK family GTPases, Cdc42, Rac1, and RhoA, which were subcloned into the BamHI and SalI sites of pGEX-4T-1, a GST gene fusion vector (Amersham Biosciences), respectively, to produce three GST-fused pGEX vectors.
Northern blot analysis of GEFT expression in multiple human tissues using multiple tissue Northern blot membrane containing premade poly(A)^+ RNA (Clontech Inc.). The membrane was hybridized with 32P-labeled GEFT and β-actin probes, respectively. A single band at ~3 kilobases (kb) was detected.

exchange reactions were initiated by the addition of excess free 400 μM GTP (final concentration). At different time points, the reactions were terminated by filtration of 20 μl of the mixtures through nitrocellulose filters. And the filters were washed twice with the ice-cold buffer B. The amount of the radionucleotides remaining bound to the Rho GTPases (RhoA, Rac1, and Cdc42) were quantified by scintillation counting, and normalized as the percentage of [3H]GDP bound at time 0. For each time point, the samples were assayed in triplicate.

Cell Culture, Transfection, and Transformation Assays—HeLa, COS-7, and NIH3T3 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Cell transfection was performed using LipofectAMINE (Invitrogen) as previously described according to the manufacturer’s instructions (49). Cells were then allowed to grow for 48 h. For each assay, control vector encoding LacZ was used as a control.

For foci formation analyses, infected NIH3T3 cells were maintained in growth medium for 12–14 days and assayed as previously described (56). Briefly, NIH3T3 cells were infected with GEFT virus or a vector (pMSCV2.1) control virus for 2 days. Cells were then washed with PBS, counted, and plated as shown in 6-well plates coated with 1 μg/ml collagen. 5 ml of Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum was added and changed every 3 days. Cells were allowed to grow for 14 days in a 37 °C incubator with a 95:5, air/carbon dioxide mixture. At the end of 14 days, cells were washed once with PBS and stained with crystal violet (0.5%), and the number of foci of transformed cells was then quantitated. Total number of foci in each well was counted, and plated as shown in 6-well plates coated with 1 μg/ml collagen.

Northern Blotting Analysis of GEFT Expression in Human Tissues—To study the expression patterns of GEFT in different human tissues, a RNA filter comprising poly(A)-selected RNAs of multiple cell lines of human tissues (Clontech, Inc.) was hybridized with specific 32P-labeled cDNAs as described previously (49, 58). In brief, human GEFT probe were radiolabeled with [α-32P]CTP by nick translation using random primers. Probes (~4 × 10^7 cpm/μg) were hybridized with the RNA filter and analyzed according to manufacturers protocol.

Immunoprecipitation, Immunoblotting, Immunocytochemistry, and Fluorescence Imaging—Immunoprecipitation of individual proteins was performed using enhanced chemiluminescence reagents from Promega as described previously (58). The reporter constructs for AP-1-Luc and SAP1-luc were obtained from Dr. K. L. Guan at the University of Michigan. The data presented are the mean of three independent assays.

Fluorescence Imaging—To study the expression patterns of GEFT in different human tissues, a RNA filter comprising poly(A)-selected RNAs of multiple times.

Northern Blotting Analysis of GEFT Expression in Human Tissues—To study the expression patterns of GEFT in different human tissues, a RNA filter comprising poly(A)-selected RNAs of multiple

Fluorescence Imaging—To study the expression patterns of GEFT in different human tissues, a RNA filter comprising poly(A)-selected RNAs of multiple

Human Complexes were resolved by SDS-PAGE and subjected to immunoblotting for interacting proteins.

FIG. 2. Expression of GEFT in human tissues. Northern blot analysis of GEFT expression in multiple human tissues using multiple tissue Northern blot membrane containing premade poly(A)^+ RNA (Clontech Inc.). The membrane was hybridized with 32P-labeled GEFT and β-actin probes, respectively. A single band at ~3 kilobases (kb) was detected.

