Activation of Ras/Erk Pathway by a Novel MET-interacting Protein RanBPM

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MET is a receptor protein-tyrosine kinase (RPTK) for hepatocyte growth factor (HGF), which is a multifunctional cytokine controlling cell growth, morphogenesis, and motility. MET overexpression has been identified in a variety of human cancers. Oncogenic missense mutations of the tyrosine kinase domain of the MET gene have been identified in human papillary renal cell carcinomas. In this study, RanBPM, also known as RanBP9, is identified as a novel interacting protein of MET through yeast two-hybrid screen. RanBPM contains a conserved SPRY (repeats in splA and RyR) domain. We demonstrate that RanBPM can interact with MET in vitro and in vivo, and the interaction can be strengthened by HGF stimulation. RanBPM interacts with the tyrosine kinase domain of MET through its SPRY domain. We show that RanBPM can induce GTP-Ras association and Erk phosphorylation and elevate serum response element-luciferase (SRE-LUC) expression, indicating that RanBPM can activate the Ras-Erk-SRE pathway. We demonstrate that RanBPM, which itself is not a guanine exchange protein, stimulates Ras activation by recruiting Sos. On the cellular level, A704 cells, a human renal carcinoma cell line, transfected with RanBPM exhibit increased migration ability. Our data suggest that RanBPM, functioning as an adaptor protein for the MET tyrosine kinase domain, can augment the HGF-MET signaling pathway and that RanBPM overexpression may cause constitutive activation of the Ras signaling pathway.

The definitive feature of all tumor cells is their deregulated growth, which often occurs as a result of deregulated signal transduction pathways controlling cell growth or differentiation. Receptor protein-tyrosine kinases (RPTKs) are important class of signaling molecules that are frequently perturbed by gene mutation, structural rearrangement, amplification, and overexpression (1). Uncontrolled and constitutive activity of these proteins can be oncogenic and cause cellular transformation (1). MET is a RPTK for the hepatocyte growth factor/scatter factor (HGF/SF), a multifunctional cytokine secreted by mesenchymal cells (2). HGF initiates signal transduction pathways controlling cell growth, morphogenesis, and motility (2). MET is a 190-kDa transmembrane protein, consisting of an extracellular α-subunit of 45 kDa linked by disulfide bonds to a transmembrane β-subunit of 145 kDa. The β-subunit contains the catalytic domain and multiple tyrosine phosphorylation sites (2). MET overexpression has been identified in a variety of human cancers (2). Missense mutations in exons 16–21 of the MET gene encoding for the tyrosine kinase domain were recently identified in human papillary renal cell carcinomas (3). Cells transfected with these naturally occurring mutants exhibit increased levels of MET tyrosine phosphorylation, constitutive activation of the Ras/Erk pathway, and increased motility (4, 5). Like most other RPTKs, activation of MET is believed to depend on intermolecular transphosphorylation of two of the tyrosine residues (Tyr1234 and Tyr1256) in the C terminus of the β-subunit resulting from receptor dimerization or oligomerization induced upon HGF stimulation (6). The phosphorylation of two other tyrosine residues (Tyr1349 and Tyr1365) serves as docking sites for several adaptor proteins, including Gab1, Grb2, She, phosphatidylinositol 3-kinase (7–12). The Ras pathway is one of the major pathways activated following the association and phosphorylation of these adaptor proteins (13, 14). The activation and inactivation of Ras are regulated by guanine exchange proteins (GEPs) and GTPase-activating proteins (15, 16). The major human GEP for Ras is Sos, which is constitutively associated with Grb2 (17). For most RPTKs, in order to activate Ras, the Grb2-Sos complex is recruited by direct association with phosphorylated RPTKs to the plasma membrane where Ras is located (18–20). For MET, indirect Shc-mediated association with the Grb2-Sos complex resulting in Ras activation has also been demonstrated (8). A number of other mammalian GEPs have been identified, including Ras GRF1, Ras GRF2, and Ras GRP, but they are predominantly expressed in the neural system (21, 22), which indicates that other similar molecules specific to other cell types might exist. Ras downstream effectors include phosphatidylinositol 3-kinase, RalGDS, AF6, and Raf-1 (23–27). Activated Raf-1 activates MEK (mitogen-activated protein kinase/extracellular signal-regulated kinase kinase), which results in the phosphorylation and activation of Erk1/2 (28, 29). One of the substrates for Erk is the transcription factor Elk-1, which upon activation, up-regulates the expression of immediate early response genes, such as c-Fos (30, 31). The Ras pathway is a common and crucial component of the signaling of many other growth factors/RPTKs (6).

