ROCK2 (Rho-associated, coiled-coil containing protein kinase 2)

Carmen Chak-Lui Wong, Irene Oi-Lin Ng

Department of Pathology and State Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong, China (CCLW, IOLN)

Published in Atlas Database: December 2012
DOI: 10.4267/2042/51038

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 2.0 France Licence. © 2013 Atlas of Genetics and Cytogenetics in Oncology and Haematology

**Identity**

**Other names:** ROCK-II
**HGNC (Hugo):** ROCK2
**Location:** 2p25.1

**DNA/RNA**

**Note**
ROCK2 was first identified as a target of active RhoA in an expression screening assay from a rat brain cDNA library (Leung et al. 1995).

**Description**

The ROCK2 gene is 4164 bp long which encodes 1388 amino acids producing a protein of 160 kDa (UniGene: Hs.681743).
The ROCK2 gene comprises 33 exons.

**Protein**

**Note**
ROCK2 is a 160 kDa serine/threonine kinase which is composed of a kinase domain (44-415 aa) at the amino-terminus, a rho-binding domain (981-1046 aa), and a pleckstrin homology (PH) domain with a cysteine-rich region at the carboxyl-terminus (1152-1350 aa).

A coiled-coil inhibitory structure was predicted to form between the kinase domain and the PH domain. ROCK2 exists in an auto-inhibitory form which could be released when active form of RhoA (RhoA-GTP) binds to the RBD domain of ROCK2. The functional domains of ROCK2 have unique roles such as protein-protein interaction, cellular localization, and regulation of ROCK2 activity. Kinase domain of ROCK2 is the key domain conferring the most important function of ROCK2 which is the phosphorylation and activation of its downstream substrates (Leung et al., 1995).

PH domain of ROCK2 is responsible for lipid binding and membrane localization of ROCK2 (Miyazaki et al., 2006).

PH domain of a ROCK2 homolog also facilitates the interaction with other proteins such as filamin A, a protein critical to actin cytoskeleton remodeling (Ueda et al., 2003).

Schematic diagram of ROCK2. RBD: rho-binding domain; PH: pleckstrin homology; C1: cysteine-rich region.
**Expression**

Very few studies have demonstrated the distinctive roles of ROCK1 and ROCK2; however, their differential tissue distribution indicates that they participate in different physiological functions. ROCK1 and ROCK2 are ubiquitously expressed in all tissues. Earlier studies using Northern blot analysis demonstrated that ROCK1 mRNA expression was especially abundant in testis, liver, and lung while ROCK2 mRNA expression was especially abundant in brain and muscle (Leung et al., 1996).

**Regulation:** The most common regulatory mechanism of ROCK2 is mediated by RhoGTPases whose activity exchanges between a GTP-bound active form and a GDP-bound inactive form. RhoA-GTP binds to the RBD domain of ROCK and releases the kinase domain of ROCK leading to the activation of ROCK (Leung et al., 1995). In addition to this classical model of activity regulation, ROCK2 can also be activated by lipids such as arachidonic acid (Feng et al., 1999). The ROCK2 kinase domain can also be released when the C-terminus of ROCK2 is cleaved by granzyme B (Sebbagh et al., 2005). Furthermore, Polo-like kinase-1 (Plk1) interacts with and phosphorylates ROCK2 at Threonine 967, Serine 1099, Serine 1133, and S1374 (Plk1) interacts with and phosphorylates ROCK2 at Thr697, Ser1099, Ser1133, and S1374 and activates ROCK2 (Lowery et al., 2007). Recent studies have demonstrated that ROCK2 is also regulated at the expression level. Two microRNAs (miRNAs), miR-139 and miR-124, have independently been shown to interact with the 3'untranslated region of ROCK2 and subsequently suppress ROCK2 expression in hepatocellular carcinoma (HCC) cell lines (Wong et al., 2011; Zheng et al., 2012). MiR-139 and miR-124 have been shown to be downregulated in human HCCs and their expression levels inversely correlated with ROCK2 protein expression in human HCC samples (Wong et al., 2011; Zheng et al., 2012).

