Histol Histopathol (2006) 21: 213-218

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

RhoB in cancer suppression

M. Huang[1] and G.C. Prendergast[1,2]

[1]Lankenau Institute for Medical Research and

[2]Department of Pathology, Anatomy, and Cell Biology, Jefferson Medical School, Thomas Jefferson University, Philadelphia PA USA

Summary. RhoB is a mainly endosomal small GTPase that regulates actin organization and vesicle trafficking. Expression of RhoB is elevated rapidly by many stimuli, including growth factors, cytokines, and genotoxic stress. In cancer, RhoB can limit cell proliferation, survival, invasion, and metastasis, and during malignant progression its levels are attenuated commonly. In support of its role as a negative modifier of cancer progression, targeted deletion of RhoB in mice can increase tumor formation initiated by Ras mutation. How RhoB acts to suppress different aspects of cancer pathophysiology has emerged as a question of significant interest.

Key words: RhoB, GTPases, Endosomes, Prenylation, Cancer

Introduction

Rho small GTPases integrate cytoskeletal actin organization with the control of many cellular processes, including cell adhesion, motility, proliferation, survival, endocytosis, and vesicle trafficking (Bishop and Hall, 2000). Beyond their diverse roles in normal physiology, Rho proteins also strongly influence pathological processes including cancer (Jaffe and Hall, 2002; Sahai and Marshall, 2002). The RhoA, RhoB, and RhoC proteins form a closely related subgroup that are ~90% identical in amino acid sequence. While each regulates actin stress fiber formation, they nevertheless have distinct physiological and pathophysiological functions, possibly derived from differences in subcellular localization that permit effector interactions to be partitioned (Adamson et al., 1992a). RhoB has received increasing attention because of emerging evidence that it may act as a negative modifier or suppressor gene in cancer. Whereas the other Rho proteins have mainly positive roles in malignant transformation, the specific role of RhoB appears to be divergent. In particular, RhoB differs from RhoA and RhoC in its unique localization to endosomes and other intracellular membranes and in its antiproliferative and proapoptotic effects in cancer cells, possibly reflecting a role in stress signaling (Prendergast, 2001). In a variety of solid tumors, RhoB levels are decreased with tumor progression (Ridley, 2004) and overexpression of RhoB can inhibit cell migration, invasion, and metastasis (Jiang et al., 2004a). Additionally, several studies indicate that RhoB is an important mediator of the cellular response to farnesyl transferase inhibitors (FTIs). RhoB responds to FTI treatment by a gain-of-function mechanism characterized by elevation of the geranylgeranylated isoform of RhoB (RhoB-GG) that mediates antiproliferation and apoptosis (Du and Prendergast, 1999; Du et al., 1999; Liu et al., 2000). Furthermore, RhoB is required for the apoptotic response of transformed cells to DNA damage or taxol (Liu et al., 2001a). Genetic analysis in mice suggests that RhoB is dispensable for normal cell physiology, but that it restrains Ras-induced skin cancer formation and modifies growth factor and adhesion signaling in transformed cells (Liu et al., 2001b). Together, these studies prompt the hypothesis that RhoB suppresses cancer, perhaps acting as negative modifier which limits the effects of oncogenic mutations. The molecular mechanisms by which RhoB links signaling pathways and endosomal trafficking pathways are of particular interest for understanding its anti-cancer function. In this review, we will describe some of the key discoveries that underpin present understanding of RhoB in cancer cells.

Offprint requests to: Lankenau Institute for Medical Research, Cancer Cell Signaling Laboratory, 100 Lancaster Avenue, Wynnewood PA USA 19096. e-mail: prendergast@limr.org

RhoB expression and localization

Several features distinguish RhoB from structurally similar Rho proteins. First, compared to RhoA and RhoC, the RhoB gene is smaller and contains only one exon, which is thought to have arisen by reverse transcription (Karnoub et al., 2004). RhoB levels have been reported to vary through the cell cycle, and the RhoB transcript has a half-life of only 30 min, which is substantially shorter than other members of Rho GTPase family (Zalcman et al., 1995). Second, in cultured cells, the RhoB gene is rapidly upregulated by a variety of stimuli such as UV irradiation, cytokines, growth factors, steroid and toxin treatments (Jahner and Hunter 1991; Fritz et al., 1995; Zalcman et al., 1995; Engel et al., 1998; Trapp et al., 2001; Chauhan et al., 2004;

Gerhard et al., 2005). On the other hand, expression of the RhoB gene is reduced by Ras via Akt/PKB, EGFR and ErbB2 (Jiang et al., 2004a,b). Together, these studies indicate that RhoB expression is highly regulated. Last, one additional level of regulation that may exist relates to a unique aspect of the RhoB protein in its existence in different geranylgeranylated (RhoB-GG) or farnesylated (RhoB-F) isoforms in cells (Adamson et al., 1992b). This difference may be a factor in differences in subcellular localization that has been reported at the plasma membrane, late endosomes, multivesicle bodies, and possibly other intracellular membranes including the nuclear membrane (Adamson et al., 1992a; Lebowitz et al., 1995; Zalcman et al., 1995; Lebowitz and Prendergast, 1998a; Michaelson et al., 2001; Adini et al., 2003; Sandilands et al., 2004; Wherlock et al., 2004).