In this study, we identified RanBPM/RanBP9 as a novel MET-interacting protein by yeast two-hybrid screening. RanBPM/RanBP9 was originally cloned as a Ran-binding protein and found to be localized in both the nucleus and cytoplasm (32, 33). RanBPM contains a very conserved SPRY domain with
unknown functions. We demonstrated that RanBPM and MET were able to interact with each other, in vitro and in vivo. We determined that the region-encoded by exons 19–21 in MET was responsible for the binding with RanBPM, and the MET-interacting region in RanBPM was mapped to its SPRY domain. We found that RanBPM could serve as an adaptor protein for MET to recruit Sos and thus stimulate Ras/Erk activation. This functional interaction between MET and RanBPM could be strengthened by HGF treatment in vivo. On the cellular level, we demonstrated that A704 human renal cancer cells transfected with RanBPM exhibited increased migration when treated with HGF. These findings suggest that RanBPM functions as an adaptor protein for MET functions.

MATERIALS AND METHODS

Yeast Two-Hybrid Screen—Yeast two-hybrid system3 (Clontech) was used in our experiments. A pretransformed human kidney cDNA library in Y187 yeast strain (Clontech) was screened by yeast mating with another yeast strain, AH109, transformed with a bait construct, pGBK7-MET1102–1408, containing MET intracellular region. The expression of GAL4-MET1102–1408 fusion protein in AH109 from the construct was confirmed by Western blotting (data not shown). This bait yeast cell did not exhibit any intrinsic activation function as demonstrated by a bait-testing assaystringent selection procedure detecting only strong interactions was used in our experiments.

Cell Culture and Transient Transfection—Cos-7, HeLa, and 293 cells were maintained in Dulbecco’s minimum essential medium containing penicillin (25 units/ml), streptomycin (25 μg/ml), and 10% fetal bovine serum (FBS). A704 cells were maintained in Eagle’s minimum essential medium (EMEM) containing 0.1 mM non-essential amino acid solution, 1 mM sodium pyruvate, penicillin (25 units/ml), streptomycin (25 μg/ml), and 10% FBS. Serum-starved cells were maintained in Dulbecco’s minimal essential medium or EMEM containing 0.2% FBS. Transient transfection was performed using SuperFect (Qiagen) transfection solution according to the manufacturer’s protocol.

