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Review

RHOBTB3 (Rho-related BTB domain containing 3)

Shuo Cai, Francisco Rivero

Cardiff China Medical Research Collaborative Institute of Cancer and Genetics, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, (SC); Centre for Cardiovascular and Metabolic Research, The Hull York Medical School, University of Hull, Cottingham Road, Hull HU6 7RX, (FR) UK. CaiS5@cardiff.ac.uk; francisco.rivero@hyms.ac.uk

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Abstract

RHOBTB3 is one of the three members of the RhoBTB family. All RhoBTB proteins are characterized by a GTPase domain followed by a proline-rich region, a tandem of two BTB domains and a C-terminal putative RING finger domain. In RHOBTB3 the GTPase domain has ATPase activity. RHOBTB3 is a putative tumour suppressor gene. Expression of RHOBTB3 has been found significantly decreased in the breast, kidney, uterus, lung, and ovary tumors and in human renal carcinomas. The mechanism of RHOBTB3 protein as a tumor suppressor may be related to its function as an adaptor of cullin 3-dependent ubiquitin ligases. RHOBTB3 targets cyclin E for degradation and facilitates entry into the G2 phase of the cell cycle. RHOBTB3 also builds a multiprotein complex that maintains HIFα (hypoxia inducible factor α) levels low by promoting its hydroxylation, ubiquitination and degradation.

Keywords
tumor suppressor, ubiquitin ligase, cullin 3, HIFα, cyclin E

Identity

HGNC (Hugo): RHOBTB3

Location: 5q15

Location (base pair): Starts at 95,713,522 bp from pter and ends at 95,824,383 bp from pter.

DNA/RNA

Description

The RHOBTB3 gene spans over 65 Kbp genomic DNA and consists of 12 exons. The first exon splits the translation initiation codon (Figure 1). The coding sequence of RHOBTB3 is 1833 nucleotides long (Ramos et al., 2002).

Transcription

There is no evidence of transcription variants.

Protein

Note
RHOBTB3 is one of the three members of the RhoBTB family in vertebrates. The RhoBTB family was identified during the study of the genes encoding Rho-related proteins in the lower eukaryote Dictyostelium discoideum (Rivero et al., 2002). All three RhoBTB proteins may be implicated in tumorigenesis (Berthold et al., 2008b).

Description

RHOBTB3 is 611 amino acids long. All RhoBTB proteins share the same domain architecture: a GTPase domain is followed by a proline-rich region, a tandem of two BTB domains and a C-terminal region (Figure 2).
The GTPase domain of RHOBTB3 is considerably divergent and unlike the GTPase domain of

RHOBTB1 and RHOBTB2, which bind GTP, it binds and hydrolyses ATP (Espinosa et al., 2009).
The proline-rich region links the GTPase to the first BTB domain. In RHOBTB1 and RHOBTB2 this region could act as a SH3 domain-binding site, however in RhoBTB3 the proline-rich region is not very prominent.
The BTB domain (broad complex, tramtrack and bric-a-brac) is an evolutionary conserved protein-protein interaction domain that participates in homomeric and heteromeric associations with other BTB domains. The BTB domain was also identified as a component of multimeric cullin 3-dependent ubiquitin ligase complexes. The first BTB domain is bipartite, being interrupted by an insertion of unknown function that is much shorter in RhoBTB3 than in the two other members of the family. The BTB domains of RhoBTB allow the formation of homodimers and of heterodimers with other proteins of the RhoBTB family (Berthold et al., 2008).
The C-terminus is a region conserved in all members of the RhoBTB subfamily. It predictably folds as 4 consecutive alpha-helices and one beta-strand and may constitute a RING finger domain (Manjarrez et al., 2014). Many RING finger domains function as ubiquitin ligases. RHOBTB3 bears a CAAX motif that is typical for classical Rho GTPases. This motif undergoes isoprenylation of the cysteine residue and proteolytic cleavage of the last three residues and serves for localization of the protein to membranes, although it's not the only determinant for the Golgi apparatus targeting of RHOBTB3 (Lu and Pfeffer, 2013).

Expression

RHOBTB3 is ubiquitously expressed, with high mRNA levels present in placenta, testis, pancreas, adrenal and salivary glands and neural and cardiac tissues. It is also expressed in fetal tissues (Ramos et al., 2002; Nagase et al., 1998).
Expression of the mouse Rhobtb3 gene has been investigated in great detail in a gene trap mouse strain that expresses β-galactosidase under the control of the endogenous Rhobtb3 promoter (Lutz et al., 2014). Histochemical detection of β-galactosidase expression revealed a profile characterized by nearly ubiquitous expression of Rhobtb3 in the embryo, with particularly high levels in bone, cartilage, all types of muscle, testis and restricted areas of the nervous system. In the adult mouse expression declines considerably, but persists at low levels in cardiac muscle, the tunica media of blood vessels, the muscularis of hollow organs and cartilage, and at high levels in the seminiferous tubules and peripheral nerves.
Expression of RHOBTB3 has been found decreased in kidney, breast, uterus, lung and ovary tumors in a cancer profiling array (Berthold et al., 2008) and in diverse renal cell carcinoma subtypes (Zhang et al., 2015).