FIG. 3. GEFT preferentially activates the guanine nucleotide exchange activities of Rac1 and Cdc42. Stimulation of GDP dissociation from RhoA, Rac1, and Cdc42 by GEFT was performed using purified bacterial expressed proteins. Time-dependent study for the dissociation of [3H]GDP from purified recombinant GST-RhoA (A), GST-Rac1 (B), and GST-Cdc42 (C) in the presence or absence of Histagged GEFT. Experiments were performed in triplicate. Shown are representatives of three independent assays.

A.

RhoA GTPases Exchange Assay

No GEF-T

GEF-T

--- [3H]GDP Remaining (%%)

0 0.1 1 2 8

Time (Min)

B.

Rac1 GTPases Exchange Assay

No GEF-T

GEF-T

--- [3H]GDP Remaining (%%)

0 0.1 1 2 8

Time (Min)

C.

Cdc42 GTPases Exchange Assay

No GEF-T

GEF-T

--- [3H]GDP Remaining (%%)

0 0.1 1 2 8

Time (Min)
4% paraformaldehyde for 20 min, blocked with 10% bovine serum albumin, and incubated with monoclonal antibody against FLAG (M2 monoclonal, Sigma). Actin filaments were labeled with rhodamine-conjugated phalloidin (Molecular Probes). Double-label immunostaining was done with appropriate fluorochrome-conjugated secondary antibodies. Fluorescent images of cells were captured on a CCD camera mounted on an Olympus inverted research microscope using Ultraview imaging software (Olympus, Inc.).

Binding of GEFT to Rho GTPases and GST-p21-binding Domain Pull-down Assays—To determine GEFT binding affinity to the Rho GTPases, 20 μg of His-tagged protein was incubated at 4 °C overnight with 20 μl of GSH-agarose beads loaded with 20 μg of each Rho GTPase, Cdc42, Rac1, or RhoA in the absence of guanine nucleotides. The beads were washed three times with PBS. The bound proteins were separated by SDS-PAGE, and His-tagged GEFT proteins were detected by Western blotting using an anti-His monoclonal antibody (Santa Cruz Biotechnology).

GTPase activation assays in the cells were performed by GST-p21-binding domain pull-down assays as described previously (59–61). Briefly, cells transfected with GEFT or a control plasmid (pCMV-LacZ) were washed and lysed on the dish in 50 mM Tris (pH 7.5), 500 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 10% glycerol, 10 μg/ml leupeptin and aprotonin, and 1 mM phenylmethylsulfonyl fluoride. GTP-bound Rac1 or Cdc42 was pulled down using the GST-p21-binding domain of PAK1 immobilized on glutathione beads. The amount of active Rac1 and Cdc42 (GTP-bound form) was detected by Western blot using specific antibodies against Rac1 and Cdc42, respectively.

Cell Proliferation Assay—Proliferation studies were carried out using the CellTiter96 AQueous One solution cell proliferation assay (Promega). Briefly, cells were transfected with GEFT or a control plasmid. Cells were plated at 500 cells/well and allowed to adhere to the plate. At the indicated time points, the AQueous One solution was added to the samples and measured at 490 nm.

Boyden Chamber Cell Migration Assays—Cell migration/motility assays were examined using modified Boyden chambers, as described previously (62, 63). Briefly, NIH3T3 cells were stably transfected with GEFT or vector (pCMV-Tag2B). The outside of the filters was coated with 1 μg/ml collagen for 1 h and then washed three times with PBS. Filters were then incubated with Dulbecco’s modified Eagle’s medium with bovine serum albumin for 1 h. Filters were then put into Dulbecco’s modified Eagle’s medium without fetal bovine serum and with 0.5% leupeptin and aprotinin, and 1 mM phenylmethylsulfonyl fluoride. GTP-bound Rac1 or Cdc42 was pulled down using the GST-p21-binding domain of PAK1 immobilized on glutathione beads. The amount of active Rac1 and Cdc42 (GTP-bound form) was detected by Western blot using specific antibodies against Rac1 and Cdc42, respectively.