Plasmid Construction—pGADT7-RanBPM was constructed by inserting the RanBPM coding sequence amplified by PCR into a pGADT7 vector (Clontech). pVP16-RanBPM was constructed by transferring RanBPM insert from pGADT7-RanBPM into a pVP16 vector (Clontech) by EcoRI and PstI digestion. pGADT7-ranBPM (truncated RanBPM, amino acids 409–729) was constructed based on pGADT7-RanBPM by restriction enzyme digestion with EcoRI and re-ligation. pGEX-6p1-SPRY was constructed by inserting the SPRY sequence amplified by PCR into a pGEX-6p1 vector (Amersham Biosciences). pGFP-C3-RanBPM containing the full-length RanBPM was kindly provided by Dr. Nishimoto (32, 33). pGBK7-MET1102–1408 was constructed by inserting MET coding sequence encoded by exons 16–21 of the MET gene amplified by RT-PCR into a pGBK7 vector (Clontech). pGEX-6p1-MET1102–1408 was constructed by transferring MET1102–1408 from pGBK7-MET1102–1408 by EcoRI and PstI digestion. pGEX-6p1-MET1102–1233 and pGEX-6p1-MET1226–1408 were constructed by inserting a MET coding sequence encoded by exons 16–18 and 19–21 into a pGEX-6p1 vector, respectively. pcDNA3-Sos was constructed in two steps. First, pcDNA3-Sos-I/1067 was constructed by subcloning human Sos (amino acids 1–1067) from a pSMs plasmid (Stratagene) to a pcDNA3 vector (Invitrogen). Second, the Sos sequence corresponding to amino acids 1067–1333 of human Sos was amplified by PCR using primers containing the BamHI restriction enzyme digestion site and fused into pcDNA3-Sos-I/1067. All sequences amplified by PCR were confirmed by complete sequencing. pUSE-H-Ras (DN) was obtained from Upstate Biotech.

Northern Blotting—The MTN blot (multiple tissue Northern blot) containing ~2 μg of poly(A) RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (Clontech) was hybridized with 32P-labeled RanBPM cDNA. The blot was subsequently probed with a β-actin cDNA probe.

GST Pull-down Assay—The assay was performed as described previously (34). Briefly, GST or GST fusion proteins were expressed in BL21 cells and purified using glutathione-Sepharose 4B MicroProtein columns (Amersham). Equal amounts of GST and GST fusion proteins were added into glutathione-Sepharose 4B MicroSpin columns and incubated for 3 h at 4 °C. Five μl of in vitro translated 32S-labeled RanBPM or ε-Myc-MET1102–1408 was then added into each reaction and incubated at 4 °C for 3 h. Bound proteins were eluted, and 20 μl of each eluted protein was resolved on an 8% SDS-PAGE gel followed by autoradiography.

In Vitro and in Vivo Co-immunoprecipitation—Antibodies including anti-ε-Myc monoclonal, anti-MET polyclonal, anti-GFP monoclonal, anti-Sos polyclonal, anti-ε-Myc polyclonal, and anti-pErk monoclonal antibodies were obtained from Santa Cruz Biotechnology Inc. In vitro and in vivo co-immunoprecipitation assays were performed as described previously (34). Briefly, for the in vitro co-immunoprecipitation assay, 20 μg of a protein A/G-agarose (Santa Cruz), 5 μl of each radiolabeled in vitro translation product, 10 μl of antibody to one of the proteins, and 470 μl of co-immunoprecipitation buffer (34) were mixed and rotated at 4 °C for 3 h. Bound proteins were eluted and separated on an 8% SDS-PAGE gel followed by autoradiography. For the in vivo co-immunoprecipitation assay, 50 μl of cell lysates (500 μg of total proteins) were incubated in co-immunoprecipitation (IP) antibody and incubated overnight at 4 °C. Fifty μl of protein A/G-agarose was then added into each mixture followed by rotating at room temperature for 2 h, centrifuged, washed three times, and resolved on an 8% SDS-PAGE gel followed by Western blot analysis.

Mammalian Two-hybrid Assay—Mammalian two-hybrid assay was performed as described before (34). COS-7 cells were co-transfected with different combinations of plasmids as indicated. After 24 h, cells were harvested, lysed, and analyzed with a chlorophenolic acetyltransferase assay.

Dual Luciferase Assay—HeLa cells were plated in 12-well dishes and transfected with different combinations of testing plasmids including reporter plasmid pRSV40-LUC, reporter plasmid p55-LUC, and luciferase reporter plasmid pRLSV40-LUC reporter plasmid plus internal control plasmid pRSV40-LUC and reporter plasmid p55-LUC. Twelve h after transfection, cells were serum-starved for 12 h and then treated with or without HGF (20 ng/ml) (Sigma) for 6 h. Cells were lysed, and the dual luciferase assay was performed using the Dual-Luciferase Reporter 1000 assay system (Promega). Mean values of luciferase activity relative to HGF-untransfected and GFP-RanBPM untransfected control (lane 1) were calculated from triplicate wells. The experiments were repeated three times to ensure consistency.