RhoB in signaling and trafficking

Rho GTPases play a pivotal role in the dynamic regulation of the actin cytoskeleton. Among their other roles, recent studies point to multiple functions for Rho proteins in endocytosis and endosomal trafficking pathways, including late stages of trafficking to lysosomes or along the recycling pathway to the plasma membrane (Ellis and Mellor, 2000). RhoB was the first member of the family to be implicated in endosomal trafficking. As determined by immunofluorescence, electron microscopy and biochemical fractionation, RhoB localizes both to the plasma membrane and the bounding membrane of multivesicular late endosomes (MVBs) (Adamson et al., 1992a; Robertson et al., 1995; Mellor et al., 1998). One caveat to these and other studies has been the use of epitope-tagged or green fluorescent protein-tagged proteins to determine localization, which may influence localization and thereby function. RhoB binds the protein kinase C-related protein kinase (PRK1) and targets it to the endosomal compartment (Mellor et al., 1998). Through activating of PRK1, RhoB regulates the kinetics of epidermal growth factor (EGF) receptor traffic from endosomes to a pre-lysosomal compartment. Expression of active RhoB causes a delay in the intracellular trafficking of the EGF receptor (Gampel et al., 1999) (Fig. 1A). In addition, RhoB and PRK1 cause recruitment of the PI3-kinase effector kinase PDK1 to endosomes (Flynn et al., 2000). Internalized receptors travel through a number of distinct endocytic sub-compartments where the decision is made to recycle to the plasma membrane or to traffic to the lysosome for degradation. One recent study to map the site of action of RhoB within the endocytic pathway has revealed that activating RhoB through drug treatment or mutation has no effect on receptor sorting into late endosomes, but that it inhibits the subsequent transfer of the receptor to the lysosome (Wherlock et al., 2004) (Fig. 1A). Like other small GTPases, RhoB has an isoprenoid anchor at its C-terminus. As mentioned above, RhoB is unique in that the isoprenoid modification can be either a farnesyl group (like Ras) or a geranylgeranyl group (like other Rho proteins). One study suggested that geranylgeranylated RhoB (RhoB-GG) resides primarily in multivesicular late endosomes, whereas farnesylated RhoB (RhoB-F) resides primarily at the plasma membrane (Wherlock et al., 2004). Driving formation of presumptive RhoB-GG by FTI treatment reduces sorting of EGF receptor to the lysosome and increases recycling to the plasma membrane. This shift in prenylated form was associated with an inhibition of cell proliferation under these circumstances.

The role of RhoB in endosomal trafficking has been extended to other important cellular signaling proteins including Akt and Src (Adini et al., 2003; Sandilands et al., 2004). In endothelial cells, RhoB is highly localized to the nuclear margin where activated Akt exhibits increased accumulation upon survival stimuli. This colocalization is functionally relevant, because depletion of RhoB caused exclusion of Akt from the nucleus and a reduction in total cellular Akt protein by a proteosome-dependent mechanism (Adini et al., 2003). Therefore, RhoB appears to be an important determinant of Akt stability and trafficking to the nucleus in this cell type (Fig. 1B). The *in vivo* function of RhoB appears to be rate limiting for endothelial cell sprouting, illustrating a novel stage-specific function to regulate endothelial cell survival during vascular development (Adini et al.,