RESULTS

Identification, Expression, and Domain Structures of GEFT—To identify genes responsible to tumorigenesis, an ERM strategy was used to screen for foci-forming genes in NIH3T3 cells (53). One of the novel genes, mutagenized by the ERM, has shown strong oncogenic activity and was identified by reverse transcriptase-PCR and direct sequencing. The gene product shows sequence homology to the Dbl family of GEFs, and was named GEFT. Subsequently, we cloned the human and mouse GEFT full-length open reading frame by reverse transcriptase-PCR and by 5’- and 3’-rapid amplification of cDNA ends. The mouse GEFT (mGEFT) sequence is 90% identical to the human GEFT (hGEFT) (Fig. 1A). In contrast to hGEFT, mGEFT possesses an extra NH2-terminal domain. Like other family members of the Dbl proteins, GEFT has an NH2-terminal Rho exchange factor domain (Dbl homology domain, called DH domain) and is followed by a PH domain (Fig. 1B). Sequence alignment of Dbl domains from hGEFT and other Dbl-containing proteins shows significant homology in this region, suggesting that GEFT is a potential exchange factor for the Rho family (RhoA, Rac1, and Cdc42) of GTPases (Fig. 1C). A data base search found that GEFT shows 35% sequence identity with human Huntingtin-associated protein-interacting protein (Duo protein) or the spectrin-like Kallirin (64, 65). GEFT also shares 35% and 60% sequence homology with the first and second DH domains of human protein Trio, respectively (66). GEFT contains 13 exons and is localized in chromosome 12q13.11, a region frequently amplified in sarcomas and brain tumors. In searching the single nucleotide polymorphisms database, we found two single nucleotide polymorphisms in the coding region of the GEFT protein: one at exon 10 with G to A substitution without an amino acid (Leu) change; and the other in exon 12 with nucleotide change A to G and an amino acid change Gln to Arg (Q401R). The potential role of these single nucleotide polymorphisms in the protein is not clear at this moment.

To examine the expression of GEFT in human tissues, a Northern blot analysis with multiple tissue membrane (Clontech) was performed. As shown in Fig. 2, we detected one main transcript of ~3 kb, with highest expression in human brain, heart, and muscle, and less extent in small intestine, colon, liver, placenta, and lung. Weak or no expression was found in the examined immune tissues (Fig. 2). In accordance with the
predicted size, a protein with a relative molecular mass of 53,000 (53 kDa) was identified with anti-FLAG tag monoclonal antibody (M2, Sigma) in cells transfected with GEFT protein (data not shown).

Specific Activation of Rac/Cdc42 by GEFT via Direct Interaction—To identify the Rho family of proteins that are activated by GEFT, we constructed, expressed, and purified a bacterially expressed hexahistidine-tagged human and mouse GEFT protein (DH-PH domain). Then, we analyzed the guanine nucleotide exchange activity of GEFT protein on the incorporation of cold GTP into [3H]GDP-loaded RhoA, Rac1, and Cdc42, respectively (Fig. 3). As shown in Fig. 3, RhoA, Rac1, and Cdc42 alone did not show significant intrinsic GDP dissociation over the time period tested (8 min). However, addition of GEFT to the reaction stimulated rapid and complete dissociation of [3H]GDP from Cdc42 and Rac1 within 2–5 min. In contrast, only 10–15% of the [3H]GDP was released from RhoA after the same time period. As a control, we also examined the GEF exchange activities of Vav2 (DH domain) and Tiam-1 (DH-PH domain), members of the Dbl family, on their respective exchange activities of GTPases, Cdc42, and Rac1 (43, 56, 67). We found that GEFT had demonstrated similar exchange activities on Rac1 and Cdc42, as did Vav2 and Tiam-1, respectively. However, GEFT had much less exchange activity to RhoA compared with Vav2 (56). No guanine nucleotide exchange activity was found for H-Ras in our control experiment (data not shown). Taken together, our data suggest that GEFT preferentially activates the release of GDP from Rac and Cdc42 proteins, and to a much less extent to RhoA.