In Vivo Ras Activation Assay—HeLa cells were transfected with pGFP-C3 or pGFP-C3-RanBPM. Eighteen h after transfection, cells were serum-starved for 12 h and treated with or without genistein (50 μM) (Sigma) for an additional 12 h. The cells were then treated with or without HGF (20 ng/ml) and lysed for a subsequent in vivo Ras activation assay. Briefly, cell lysates prepared from HeLa cells were incubated with 5 μg of Raf-1 RBD agarose (Upstate Biotech) at 4 °C for 1.5 h. The agarose beads were collected, washed, resuspended in the sample buffer, and resolved on a SDS-PAGE gel followed by Western blotting with anti-Ras monoclonal antibody (Upstate Biotech). The cell lysates incubated with both GDP and GTPyS (Upstate Biotech) were served as negative and positive controls, respectively.

In Vitro GDP Dissociation Assay—Effects of RanBPM on the dissociation of [3H]GDP (PerkinElmer Life Sciences) from Ras were assayed as described before (33). Briefly, the [3H]GDP-bound form of Ras was obtained by incubating 20 pmol of GST-Ras protein (cytoskeleton) with 1 μM [3H]GDP at 30 °C for 20 min in the reaction buffer 1 (20 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 10 mM MgCl2, and 2.9 mM EDTA) at 30 °C. To prepare the GFP or GFP-RanBPM IP complex, cell lysates from two 10-cm diameter dishes of 293 cells transfected with pGFP-C3 vector or pGFP-C3-RanBPM and serum-starved for 18 h were precipitated with anti-GFP antibody. Total IP proteins were resuspended in 100 μl of reaction buffer II (50 mM Tris-HCl, pH 8.0, 1 mM diithiothreitol, 10 mM MgCl2, and 2.9 mM EDTA) and increasing amounts (0, 1, 5, 10, 25, and 50 μl) in proportion to the amounts of GST-RanBPM were added into reactions. The dissociation of [3H]GDP from Ras was assayed in reaction buffer II at 25 °C by adding a 200-fold excess of unlabeled GTP (Sigma) and increasing amounts of GST-RanBPM, GFP alone, GFP-RanBPM, or GST-cdc25 (a positive control) (16). The reaction was stopped at 1 h after incubation by adding 2 ml of ice-cold stop buffer (20 mM Tris-HCl, pH 7.5, 20 mM MgCl2, and 100 mM NaCl). The mixtures were then filtered through nitrocellulose filters, washed, and counted for radioactivity. The concentration of GST-RanBPM and GST-cdc25 was measured using the Bradford assay (Bio-Rad).

Cell Migration Assay—Migration of A704 cells was analyzed by a BD Biosciences Matrigel invasion chamber. Briefly, A704 cells were transfected with pGFP-C3 or pGFP-C3-RanBPM. Twenty-four h after transfection, 2 × 104 cells were seeded onto the bottom well chamber in EMEM + 0.2% FBS. Chambers were then placed into 24-well companion plates containing EMEM + 0.2% FBS alone, or with HGF (20 ng/ml), and incubated at 37 °C for 22 h. Cells migrated through the membrane were fixed and stained with 1% methanol and 1% toluidine blue and counted using bright field microscopy. Mean values of numbers of cells migrated from 10 randomly selected unit
areas were calculated for each well plated in triplicate. The experiments were repeated three times to ensure consistency. Representative fields were photographed.