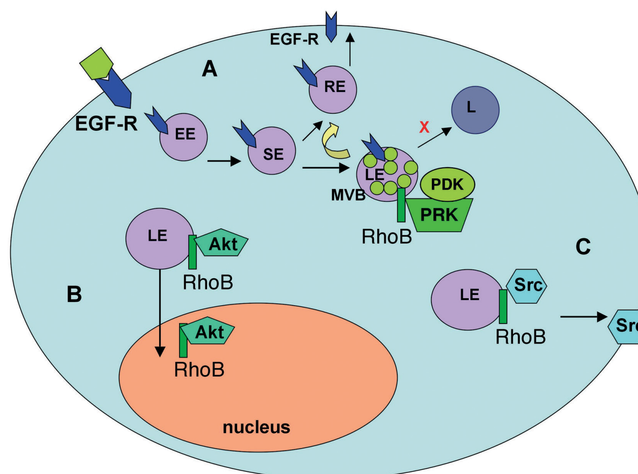

Fig. 1. RhoB and endocytic traffic. **A.** RhoB controls epidermal growth factor receptor (EGF-R) trafficking. RhoB binds the protein kinase C-related protein kinase (PRK1) and targets PRK1 and PI3-kinase effector PDK1 to the multivesicular body late endosomal compartment. Through activating PRK1, RhoB regulates the kinetics of EGF-R traffic from endosomes to lysosome, by inhibiting sorting of EGF-R to the lysosome and increasing recycling to the plasma membrane. **B.** RhoB regulates nuclear trafficking of Akt in endothelial cells. RhoB and Akt are colocalized in the nuclear periphery, and loss of nuclear Akt occurs upon RhoB inhibition (Adini et al., 2003). **C.** Trafficking of active Src to the plasma membrane is RhoB-dependent. RhoB colocalizes with active Src in the cytoplasmic endosomes and translocates this active kinase to peripheral membrane structures (Sandilands et al., 2004). Abbreviations: EE, early endosome; SE, sorting endosome; RE, recycling endosome, LE, late endosome; L, lysosome; MVB, multivesicular body.

2003). RhoB has also been shown to control trafficking of Src (Sandilands et al., 2004). In this work, RhoB was identified as a component of "outside-in" signaling pathways that coordinate Src activation with its translocation to transmembrane receptors (Fig. 1C). Notably, Src is activated during transit, particularly in RhoB-containing cytoplasmic endosomes associated with the perinuclear recycling compartment. Knocking out RhoB suppresses both the catalytic activation of Src as well as translocation of the active kinase to peripheral membrane structures (Sandilands et al., 2004). A common theme in regulation of Akt and Src by RhoB is that its control of kinase trafficking is distinct but linked to other events that influence the signaling capacity of the kinase. In this sense, RhoB may act to modify kinase action by influencing the efficiency of its localization. Another cell survival regulator that might mediate downstream effects of RhoB is NF-κB, based on the finding that RhoB can suppress NF-κB by interfering with IkB turnover (Fritz and Kaina, 2001). However, the precise effector pathways that RhoB uses to modulate Akt, Src, and NF-κB in cells remain undefined at present.

RhoB in cancer

Some of the clearest illustrations of how RhoB differs in function from other Rho GTPases has been in cancer. RhoA and RhoC, like other GTPase family members such as Ras, Rac1, and Cdc42, promotes oncogenesis, invasion, and metastasis (Khosravi-Far et al., 1995; Westwick et al., 1997; Pruitt and Der 2001; Ridley, 2004). In contrast, RhoB has a variety of properties that indicate a cancer suppressive role (Du and Prendergast, 1999; Du et al., 1999; Liu et al., 2000, 2001a,b; Delarue et al., 2001), possibly through its ability to compete for binding to Rho effector proteins and thereby to interfere with their ability to mediate pro-oncogenic signaling by Rho (Zeng et al., 2003). Ectopic expression of RhoB can inhibit tumor growth and metastasis (Chen et al., 2000; Jiang et al., 2004a). Notably, transformed cells lacking RhoB are more aggressive at forming tumors and knockout mice are more sensitive to induction of chemically-induced tumors (Liu et al., 2001b) (Fig. 2). In most tumor-derived cell lines, transcription of RhoB is generally attenuated, consistent with the idea that reduced levels of expression facilitates cell division (Wang et al., 2003). Many oncogenes such as the EGF receptor, Ras, and Akt appear to suppress the expression of RhoB (Jiang et al., 2004a,b). Furthermore, study of patient biopsies exhibited that RhoB expression levels are dramatically decreased in lung, head and neck and brain cancer, when tumors become more aggressive (Adnane et al., 2002; Forget et al., 2002; Mazieres et al., 2004). Taken together, these observations establish that RhoB as having a suppressor or negative modifier function in cancer.

Recent investigations have provided some insights into how RhoB is regulated in cancer cells. By using a genomic approach to identify the transcriptional consequences of HDAC inhibition, a link between HDAC activity and RhoB suppression was elucidated (Wang et al., 2003). Transcriptional suppression by HDAC was found to be mediated by an inverted CCAAT element in the RhoB promoter (Wang et al., 2003). Induction of RhoB by environment stresses, such as UV irradiation, is mediated by changes in gene transcription and mRNA stabilization, the latter of which is mediated by the RNA-binding protein HuR which stabilizes messages (Westmark et al., 2005). Other work has revealed suppression of RhoB by oncogenic signals mediated by the Ras/PI3K/Akt pathway (Jiang et al., 2004a). Like other Rho proteins, post-translational modification is critical for function, and site-directed mutagenesis studies have defined the crucial requirement for palmitoylated cysteine 192 and prenylated cysteine 193 for the tumor suppressive and proapoptotic activities of RhoB (Du et al., 1999; Wang and Sebti, 2005).