Localisation

The localisation of endogenous RHOBTB3 has not been investigated. Available antibodies fail to recognise any endogenous RHOBTB3 in fixed cells and tissues. In cells expressing epitope tagged RHOBTB3 ectopically the protein tends to form aggregates in a paranuclear pattern (Berthold et al., 2008). When expressed at moderate levels RHOBTB3 displays a vesicular pattern predominantly surrounding the centrosome. RHOBTB3 co-localises with Golgi apparatus markers. Some vesicles co-localize with early endosome markers or in close vicinity to microtubules or stress fibres (Berthold et al., 2008; Espinosa et al., 2009).

Function

Following functions have been proposed for RHOBTB3. The molecular mechanisms by which RhoBTB3 exerts those roles are beginning to be elucidated and in most cases may be related to its role in ubiquitination.

1. RHOBTB3 as adaptor of cullin 3-dependent ubiquitin ligases.

The first BTB domain binds to the N-terminal region of CUL3 (cullin 3), but not other cullins. RHOBTB3 is itself a substrate for the cullin 3-based ubiquitin ligase complex (Berthold et al., 2008). RhoBTB proteins appear to exist in an inactive state through an intramolecular interaction of the BTB domain region with the GTPase domain (Berthold et al., 2008).
Several potential substrates of RHOBTB3-dependent ubiquitin ligase complex have been described, including LRRC41 (MUF-1) and cyclin E and RHOBTB3 also participates in the degradation of HIF1A (HIFα hypoxia inducible factor α). They have implications in tumorigenesis and are described below.
RHOBTB3 also interacts with the 5-HT7a receptor, the most common splice variant of HTR7, the serotonin receptor 7. This receptor is involved in a wide variety of pathophysiological processes of the central nervous system. Interestingly, the 5-HT7a receptor appears to interact with cullin 3 independently of RHOBTB3, and RHOBTB3 apparently inhibits proteasomal degradation of the receptor (Mathys et al., 2012)

2. RHOBTB3 roles in cell cycle regulation and tumorigenesis.

RHOBTB3, like RHOBTB1 and RHOBTB2, interacts with MUF1 (LLRC41, leucine rich repeat containing 41). MUF1 is a nuclear protein and carries a BC-box that functions as a linker in multicomponent cullin 5-dependent ubiquitin ligase complexes (Schenkov et al., 2012). MUF1 may be a

substrate for RhoBTB-Cullin 3 ubiquitin ligase complexes. The function of MUF1 is unknown, but it is suspected to be involved in the DNA damage response.

RHOBTB3 binds cyclin E1 (CCNE1) (and to a lesser extent CCNB1 (cyclin B1)), uncoupled from its dependent kinaseCDK2. Cyclin E regulates the cell cycle transition from G1 to S phase and is degraded before entry into G2 phase. RHOBTB3 targets cyclin E for ubiquitination by a cullin 3-dependent ubiquitin ligase that localizes at the Golgi apparatus (Lu and Pfeffer, 2013). RhoBTB3 protein accumulates during the S phase after the plateau of cyclin E. Depletion of RHOBTB3 causes S-phase arrest in cultured cells accompanied by increased levels of cyclin E and increased activity of CDK2. Therefore RHOBTB3 regulates the S/G2 transition of the cell cycle by targeting cyclin E for ubiquitination. The RHOBTB3-CUL3 pathway constitutes an alternative ubiquitination route to the KITLG (SCF)- FBXW7 pathway, but these two pathways may target different pools of cyclin E. This mechanism may contribute to the role of RHOBTB3 as a tumor suppressor. Deregulation of cyclin E levels can have a significant impact on cell proliferation, as shown in a significant percentage of breast cancers where high cyclin E correlates with tumor stage and grade.

Regulation of HIFα levels constitutes another case of cross-talk between different ubiquitination pathways. HIFs are key regulators of adaptive responses to low oxygen concentration. In the presence of oxygen their α-subunits are rapidly degraded through an ubiquitination-dependent proteasomal pathway after hydroxylation. Under hypoxia conditions HIFs accumulate and bind to hypoxia responsive elements of various genes, in many cases related to aspects of cancer growth. In fact, aberrant accumulation or activation of HIFs is closely linked to many types of cancer. Hydroxylated HIFα is targeted for ubiquitination by the von Hippel-Lindau (VHL) protein, a component of a CUL2 (cullin 2)-dependent ubiquitin ligase (Tanimoto et al., 2000).