RESULTS

Identification of RanBPM as a MET-interacting Protein by Yeast Two-hybrid Screening—Using the MET intracellular portion (amino acids 1102–1408 encoded by exons 16–21 of the MET gene) as a bait (Fig. 1A), a human kidney cDNA library was screened via yeast two-hybrid screen. Five positive clones were identified after screening 1.2 × 10^7 clones. DNA sequencing revealed that three of the positive clones encoded a partial sequence of RanBPM/RanBP9 (Fig. 1A), a protein originally cloned as a Ran-binding protein with unknown function (32, 33). RanBPM contains a SPRY domain (amino acids 212–333 of RanBPM), a structural motif that was originally identified in ryanodine receptors, and a Dictyostelium discoideum dual-specificity kinase termed splA (36). A yeast two-hybrid assay using independent co-transformation of the bait construct and the candidate clones, followed by growth selection and a β-galactosidase assay, further confirmed the direct interaction between MET and RanBPM in yeast (data not shown). A BLASTP search comparing RanBPM orthologues of different organisms revealed that the SPRY domain in RanBPM from human, mouse, and Xenopus are very conserved. Increasing intensity of shading corresponds to increasing conservation. The SPRY domain consensus is derived from proteins containing SPRY domains other than RanBPM. C, mRNA expression of RanBPM in human tissues. MTN blot was hybridized with a 32P-labeled RanBPM cDNA probe followed by autoradiography. The membrane was then stripped and rehybridized with a β-actin probe.
human RanBPM is expressed in whole embryo, fetal eye, placenta, uterus, ovary, germ cell, testis, breast, aorta, marrow, natural killer cell, lymph node, tonsil, thymus, spleen, heart, lung, kidney, pancreas, stomach, colon, brain and nervous tissue, parathyroid, prostate, skeletal muscle, skin, and tumor tissues or cell lines including carcinoid, choriocarcinoma, chronic myelogenous leukemia, glioblastoma with epidermal growth factor receptor amplification, hepatocellular carcinoma, insulinoma, lymphoma, mammary adenocarcinoma, mucoepidermoid carcinoma, nervous tumor, renal cell adenocarcinoma, rhabdomyosarcoma, and uterine tumor.

RanBPM Interacts with MET Directly in Vitro and in Vivo—The interaction of RanBPM with MET was first tested in vitro using a GST pull-down assay. The in vitro translated 35S-labeled RanBPM was incubated with GST-MET1101–1408 or GST-coated beads. Eluted samples and 10% input of 35S-labeled protein were resolved on a SDS-PAGE gel followed by autoradiography. In vitro co-immunoprecipitation of MET with RanBPM. In vitro translated 35S-labeled c-Myc-MET1102–1408 and RanBPM (lane 3) or RanBPM protein only (lane 2) was immunoprecipitated with an anti-c-Myc antibody and analyzed on a SDS-PAGE gel followed by autoradiography. In lane 1, 0.5 μl of RanBPM was loaded as a marker. C, interaction of RanBPM and MET protein in a mammalian two-hybrid assay. In lane 5, pM-MET1102–1408 and pVP16-RanBPM were co-transfected into COS-7 cells together with reporter plasmid pG5-CAT. Other lanes served as negative controls, including transfection with pG5-CAT reporter plasmid alone (lane 1), pM and pVP16 empty vectors (lane 2), pM and pVP16-RanBPM (lane 3), pVP16 and pM-MET1102–1408 (lane 4). D, in vivo co-immunoprecipitation of MET with GFP-RanBPM fusion protein. Lysates prepared from HeLa cells transfected with pGFP-C3 or pGFP-C-RanBPM were subjected to IP with an anti-MET antibody followed by anti-GFP or anti-MET immunoblotting.

The SPRY Domain of RanBPM Interacts with the MET Tyrosine Kinase Domain—A SPRY domain was found in the N terminus of RanBPM in a domain search (Pfam protein families data base) of RanBPM. The function of the SPRY domain has not been established. We speculated that it might be involved in protein-protein interactions. As we expected, yeast AH109 cells co-transformed with pGAD77-tRanBPM and pGBK7-MET1102–1408 were selected on the nutrition-deficient plates. Co-transformation of pGBK7-t-p53 and pGAD77-T was served as a positive control and co-transformation of pGBK7-LamC and pGAD77-T as a negative control. B, mapping the MET-interacting region in RanBPM by GST pull-down assay. C, mapping the RanBPM-interacting region in MET by GST pull-down assay.