Emerging evidence suggests that there are differences in the action of the RhoB-F and RhoB-GG isoforms in some settings. In one recent study, RhoB and RhoB-GG, but not RhoB-F, displayed anti-transforming, anti-proliferative, and proapoptotic activity in Ras-transformed NIH3T3 mouse fibroblasts (Mazieres et al., 2005). These findings support evidence that RhoB-F can selectively support the survival of sprouting endothelial cells and irradiated cells (Adini et al., 2003; Milia et al., 2005). In contrast, in several human cancer cell lines RhoB-F and RhoB-GG each appear to exert anti-proliferative activity (Chen et al., 2000). These differences illustrate contextual differences that might be expected of a modifier function that also highlight the important role of different prenyl groups in determining

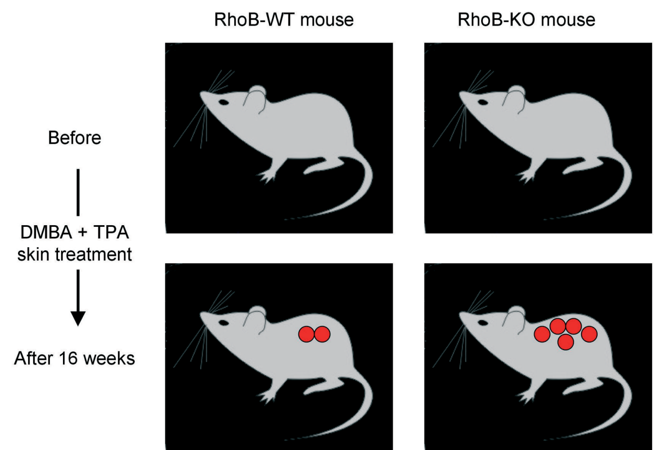

Fig. 2. RhoB deletion promotes tumor formation. Treatment of mouse dorsal epidermis with a single dose of 7,12-dimethylbenz[a]anthracene (DMBA) followed by twice weekly application of the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) induced skin tumors on each mouse within 16 weeks. RhoB KO mice were more susceptible to the formation of benign skin papillomas, which predominate in the mouse at this time point (Liu et al., 2001b).

protein function (Mazieres et al., 2005). In addition, RhoB and RhoB-GG inhibit Ras activation of Akt (Liu and Prendergast, 2000). The above studies suggest that various levels of cellular control of RhoB are important in contributing to malignant transformation and tumorigenesis.

RhoB in cell suicide

Cell suicide processes are thought to play an important role in limiting cancer progression and therapeutic response. RhoB has been shown to be required for the apoptotic response of transformed cells to FTI, DNA damaging agents, and paclitaxel (Liu et al., 2000, 2001a). Recently, microarray studies identified Cyclin B1 as a major target for downregulation by RhoB in transformed mouse cells fated to undergo FTI-induced apoptosis (Kamasani et al., 2003). Further study demonstrated that Cyclin B1 is a crucial target for suppression by RhoB in this death program. Steady-state levels of Cyclin B1 and its associated kinase Cdk1 were suppressed in a RhoB-dependent manner in cells fated to undergo FTI-induced apoptosis. Mechanistic investigations indicated that RhoB mediated transcriptional suppression but also accumulation of Cyclin B1 in the cytosol at early times after FTI treatment, before subsequent reduction in steady-state protein levels (Kamasani et al., 2004). Further study is needed to identify the effector pathway(s) used by RhoB to control Cyclin B1.