To further confirm that GEFT activates Rac1 and Cdc42 in the cells, we compared the amount of GTP-bound forms (active status) of Rac1 and Cdc42 in cells transfected with GEFT or a control plasmid. To determine the level of GTP-bound Rac1 and Cdc42 in the cells, we utilized a GST-PAK1 fusion protein containing the Rac1/Cdc42-binding domain as an affinity reagent in a GST pull-down assay (59–61). PAK1 is a downstream effector of Rac1 and Cdc42, and PAK1 binds preferentially to the active, GTP-bound forms of Rac1 and Cdc42 GTPases. As shown in Fig. 4A, transfection of GEFT in COS-7 cells increased the Rac1-GTP level at least 5-fold compared with cells transfected with a control plasmid (pCMV-tag2B). We also found a ~3-fold increase in Cdc42-GTP levels in cells transfected with expression plasmid encoding GEFT (Fig. 4A, right). Together, these results suggest that GEFT activate Rac1 and

Fig. 5. GEFT induces the formation of membrane microspikes, filopodia, and lamellipodia in the cells. A and B, formation of membrane microspikes in cells transfected with FLAG-tagged GEFT. C and D, induction of filopodia and lamellipodia in transfected HeLa cells. Immunostaining of GEFT and actin cytoskeleton in HeLa cells transfected with pCMV-Flag-GEFT are shown. Cells were plated on fibronectin-coated coverslips, then fixed and immunostained with specific anti-FLAG monoclonal antibody M2 (Sigma) for the FLAG-GEFT (left). Actin cytoskeleton was stained with rhodamine-phalloidin (middle). Shown in the right are overlay pictures of GEFT (left) and actin cytoskeleton (middle).
Cdc42 in the cell by stimulating the guanine nucleotide exchange of the two GTPases (Rac1 and Cdc42).

GEFs can be distinguished from other GTPase-interacting proteins by their ability to bind preferentially to the nucleotide-depleted state of small GTPases compared with GTP- or GDP-bound states (29, 36). To test whether GEFT can directly bind to Rac1, Cdc42, and RhoA, GST fusion protein pull-down assays were performed with GST-Rac1, GST-Cdc42, and GST-RhoA, in the absence of GTP. As shown in Fig. 4B, Cdc42 and Rac1 bound His-tagged human GEFT protein in the absence of GTP, but not GST-RhoA protein. These data suggest that GEFT activate the guanine nucleotide exchange activities of Rac1 and Cdc42 through direct protein-protein interactions.

GEFT induces formation of Filopodia and Lamellipodia, and Actin Cytoskeleton Rearrangement by Activating Cdc42 and Rac1 GTPases in the Cells—Previous studies demonstrate that in fibroblasts, Rac and Cdc42 induced the formation of lamellipodia and filopodia, respectively, whereas RhoA promotes stress fiber formation (3). We examined the effects of GEFT overexpression on the actin cytoskeleton reorganization in HeLa cells. As shown in Fig. 5, overexpression of GEFT in HeLa cells caused actin cytoskeleton rearrangement, an induction of membrane spikes and filopodia, a characteristic of Cdc42 activation by the GEFT protein (Fig. 5, A–C). In addition, cells expressing GEFT displayed some membrane ruffling and formation of lamellipodia (Fig. 5D), suggesting activation of the Rac1 protein in the GEFT-transfected cells. The fact that GEFT caused the induction of filopodia, microspikes, and lamellipodia supported the possibility that overexpression of
GEFT is associated with constitutive up-regulation of Cdc42 and Rac1 function.