Fig. 2. RanBPM interacts with MET directly in vitro and in vivo. A, analysis of the interaction between MET and RanBPM by GST pull-down assay. In vitro translated 35S-labeled RanBPM was incubated with GST-MET1101–1408 or GST-coated beads. Eluted samples and 10% input of 35S-labeled protein were resolved on a SDS-PAGE gel followed by autoradiography. B, in vitro co-immunoprecipitation of MET with RanBPM. In vitro translated 35S-labeled c-Myc-MET1102–1408 and RanBPM (lane 3) or RanBPM protein only (lane 2) was immunoprecipitated with an anti-c-Myc antibody and analyzed on a SDS-PAGE gel followed by autoradiography. In lane 1, 0.5 μl of RanBPM was loaded as a marker. C, interaction of RanBPM and MET protein in a mammalian two-hybrid assay. In lane 5, pM-MET1102–1408 and pVP16-RanBPM were co-transfected into COS-7 cells together with reporter plasmid pG5-CAT. Other lanes served as negative controls, including transfection with pG5-CAT reporter plasmid alone (lane 1), pM and pVP16 empty vectors (lane 2), pM and pVP16-RanBPM (lane 3), pVP16 and pM-MET1102–1408 (lane 4). D, in vivo co-immunoprecipitation of MET with GFP-RanBPM fusion protein. Lysates prepared from HeLa cells transfected with pGFP-C3 or pGFP-C-RanBPM were subjected to IP with an anti-MET antibody followed by anti-GFP or anti-MET immunoblotting.

Fig. 3. The SPRY domain of RanBPM interacts with MET C terminus encoded by exons 19–21 of the MET gene. A, mapping the MET-interacting region in RanBPM by yeast two-hybrid assay. Yeast AH109 cells co-transformed with pGAD77-tRanBPM and pGBK7-MET1102–1408 were selected on the nutrition-deficient plates. Co-transformation of pGBK7-t-p53 and pGAD77-T was served as a positive control and co-transformation of pGBK7-LamC and pGAD77-T as a negative control. B, mapping the MET-interacting region in RanBPM by GST pull-down assay. C, mapping the RanBPM-interacting region in MET by GST pull-down assay.
lysates were then immunoblotted with anti-pErk antibody or anti-Erk2 by treatment with or without HGF (20 ng/ml) for 15 min. The cell BPM protein could be pulled down by GST-MET1226 or pGFP-C3 and BPM alone without HGF stimulation cannot promote the cell migration. A704 cells transfected with pGFP-C3-RanBPM without HGF treatment showed almost no cell migration through the three-dimensional collagen matrix than cells transfected with the empty pGFP-C3 vector (Fig. 6A, panels 2 and 4, and Fig. 6B), suggesting that RanBPM can enhance HGF-induced cell migration. A704 cells transfected with pGFP-C3-RanBPM without HGF treatment exhibited a significant increase in cell migration through the three-dimensional collagen matrix than cells transfected with the empty pGFP-C3 vector (Fig. 6A, panels 2 and 4, and Fig. 6B), suggesting that RanBPM can enhance HGF-induced cell migration. A704 cells transfected with pGFP-C3-RanBPM without HGF treatment showed almost no cell migration through the three-dimensional collagen matrix (Fig. 6A, panel 3), similar to the control well (Fig. 6A, panel 1). This indicates that although RanBPM can activate the Ras pathway, RanBPM alone without HGF stimulation cannot promote the cell migration. Thus, HGF-stimulated cell migration might require a more orchestrated molecular activation than just the Ras pathway.