RhoB in FTI anticancer activity

FTIs are a new experimental class of cancer therapeutics originally strategized to target Ras, which is frequently activated in tumor cells either indirectly or directly through oncogenic mutations that lock the protein in a constitutively active state (Shields et al., 2000). FTIs can inhibit the function of mutant H-Ras protein by blocking its post-translational farnesylation, which is essential for proper membrane localization and oncogenic activity. Early preclinical studies showed that FTIs were selective antagonists of Ras-dependent neoplastic transformation, with few significant effects on untransformed cells. However, mechanistic investigations showed that Ras inhibition could not entirely explain their antitransforming properties (Cox and Der, 1997; Lebowitz and Prendergast, 1998b). So, although FTIs inhibit Ras farnesylation, their biological effects could be traced beyond this effect. Several other farnesylated proteins have been suggested as potential relevant FTI targets (Sebti and Hamilton, 2000; Prendergast, 2001; Cox and Der, 2002), of which RhoB is the best characterized to date. As noted above, RhoB is unique in being the only cellular protein that is known to exist in both farnesylated and geranylgeranylated forms within the cell (Adamson et al., 1992b). Treatment of cells with FTIs causes a loss of RhoB-F and a consequent increase in RhoB-GG, as newly synthesized RhoB protein can be efficiently prenylated by the geranyl geranyltransferase-I enzyme which is not affected by FTI (Lebowitz et al., 1997). This shift therefore represents a gain-of-function action of FTI treatment in cells. Whereas RhoB-F may have either pro-growth or anti-growth activities in different settings, RhoB-GG appears to uniformly act as an anti-growth or pro-apoptotic mediator that is induced by FTI treatment (Du and Prendergast, 1999; Du et al., 1999; Liu et al., 2000). Significantly, induction of RhoB-GG is an event that is sufficient to mediate phenotypic reversion, growth inhibition, cytoskeletal actin reorganization and apoptosis of Ras-transformed rodent cells (Du and Prendergast, 1999; Du et al., 1999; Prendergast, 2000). Consistent with the function of RhoB-GG in FTI-treated cells, a recent study with RhoB mutants that are exclusively either farnesylated or geranylgeranylated demonstrated that RhoB-GG, but not RhoB-F, suppresses the growth and tumor formation by Ras-transformed NIH3T3 cells (Mazieres et al., 2005). Conversely, transformed cells that are genetically null for RhoB show a variety of defects in their FTI response, including partially defective growth inhibition and complete ablation of actin reorganization and apoptosis induced by drug treatment (Liu et al., 2000). Thus, perhaps the most interesting and important feature of FTIs, their ability to selectively activate apoptosis in transformed cells, involves a gain of RhoB function that is based on an alteration of its prenylation and an accentuation of its expression and activity.

Other studies support the initial work linking RhoB to the antineoplastic effect of FTIs. The role of RhoB in transformed epithelial cells treated with FTI was addressed by using rat intestinal epithelial cells transformed with activated K-Ras or Rac1 (Zeng et al., 2003). These cells were highly sensitive to FTI-induced actin reorganization and growth inhibition, and ectopic expression of the RhoB-GG in cells phenocopied these effects. Analysis of RhoB effector domain mutants support a role for interactions with the Rho effector protein PRK in mediating RhoB-dependent facets of the FTI response that relate to growth inhibition. As noted above, PRK is a critical effector for the physiological function of RhoB in intracellular receptor trafficking (Mellor et al., 1998). This observation is consistent with a link between trafficking function and the antiproliferative effects of RhoB. The study on the role of RhoB in the regulation of endocytic traffic has indicated that FTI treatment disrupts EGF receptor traffic through modulation of RhoB (Wherlock et al., 2004). At early times, FTI treatment leads to production of two cellular pools of RhoB, with RhoB-GG localizing to MVB endosomes and RhoB-F localizing to the plasma membrane. FTI-induced redistribution of RhoB leads to retention of the internalised receptor within MVB endosomes (Wherlock et al., 2004). Together, these studies offer additional support for the concept that RhoB alteration contributes to FTI action in cells.

Recently, a mouse genetic study has revealed that FT is not required for tumor induction by ras oncogenes (Mijimolle et al., 2005). K-Ras-induced tumor formation

occurred in the absence of FT activity due to alternative prenylation of K-Ras that can occur. However, despite loss of prenylation, H-Ras also associated with membraneous structures and retained its transforming activity when expressed in cells devoid of FT activity. This radical observation argues that Ras proteins including surprisingly H-Ras can not be critical targets of FTI. Another recent study has shown that FTI can induce oxidative DNA damage, thereby initiating a DNA damage response that includes RhoB induction (Pan et al., 2005). Consistent with studies that DNA damage agents cause elevation of RhoB level (Fritz et al., 1995; Liu et al., 2001a), it was found that RhoB protein is increased after treatment with FTI. Quenching ROS prevented induction of RhoB by FTI, suggesting that RhoB was induced as an indirect result of ROS-mediated DNA damage. This study argues that FTI may elevate RhoB by affecting both prenylation status and gene transcription.