**GEFT Induces Foci Formation and Contact-independent Colony Growth in NIH3T3 Cells**—By abrogating normal contact inhibition, some of the tumorigenic proteins have the ability to form foci of piled up transformed cells on a background monolayer of untransformed cells. To determine whether GEFT protein has the transformation activity, we infected NIH3T3 cells with retrovirus vector-encoding GEFT. As shown in Fig. 6, overexpression of GEFT induces the transformed foci formation in NIH3T3 cells, whereas vector alone has no effect on the cells (Fig. 6A). The GEFT-induced foci contain densely packed non-refractile cells, which is different from foci induced by Ras, similar to foci formed by active Rho family of proteins (Fig. 6B and C). Similar to the foci induced by another guanine nucleotide exchange factor, Vav, we observed no multinucleated giant cells for GEFT-induced foci. The number of foci formed by GEFT in NIH3T3 cells was examined at three different cell densities. As expected, GEFT induced foci formation at all three densities (Fig. 6D), suggesting a stronger transforming ability for the GEFT protein. The transforming activity of GEFT protein, together with its ability in activation of transcription factors, suggest that this protein may play an important role in cell proliferation and tumorigenesis.

**GEFT Expression Leads to Cell Proliferation and an Increase in Cell Motility**—Proliferative signaling has been associated with polypeptide growth factor receptors that possess an intrinsic protein-tyrosine kinase activity as well as many G protein-coupled receptors, including thrombin, bombesin, bradykinin, substance P, endothelin, serotonin, acetylcholine, prostaglandin F2α, and lysophosphatidic acid, in a variety of cell types (reviewed by Gutkind (77)). The effect of GEFT on cell transformation prompted us to examine the role of GEFT in cell proliferation and cell motility/migration. To understand the potential role of GEFT in cell proliferation and tumorigenesis, we examined cell proliferation in cells stably transfected with the mouse GEFT vector using the CellTiter 96 (Promega) assay. Fig. 7A shows that expression of GEFT in NIH3T3 cells significantly increased cell proliferation compared with cells expressed in vector only, suggesting that GEFT can induce cell proliferation and tumorigenesis.

The effect of GEFT on cell morphology also prompted us to examine whether expression of this protein changes cell migration and leads to an increase in cell motility. In our experiments, we generated NIH3T3 cells stably transfected with GEFT and a control vector. When the cells were placed in modified Boyden chambers coated with collagen, the cells expressing GEFT migrated much faster than the ones expressing the vector alone (Fig. 7B). The number of cells that migrated increased 2–3-fold in NIH3T3 cells expressing GEFT, suggesting that GEFT mediates cell motility via the activation of Rac/Cdc42 proteins.

**GEFT Activate Rac/Cdc42-mediated Transcriptional Activities**—To further examine the signaling pathways activated by GEFT, we examined the ability of GEFT to stimulate Rac1- and Cdc42-mediated signaling pathways and transcription factors. It has been shown that activation of the Rho family of small GTPases leads to the activation of a number of transcriptional factors in cell growth and proliferation, including the c-fos serum response element (SRE) and other transcription factors. The SRE forms a ternary complex with the transcription factor serum response factor and ternary complex factors, such as Elk1 and SAP1. Activation of the Rho family of GTPases, RhoA, Rac1, and Cdc42 leads to transcriptional activation via serum response factor and act synergistically at the SRE with signals that activate ternary complex factors (12). To test whether GEFT directly affects the activation of transcription factors at the SRE, we measured the activation of GEFT on transcription factors in our cell-based transfection assays. COS-7 cells were transfected with luciferase reporter genes controlled by SRE, Elk1, and SAP1, together with the expression plasmids encoding RhoA, Rac1, and Cdc42. As shown in Fig. 8A, transfection of GEFT, together with Cdc42 and Rac1, dramatically increased SRE-luciferase activity ~100- and ~150-fold, respectively. GEFT also moderately activated RhoA mediated SRE activity in our transcriptional reporter assay (Fig. 8A). To further examine the effects of GEFT on ternary complex factor-linked signaling pathway, we measured the activation of Ets domain transcription factors, Elk1 and SAP1, members of the ternary complex factor complex. Similar to the activation of SRE, cotransfection of GEFT with Rac1 and Cdc42 significantly stimulated the transcriptional activities of Elk1 and SAP1 (data not shown). Whereas the stimulation of GEFT on RhoA-mediated activation was significantly lower compared with Rac1 and Cdc42.