RHOBTB3 acts as a scaffold for a multicomponent complex that regulates the degradation of HIFα. RHOBTB3 binds the prolyl hydroxylase EGLN1 (PHD2) that promotes hydroxylation of HIFα (Zhang et al., 2015). The complex also binds VHL protein and facilitates ubiquitination of HIFα. Additionally RHOBTB3 appears to heterodimerize with LIMD1, an adaptor for PHD2 and VHL, and this interaction enhances the activity of the complex. The chaperone HSP90AA1 (Hsp90) is incorporated to the complex through interaction with HIFα and does not seem to interact with RHOBTB3. Hsp90 may contribute to relieve the autoinhibitory conformation of RHOBTB3, as it has been proposed for RHOBTB2 (Manjarrez et al., 2014). Hypoxia reduces the formation of the RHOBTB3-dependent multicomponent complex, resulting in an accumulation of HIFα.

A tumor suppressor role for RHOBTB3 has been shown in xenograft experiments with Ras-transformed embryonic fibroblasts isolated from Rhobtb3 deficient mice or HeLa cells in which Rhobtb3 was silenced. The xenografts were larger and had increased levels of HIFα and its gene targets. It has been proposed that RHOBTB3 inhibits tumorigenesis by maintaining low HIFα levels and consequently suppressing the Warburg effect (Zhang et al., 2015).

3. RHOBTB3 and vesicle trafficking.

RHOBTB3 is a component of a complex required for retrograde transport to the Golgi complex that contains RAB9A and the cargo selection protein PLIN3 (TIP47), with which RHOBTB3 interacts (Espinosa et al., 2009). When RHOBTB3 is depleted by gene silencing the Golgi apparatus becomes fragmented (Lu and Pfeffer 2013) and the mannose-6-P receptor adopts a disperse localization in Rab9 positive vesicles, indicative of altered retrograde transport, but endocytosis and exocytosis are not changed (Espinosa et al., 2009). A model has been proposed in which Rab9 on vesicles travelling from late endosomes to the Golgi relieves the autoinhibitory conformation of RHOBTB3 and allows maximal ATP hydrolysis. Activation of RHOBTB3 releases TIP47, facilitating vesicle uncoating and membrane fusion (Pfeffer 2009).

4. Other roles.

A case of a male carrying a balanced paracentric inversion of chromosome 5 that disrupts RHOBTB3 has been reported. This patient showed asymmetric leg growth and large hands and behavior problems. It hasn't been determined whether disruption of RHOBTB3 is the cause of those alterations (Chen et al., 2010).

The characterization of a gene trap knockout mouse strain has shown that disruption of the Rhobtb3 gene causes reduced perinatal viability, a postnatal growth defect that persists in males after weaning and reduced testis size (Lutz et al., 2014). Ablation of Rhobtb3 only caused very modest changes in the pattern of gene expression of adult heart and brain. Lack of Rhobtb3 did not affect the rate of proliferation of primary lung fibroblasts isolated from 10-week-old animals (Lutz et al., 2014) but higher proliferation rates have been reported in mouse embryonic fibroblasts (Zhang et al., 2015).

RHOBTB3 has been identified as a candidate blood biomarker for hallucinations. Gene expression was found decreased in high hallucination states (Kurian et al., 2011). RHOBTB3 has also been proposed as a candidate vulnerability gene for Alzheimer's disease (Miller et al., 2013).

Like other members of the RhoBTB family, RHOBTB3 has no apparent influence on cell

morphology and actin organization (Berthold et al., 2008).

Homology

There are three RhoBTB proteins in vertebrates: RHOBTB1, RHOBTB2 and RhOBTB3 (Figure 2). RHOBTB2 is very similar to RHOBTB1, while RHOBTB3 displays very low similarity to these. Orthologues have been found in amoebae and in insects but they are absent in plants and fungi.

Mutations

No pathogenic mutations have been identified to date.

Implicated in

Various cancers, including kidney, breast, uterus, lung and ovary

Expression of RHOBTB3 was found moderately but significantly decreased in breast, kidney, uterus, lung, and ovary tumour samples in a cancer profiling array. The decrease affected to 80% of kidney and to 56% of breast cancer samples. The expression changes correlated with those of CUL3 in the same samples (Berthold et al., 2008)

Renal cell carcinoma

RHOBTB3 expression is significantly decreased in clear cell renal cell carcinoma, papillary renal cell carcinoma, hereditary clear cell renal cell carcinoma and non-hereditary clear cell renal cell carcinoma subtypes (Zhang et al., 2015).

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