Future perspectives

It has become apparent that RhoB has a strikingly different function from RhoA and RhoC despite the very close sequence relationship of these proteins. The role for RhoB in cancer suppression raises the question of what its key targets on endosomes are, and to what extent effects of RhoB on endocytic trafficking link changes in pathways affecting cell survival, proliferation, stress signaling, and tumorigenesis. Additionally, further studies of the role of RhoB in the FTI response in settings that have become relevant to clinical application of these drugs for treatment of breast cancer and acute myeloid leukemias may improve the application of this novel class of cancer therapeutics.

References

Adamson P., Paterson H.F. and Hal l A. (1992a) Intracellular localization of the p21rho proteins. J. Cell Biol.119, 617-627.

Adamson P., Marshall C.J., Hall A. and Tilbrook P.A. (1992b) Post-translational modification of p21rho proteins. J. Biol. Chem. 267, 20033-20038.

Adini I., Rabinovitz I., Sun J.F., Prendergast G.C. and Benjamin L.E. (2003) RhoB controls Akt trafficking and stage-specific survival of endothelial cells during vascular development. Genes Dev. 17, 2721-2732.

Adnane J., Muro-Cacho C., Mathews L., Sebti S.M. and Munoz-Antonia T. (2002) Suppression of rho B expression in invasive carcinoma from head and neck cancer patients. Clin. Cancer Res. 8, 2225-2232.

Bishop A.L. and Hall A. (2000). Rho GTPases and their effector proteins. Biochem. J. 348, 241-255.

Chauhan S., Kunz S., Davis K., Roberts J., Martin G., Demetriou M.C., Sroka T.C., Cress A.E. and Miesfeld R.L. (2004) Androgen control of cell proliferation and cytoskeletal reorganization in human fibrosarcoma cells: role of RhoB signaling. J. Biol. Chem. 279, 937-944.

Chen Z., Sun J., Pradines A., Favre G., Adnane J. and Sebti S.M. (2000) Both farnesylated and geranylgeranylated RhoB inhibit malignant transformation and suppress human tumor growth in nude mice. J. Biol. Chem. 275, 17974-17978.

Cox A.D. and Der C.J. (1997) Farnesyltransferase inhibitors and cancer treatment: targeting simply Ras? Biochim. Biophys. Acta, 1333, F51-F71.

Cox A.D. and Der C.J. (2002) Farnesyltransferase inhibitors: promises and realities. Curr. Opin. Pharmacol, 2, 388-393.

Delarue F.L., Taylor B.S. and Sebti S.M. (2001) Ras and RhoA suppress whereas RhoB enhances cytokine-induced transcription of nitric oxide synthase-2 in human normal liver AKN-1 cells and lung cancer A-549 cells. Oncogene 20, 6531-6537.

Du W. and Prendergast G.C. (1999) Geranylgeranylated RhoB mediates inhibition of human tumor cell growth by farnesyltransferase inhibitors. Cancer Res. 59, 5924-5928.

Du W., Lebowitz P. and Prendergast G.C. (1999) Cell growth inhibition by farnesyltransferase inhibitors is mediated by gain of geranylgeranylated RhoB. Mol. Cell. Biol.19, 1831-1840.

Engel M.E., Datta P.K. and Moses H.L. (1998). RhoB is stabilized by transforming growth factor b and antagonizes transcriptional activation. J. Biol. Chem. 273, 9921-9926.

Ellis S. and Mellor H. (2000) Regulation of endocytic traffic by rho family GTPases. Trends Cell Biol. 10, 85-88.

Flynn P., Mellor H., Casamassima A. and Parker P.J. (2000) Rho GTPase control of protein kinase C-related protein kinase activation by 3-phosphoinositide-dependent protein kinase. J. Biol. Chem. 275, 11064-11070.

Forget M.A., Desrosiers R.R., Del M., Moumdjian R., Shedid D., Berthelet F. and Beliveau R. (2002) The expression of rho proteins decreases with human brain tumor progression: potential tumor markers. Clin. Exp. Metastasis 19, 9-15.

Fritz G. and Kaina B. (2001) Ras-related GTPase RhoB represses NF-kappaB signaling. J. Biol. Chem. 276, 3115-3122.

Fritz G., Kaina B. and Aktories K. (1995) The ras-related small GTP-binding protein RhoB is immediate-early inducible by DNA damaging treatments. J. Biol. Chem. 270, 25172-25177.

Gampel A., Parker P.J. and Mellor H. (1999) Regulation of epidermal growth factor receptor traffic by the small GTPase RhoB. Curr. Biol. 9, 955-958.

Gerhard R., Gerhard R., Tatge H., Genth H., Thum T., Borlak J., Fritz G. and Just I. (2005) Clostridium difficile toxin A induces expression of the stress-induced early gene product RhoB. J. Biol. Chem. 280, 1499-1505.