In most cell types, activation of Rac1 and Cdc42, but not...
RhoA, leads to the stimulation of JNK activity, and consequently, the activation of the AP-1 and c-Jun transcription factors (11, 22). To investigate the effects of GEFT on the stress-regulated JNK signaling pathway, we examined the ability of GEFT to stimulate c-Jun and AP-1 transcriptional activities using a transient transcriptional reporter assay in COS-7 cells. We transfected the control vector or vectors encoding wild type RhoA, Rac1, and Cdc42 in the presence or absence of GEFT, and then assessed the transcriptional activation of c-Jun and AP-1. As shown in Fig. 8, coexpression of GEFT with Rac1 and Cdc42 significantly induced the activation of the c-Jun luciferase reporter gene, whereas cotransfection of GEFT with RhoA had little stimulation of the reporter genes. Also, similar activation of AP-1 was obtained with AP1-luciferase reporter assays (data not shown). Together, these data suggest that GEFT strongly stimulates the JNK signaling pathway and its related transcriptional factors by activating the small GTPases, Rac1 and Cdc42 in the cells.

DISCUSSION

GEFs regulate GTP-binding and regulatory proteins by converting GTPases to their biologically active state by catalyzing the exchange of bound GDP for GTP. The Dbl family of proteins are Rho-specific GEFs that contain a Dbl domain followed by a PH domain. In our search of genes involved in tumorigenesis using the ERM strategy, we identified a new partial sequence encoding a new guanine nucleotide exchange factor and strongly promoting foci formation in NIH3T3 cells, and the gene was named GEFT (53). In this research, we cloned the full-length genes encoding the human and mouse GEFT and characterized the roles of this protein in the Rho family of small GTPases and their signaling pathways.

We found that GEFT preferentially activated Rac1 and Cdc42 and promoted the guanine nucleotide exchange of Rac1 and Cdc42 GTPases whereas relatively low activity was observed with RhoA GTPase in our in vitro exchange assays. Moreover, GEFT activated Rac1/Cdc42-mediated transcriptional activities of SRE, Elk1, and SAP1 in our transcriptional reporter gene assays. Furthermore, we demonstrated that GEFT significantly induced the activation of c-Jun and AP-1 transcription factors, downstream targets of the JNK mitogen-activated signaling pathway that are activated by Rac1 and Cdc42, but not RhoA (11, 22). Therefore, GEFT may function as a specific activator for Rac1 and Cdc42 small GTPases in the cells.

The Rho family proteins have been shown to regulate actin cytoskeletal re-organization, and therefore, influencing cell shape, morphology, adhesion, cell migration, and motility (3, 6). For example, RhoA promotes the formation of actin stress fibers and focal adhesions, whereas Rac1 induces the formation of lamellipodia and membrane ruffling. On the other hand, Cdc42 promotes formation of microspikes on cell surface and filopodia development in the cells. In our experiments, we observed that expression of GEFT promotes cell morphology change and actin cytoskeleton reorganization. Like activation of Cdc42 and Rac1, we found that GEFT induced the formation of microspike, filopodia, and lamellipodia structure in cells overexpressing the proteins, suggesting that GEFT activates Rac1- and Cdc42-mediated signal pathways in the cells.

The fact that GEFT is highly expressed in the brain and the heart, and that GEFT is localized on chromosome 12q13.11, a region frequently amplified in sarcomas and brain tumors, suggests that the protein may play a potential role in brain tumor and other tumors. In this study, we demonstrated that overexpression of GEFT in NIH3T3 cells strongly induced the formation of foci, similar in morphologic appearance to the ones induced by activated Rac and Cdc42 signaling pathway, suggesting the tumorigenic potential of the GEFT protein. We also demonstrated that GEFT strongly activated a number of transcription factors that mediated the expression of genes involved in cell growth, proliferation, and survival. These cellular functions are important in tumorigenesis. The exact roles of this protein in brain tumor and other tumors are under further investigation.