**DISCUSSION**

Growth factors and their RPTKs play a very important role in tumorigenesis. Elucidation of their physiological signaling pathways holds the key to understanding the molecular mechanisms of their oncogenic activation. In this study, we have
identified a novel MET-interacting protein, RanBPM/RanBP9, by yeast two-hybrid screening. The RanBPM-interacting region in MET is within its tyrosine kinase domain and is encoded by exons 19–21 of the MET gene. MET interacts with the SPRY domain located in the N terminus of RanBPM. Unlike most known MET-interacting adaptor proteins, whose bindings to MET require the phosphorylation of the so-called docking site induced upon HGF stimulation, RanBPM can interact with MET in vitro and in vivo without HGF stimulation. In our in vivo co-immunoprecipitation assay, we have also shown that in the presence of HGF, more RanBPM can be co-immunoprecipitated with anti-MET antibody than in the absence of HGF. This favors the idea that the phosphorylation of MET induced by HGF stimulation enhances the direct interaction between MET and RanBPM, although it is possible that phosphorylated MET may indirectly recruit more RanBPM via other adaptor proteins. Recently, a novel protein named FAP68 was identified to interact specifically with the inactive form of MET, and HGF abrogated the interaction (37). Therefore, in terms of the effects
of HGF on the interaction, MET-interacting proteins can be classified into three groups. The first group of proteins, exemplified by Grb2, Shc, and most other MET-interacting proteins, are the ones whose interaction with MET is HGF-dependent (Fig. 7). FAP68 belongs to the second group of proteins, whose interaction with MET is negatively regulated by HGF (Fig. 7). The third group of proteins, such as RanBPM, interacts with MET independent of HGF, but the interaction is positively regulated by HGF (Fig. 7). The complexity of the interactions between MET and its interacting molecular partners in response to HGF might explain the molecular bases for the complicated and finely coordinated cellular functions of HGF.

Our studies also provide new insights into the activation of the Ras signaling pathway via RPTKs. We have found that overexpression of RanBPM induces activation of Ras signaling, reflected by the elevated levels of SRE-LUC expression, phosphorylated Erk, and the GTP-bound form of Ras without the need for growth factor stimulation. The fact that this activation is significantly repressed by co-transfection with dominant-negative Ras and is not affected by genistein, a tyrosine kinase inhibitor, demonstrates that the Ras pathway activation by RanBPM is achieved at the step of Ras activation itself. The Ras pathway is a crucial one in any cells for proliferation, differentiation, and apoptosis as well as migration. Multiple molecular networks are probably needed to achieve the fine and accurate regulation of Ras activation in different developmental stages or different environments. The current model for Ras activation via RPTKs is that the phosphorylated tyrosine residue(s) on activated receptors serve as docking sites for Grb2, which constantly associates with Sos. Indirect Shc-mediated association of the Grb2-Sos complex with phosphorylated Sos has also been demonstrated (8). Thus, Sos is recruited near Ras and catalyzes the GDP-GTP exchange reaction that activates Ras. However, at least for MET, there is evidence for the existence of adapter proteins capable of recruiting Sos other than Shc-Grb2 or Grb2. For example, a MET mutant, which loses its interaction with both Shc and Grb2, was found to be still able to activate the Ras pathway (38). Although it is not yet clear whether RanBPM can also associate with other RPTKs and act as a general Sos-recruiting protein, we provide evidence that RanBPM is able to serve as an adapter protein of MET to recruit Sos, activate the Ras/Erk pathway, and stimulate cell motility. RanBPM is widely expressed in many human tissues at variable levels as demonstrated by our Northern blot analysis and EST data base search. It is reasonable to think that in different tissues the quantity of RanBPM is tightly regulated so that, in the absence of HGF, the level of protein recruited by RanBPM is highly controlled. In any case when RanBPM expression is deregulated, its overexpression may cause constitutive activation of the Ras signaling pathway.

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