Jaffe A.B. and Hall A. (2002). Rho GTPases in transformation and metastasis. Adv. Cancer Res. 84, 57-80.

Jahner D. and Hunter T. (1991) The ras-related gene rhoB is an immediate-early gene inducible by v-Fps, epidermal growth factor, and platelet-derived growth factor in rat fibroblasts. Mol. Cell. Biol. 11, 3682-3690.

Jiang K., Sun J., Cheng J., Djeu J.Y., Wei S. and Sebti S. (2004a) Akt mediates Ras downregulation of RhoB, a suppressor of transformation, invasion, and metastasis. Mol. Cell Biol. 24, 5565-5576.

Jiang K., Delarue F.L. and Sebti S.M. (2004b) EGFR, ErbB2 and Ras but not Src suppress RhoB expression while ectopic expression of RhoB antagonizes oncogene-mediated transformation. Oncogene 23, 1136-1145.

Kamasani U., Liu A. and Prendergast G.C. (2003) Genetic response to farnesyltransferase inhibitors: proapoptotic targets of RhoB. Cancer Biol. Therapy 2, 273-280.

Kamasani U., Huang M., Duhadaway J.B., Prochownik E.V., Donover

P.S. and Prendergast G.C. (2004) Cyclin B1 is a critical target of RhoB in the cell suicide program triggered by farnesyl transferase inhibition. Cancer Res. 64, 8389-8396.

Karnoub A.E., Symons M., Campbell S.L. and Der C.J. (2004) Molecular basis for Rho GTPase signaling specificity. Breast Cancer Res. Treat 84, 61-71.

Khosravi-Far R., Solski PA., Clark GJ., Kinch M.S. and Der C.J. (1995) Activation of Rac1, RhoA, and Mitogen-Activated Protein Kinase are required for Ras transformation. Mol. Cell. Biol. 15, 6443-6453.

Lebowitz, P. and Prendergast G.C. (1998a) Functional interaction between RhoB and the transcription factor DB1. Cell Adhes. Comm. 4, 1-11.

Lebowitz P.F. and Prendergast G.C. (1998b) Non-Ras targets for farnesyltransferase inhibitors: focus on Rho. Oncogene 17, 1439-1447.

Lebowitz P.F., Davide J.P. and Prendergast G.C. (1995) Evidence that farnesyl transferase inhibitors suppress Ras transformation by interfering with Rho activity. Mol. Cell. Biol. 15, 6613-6622.

Lebowitz P.F., Casey P.J., Prendergast G.C. and Thissen J.A. (1997) Farnesyltransferase inhibitors alter the prenylation and growth-stimulating function of RhoB. J. Biol. Chem. 272, 15591-15594.

Liu A. and Prendergast G.C. (2000) Geranylgeranylated RhoB is sufficient to mediate tissue-specific suppression of Akt kinase activity by farnesyltransferase inhibitors. FEBS Lett. 481, 205-208.

Liu A., Du W., Liu J.P., Jessell T.M. and Prendergast G.C. (2000). RhoB alteration is necessary for the apoptotic and antineoplastic responses to farnesyltransferase inhibitors. Mol. Cell Biol. 20, 6105-6113.

Liu A., Cerniglia G.J., Bernhard E.J. and Prendergast G.C. (2001a). RhoB is required for the apoptotic response of neoplastically transformed cells to DNA damage. Proc. Natl. Acad. Sci. USA 98, 6192-6197.

Liu A., Rane N., Liu J.P. and Prendergast G.C. (2001b). RhoB is dispensable for mouse development, but it modifies susceptibility to tumor formation as well as cell adhesion and growth factor signaling in transformed cells. Mol. Cell. Biol. 21, 6906-6912.

Mazieres J., Antonia T., Daste G., Muro-Cacho C., Berchery D., Tillement V., Pradines A., Sebti S. and Favre G. (2004) Loss of RhoB expression in human lung cancer progression. Clin. Cancer Res. 10, 2742-2750.

Mazieres J., Tillement V., Allal C., Clanet C., Bobin L., Chen Z., Sebti S.M., Favre G. and Pradines A. (2005) Geranylgeranylated, but not farnesylated, RhoB suppresses Ras transformation of NIH-3T3 cells. Exp. Cell Res. 304, 354-364.

Mellor H., Flynn P., Nobes C.D. and Hall A. (1998) Parker PJ. PRK1 is targeted to endosomes by the small GTPase, RhoB. J. Biol. Chem. 273, 4811-4814.

Michaelson D., Silletti J., Murphy G., D'Eustachio P., Rush M. and Philips M.R. (2001) Differential localization of Rho GTPases in live cells: regulation by hypervariable regions and RhoGDI binding. J. Cell Biol. 152, 111-126.

Mijimolle N., Velasco J., Dubus P., Guerra C., Weinbaum C.A., Casey P.J., Campuzano V. and Barbacid M. (2005) Protein farnesyltransferase in embryogenesis, adult homeostasis, and tumor development. Cancer Cell 7, 313-324.

Milia J., Teyssier F., Dalenc F., Ader I., Delmas C., Pradines A., Lajoie-Mazenc I., Baron R., Bonnet J., Cohen-Jonathan E., Favre G. and Toulas C. (2005) Farnesylated RhoB inhibits radiation-induced mitotic cell death and controls radiation-induced centrosome overduplication. Cell Death Differ. 12, 492-501.

Pan J., She M., Xu Z.X., Sun L. and Yeung S.C. (2005) Farnesyltransferase inhibitors induce DNA damage via reactive oxygen species in human cancer cells. Cancer Res. 65, 3671-3681.

Prendergast G.C. (2000) Farnesyltransferase inhibitors: antineoplastic mechanism and clinical prospects. Curr. Opin. Cell Biol. 12, 166-173.

Prendergast G.C. (2001) Actin' up: RhoB in cancer and apoptosis. Nature Rev. Cancer 1, 162-168.

Pruitt K. and Der C.J. (2001) Ras and Rho regulation of the cell cycle and oncogenesis. Cancer Lett. 171, 1-10.

Ridley A.J. (2004) Rho proteins and cancer. Breast Cancer Res Treat, 84, 13-19.

Robertson D., Paterson H.F., Adamson P., Hall A. and Monaghan P. (1995) Ultrastructural localization of ras-related proteins using epitope-tagged plasmids. J. Histochem. Cytochem. 43, 471-480.

Sahai E. and Marshall C.J. (2002) Rho GTPases and cancer. Nature Rev. Cancer 2, 133-142.

Sandilands E., Cans C., Fincham V.J., Brunton V.G., Mellor H., Prendergast G.C., Norman J.C., Superti-Furga G. and Frame M.C. (2004) RhoB and actin polymerization coordinate Src activation with endosome-mediated delivery to the membrane. Dev. Cell 7, 855-869.

Sebti S.M. and Hamilton A.D. (2000) Farnesyltransferase and geranylgeranyltransferase I inhibitors in cancer therapy: important mechanistic and bench to bedside issues. Exp. Opin. Invest. Drugs 9, 2767-2782.

Shields J.M., Pruitt K., McFall A., Shaub A. and Der C.J. (2000) Understanding Ras: 'it ain't over 'til it's over'. Trends Cell Biol. 10, 147-154.

Trapp T., Olah L., Holker I., Besselmann M., Tiesler C., Maeda K. and Hossmann K.A. (2001). GTPase RhoB: an early predictor of neuronal death after transient focal ischemia in mice. Mol. Cell. Neurosci.17, 883-894.

Wang D.A. and Sebti S.M. (2005) Palmitoylated cysteine 192 is required for RhoB tumor-suppressive and apoptotic activities. J. Biol. Chem. 280, 19243-19249.

Wang S., Yan-Neale Y., Fischer D., Zeremski M., Cai R., Zhu J., Asselbergs F., Hampton G. and Cohen D. (2003) Histone deacetylase 1 represses the small GTPase RhoB expression in human nonsmall lung carcinoma cell line. Oncogene 22, 6204-6213.

Westmark C.J., Bartleson V.B. and Malter J.S. (2005) RhoB mRNA is stabilized by HuR after UV light. Oncogene 24, 502-511.

Westwick J.K., Lambert Q.T., Clark G.J., Symons M., Van Aelst L., Pestell R.G. and Der C.J. (1997) Rac regulation of transformation, gene expression, and actin organization by multiple, PAK-independent pathways. Mol. Cell Biol. 17, 1324-1335.

Wherlock M., Gampel A., Futter C. and Mellor H. (2004) Farnesyltransferase inhibitors disrupt EGF receptor traffic through modulation of the RhoB GTPase. J. Cell Sci.117, 3221-3231.

Zalcman G., Zalcman G., Closson V., Linares-Cruz G., Lerebours F., Honore N., Tavitian A. and Olofsson B. (1995) Regulation of Ras-related RhoB protein expression during the cell cycle. Oncogene 10, 1935-1945.

Zeng P.Y., Rane N., Du W., Chintapalli J. and Prendergast G.C. (2003) Role of RhoB and PRK in the suppression of epithelial cell transformation by farnesyltransferase inhibitors. Oncogene 22, 1124-1134.

Accepted August 5, 2005