There is a great deal of evidence that the Rho family of GTPases play an important role in neuronal morphogenesis (7, 22). During neuronal development, neuronal precursor cells migrate and then differentiate, extending axons and dendrites to specific regions to form synapses with appropriate target cells. Several GEFs, such as Tiam-1, Trio, Ephexin, and Kali-rin, have been implicated in neuronal morphogenesis, growth cone guidance, and neuronal dendritic spines (68–72). We have demonstrated that GEFT is highly expressed in human brain, and GEFT can induce the formation of lamellipodia, microspikes, and filopodia in the cells. The potential roles of this brain-specific GEF protein in neuronal morphogenesis and differentiation, axon guidance, and dendritic spines are under investigation.

A variety of extracellular stimuli have been found to activate the Rho family of GTPases, including growth factors, cytokines, lysophosphatidic acid, interleukins, and matrix components via receptor tyrosine kinases, G protein-coupled receptors, and integrin receptors, respectively. How different signaling pathways directly link the extracellular stimuli to the intracellular signal change and consequently, gene expression, is one of the key questions. Protein phosphorylation or membrane association and protein-protein interaction through the PH domain of GEFs has been shown to activate GEF proteins upon stimulation by extracellular stimuli (44, 45, 73–75). Domain and motif analysis of the GEFT protein indicates that GEFT protein has a PH domain directly after the guanine nucleotide exchange domain and several potential protein phosphorylation sites that can be regulated by pathways coupled to different cellular protein kinases.

In summary, we have identified and characterized a guanine nucleotide exchange factor, preferentially for Rac1 and Cdc42, named GEFT. The protein is highly expressed in human brain and heart, but very weakly in other tissues. Overexpression of GEFT in the cell induces cell morphology change, cytoskeleton reorganization, and the formation of microspikes, filopodia, and lamellipodia, characteristic of Rac1/Cdc42 activation. Furthermore, overexpression of GEFT in NIH3T3 cells promotes the induction of foci formation and tumorigenesis, possibly by activating Rac1 and Cdc42 signaling pathways and transcription factors in the cells. The mechanism of GEFT activation and its roles in neuronal cells and tumors need to be established in future studies.

Acknowledgements—We thank members of the Center for Cancer Biology and Nutrition for insightful discussions and Dr. Kuanliang Guan for SAP1 and Elk1 reporter plasmids.

REFERENCES

1. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. (1992) Cell 70, 401–410
2. Hall, A. (1998) Science 279, 509–514
3. Bar-Sagi, D., and Hall, A. (2000) Cell 103, 227–238
4. Price, L. S., and Collard, J. G. (2001) Semin. Cancer Biol. 11, 167–173
5. Jaffe, A. B., and Hall, A. (2002) Adv. Cancer Res. 84, 57–80
6. Takai, Y., Sasako, T., and Matsuzaki, T. (2003) Physiol. Rev. 81, 153–208
7. Luo, L. (2000) Nat. Rev. Neurosci. 1, 173–180
8. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
9. Nöges, C. D., and Hall, A. (1995) Cell 81, 53–62
10. Kozma, R., Ahmed, S., Best, A., and Lim, L. (1995) Mol. Cell. Biol. 15, 1942–1952
11. Coso, O. A., Chiariello, M., Yu, J. C., Terramoto, H., Crespo, P., Xu, N., Miki, T., and Gutfeld, J. S. (1995) Cell 81, 1137–1146
12. Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170
13. Persson, R., Montaner, S., Saniger, L., Sanchez-Perez, I., Bravo, R., and Lecal,
A Rac/Cdc42-specific Exchange Factor, GEFT, Induces Cell Proliferation, Transformation, and Migration
Xiangrong Guo, Lewis Joe Stafford, Brad Bryan, Chunzhi Xia, Wenbin Ma, Xiushan Wu, Dan Liu, Zhou Songyang and Mingyao Liu

J. Biol. Chem. 2003, 278:13207-13215.
doi: 10.1074/jbc.M208896200 originally published online January 23, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M208896200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 77 references, 41 of which can be accessed free at
http://www.jbc.org/content/278/15/13207.full.html#ref-list-1