**Localisation**

ROCK2 is mainly found in the cytoplasm in most cell types. It was reported that overexpression of a dominant-active form of RhoA re-localized ROCK2 to the membranous actin filaments in a cervical cancer cell line, HeLa (Leung et al., 1995). A study has also shown that ROCK2 could be found in the nuclei in multiple cell types such as human keratinocytes, mouse mammary epithelial cells, osteoblasts, and mouse fibroblasts (Tanaka et al., 2006). ROCK2 was also found to be located in the centrosome in fibroblasts (Ma et al., 2006; Wang et al., 2011).

**Function**

ROCK2 are responsible for many key cellular processes including cell migration and invasion (Croft et al., 2004), apoptosis (Sebbagh et al., 2005), centrosome duplication (Ma et al., 2006), and cytokinesis (Lowery et al., 2007). The best well-characterized function of ROCK is contributed by the kinase domain for its ability to phosphorylate the serine/threonine residues of downstream substrates that are important for actin cytoskeleton organization. The most well-known substrates of ROCK include myosin light chain 2 (MLC2) and myosin phosphatase 1 (MYPT1) which regulate actomyosin contractility in cells (Amano et al., 1996). Other ROCK substrates include coflin and LIM kinases, responsible for actin polymerization and depolymerization (Yang et al., 1998; Sumi et al., 1999; Ohashi et al., 2000; Sumi et al., 2001); vimentin (Goto et al., 1998), responsible for actin filament formation; adducin, responsible for membrane ruffles formation (Fukata et al., 1999); calponin, responsible for actin filament binding (Kaneko et al., 2000); ezrin, responsible for anchoring the cytoskeleton to the cell membrane and the formation of focal adhesion molecules (Matsui et al., 1998; Tran Quang et al., 2000).

In addition to cell movement, ROCK2 was shown to be important in keratinocyte differentiation (McMullan et al., 2003). Also, Plk1-mediated phosphorylation and activation of ROCK2 induced cytokinesis (Lowery et al., 2007).

Nuclear function of ROCK2 was first reported when ROCK2 was found to be a binding partner of p300 acetyltransferase (Tanaka et al., 2006). Nuclear ROCK2 phosphorylated p300 and activated p300-mediated transcription (Tanaka et al., 2006). Centrosomal ROCK2 interacted with nucleosomin/B23 and BRCA2 proteins for centrosome duplication (Ma et al., 2006; Wang et al., 2011).

ROCK2 also regulates the extracellular matrix. Expression of conditional active ROCK2 construct has been shown to activate MYPT and MLC and drive actomyosin contraction in mouse skin, thereby modifying the ECM through increased collagen deposition and tissue stiffness (Samuel et al., 2011). Along with the ECM modification, activation of ROCK2 may also increase nuclear accumulation and activity of β-catenin. Activation of β-catenin can elevate epidermal cell proliferation rate, subsequently contributing to epidermal hyperplasia and tumor growth (Samuel et al., 2011).

**Homology**

ROCK2 shares close homology with another protein called ROCK1. ROCK1 and ROCK2 are 65% homologous in human; particularly, their kinase domains are 87% homologous (Leung et al., 1996). Due to their high degree of homology, ROCK1 and ROCK2 are regulated by common mechanisms and share many common substrates.
ROCK2 (Rho-associated, coiled-coil containing protein kinase 2) Wong CCL, Ng IOL

Atlas Genet Cytogenet Oncol Haematol. 2013; 17(7) 443

Implicated in

**Solid cancers**

**Note**

ROCK, in general, has been shown to be implicated in various cancer cell line models; however, only a few studies focus on the expression and roles of ROCK2 in cancers.

**Colorectal cancer**

**Note**

In a subcutaneous tumor model in nude mice, conditional expression of active ROCK2 construct in colorectal cancer cell lines has been demonstrated to enhance angiogenesis and cancer cell invasion into the surrounding stromal tissues (Croft et al., 2004).

**Hepatocellular carcinoma**

**Note**

ROCK2 protein was shown to be over-expressed in 54% (22/41 cases) of human hepatocellular carcinoma (HCC) as compared with their corresponding non-tumorous liver tissues by Western blot analysis (Wong et al., 2009). Over-expression of ROCK2 in HCC was associated with the presence of tumor microsatellite formation, an important clinicopathological feature of aggressive HCC and an indicator of intrahepatic metastasis in human HCC (Wong et al., 2009). Knockdown of ROCK2 in human HCC cell lines suppressed stress fiber and focal adhesion formation, as well as actomyosin contractility in HCC cells, thereby retarding HCC cell invasion in vitro and in vivo (Wong et al., 2009).

**Squamous skin carcinomas**

**Note**

Immunohistochemical study using an antibody with reactivity towards both ROCK1 and ROCK2 indicates that high ROCK expression was detected in 40 cases of human squamous skin carcinomas (Samuel et al., 2011). Along with this finding, phosphorylation of MYPT, as a reflection of ROCK activity, was also detected in these cases of carcinoma (Samuel et al., 2011).

**Testicular cancer**

**Note**

ROCK2 protein was shown in Western blot analysis to be overexpressed in testicular cancers as compared to non-tumorous tissues in a cohort of 57 patients (Kamai et al., 2004). ROCK2 protein expression was significantly higher in patients with recurrent testicular cancers than those without sign of recurrence after treatment (Kamai et al., 2004).

**Breast cancer**

**Note**

It has long been challenging to detect the ROCK activity in human clinical specimens owing to the fact that ROCK activity declines rapidly in clinical specimens after resection. Also, the phosphorylation sites in ROCK, which truly reflect its activity status, have not been identified or extensively studied. Recently, a study has demonstrated that the activity status of ROCK2 can be reflected by the phosphorylation level of ROCK2 at Ser"^{366}. Immunohistochemical staining shows that phosphorylation of ROCK2 at Ser"^{366} was increased in 2 cases of breast cancer tissues as compared to their normal counterparts (Chuang et al., 2012).

**To be noted**

**Drugs**

Multiple ROCK inhibitors are available; but none of these inhibitors specifically discriminate the two homologs. The common commercially available ROCK inhibitors are pyridine or isoquinoline-based small molecule inhibitors. Both inhibitors compete for the ATP-binding sites of ROCK. Y27632 is the most commonly used pyridine-based ROCK inhibitor and has been shown to possess significant selectivity towards ROCK than other protein kinases (Davies et al., 2000; Ishizaki et al., 2000). Fasudil, the most common isoquinoline-based ROCK inhibitor, was first used in the clinic in 1993 for the treatment of cerebral vasospasm in Japan and was later discovered to possess inhibitory effect on ROCK (Shibuya and Suzuki, 1993). Clinical experience of fasudil shows that it is well tolerated in the human body; however, it is less selective than Y27632. Therefore, Y27632 is more widely used in basic research whilst fasudil is more widely used in the clinic. The half-life of Y27632 in vivo is around 2 hours (Takamura et al., 2001). Studies have demonstrated that continuous delivery of Y27632 by osmotic pump profoundly suppressed the formation of metastases in syngeneic rats that were peritoneally implanted with rat hepatoma cells or in SCID mice that were orthotopically implanted with human HCC cells (Itoh et al., 1999; Takamura et al., 2001). So far, ROCK inhibitors have not been used in the clinic for cancer treatment. Further investigation on the efficacy of ROCK inhibitors in cancer treatments is much warranted.

**References**

Shibuya M, Suzuki Y. [Treatment of cerebral vasospasm by a protein kinase inhibitor AT 877]. No To Shinkei. 1993 Sep;45(9):819-24

Leung T, Manser E, Tan L, Lim L. A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. J Biol Chem. 1995 Dec 8;270(49):29051-4

Keratinocyte differentiation is regulated by the Rho and ROCK

McMullan R, Lax S, Robertson VH, Radford DJ et al..

hepatocellular carcinoma by Rho-associated protein kinase

Hirohashi S. Inhibition of intrahepatic metastasis of human

Takamura M, Sakamoto M, Genda T, Ichida T, Asakura H,

5;276(1):670-6

Rho-dependent protein kinase 2 via phosphorylation of threonine 505 by ROCK, a

Sumi T, Matsumoto K, Nakamura T. Specific activation of LIM

24;273(1):110-6

Rho-kinase ROK alpha is a member of a kinase family and is


5;393(6687):809-12


This article should be referenced as such:


Ueda K, Ohta Y, Hosoya H. The carboxy-terminal pleckstrin homology domain of ROCK interacts with filamin-A. Biochem Biophys Res Commun. 2003 Feb 21;301(4):886-90


Wong CCL, Wong CM, Tung EK, Man K, Ng IO. ROCK2 is involved in tumor invasion. Hepatology. 2009 May;49(5):1583-94


This article should be referenced as such: