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Gene Section
Review

RHOBTB2 (Rho-related BTB domain containing 2)

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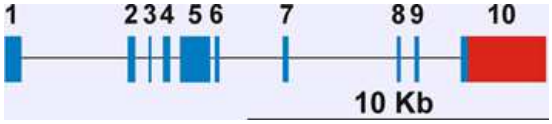
Identity

Other names: DBC2 (Deleted in breast cancer 2 gene protein); KIAA0717

HGNC (Hugo): RHOBTB2

Location: 8p21.3

DNA/RNA



Gene structure of RHOBTB2. Boxes represent exons. The coding region is represented in blue.

Description

The RHOBTB2 gene spans over 20 Kbp genomic DNA and consists of 10 exons, 9 coding exons and one exon in the 5'UTR (Figure 1). The coding sequence of RHOBTB2 is 2184 nucleotides long. The promoter region of RHOBTB2 has CpG islands.

Transcription

There is no evidence of alternatively spliced transcripts.

Protein

Note

RhoBTB2 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 tumourigenesis.

Description

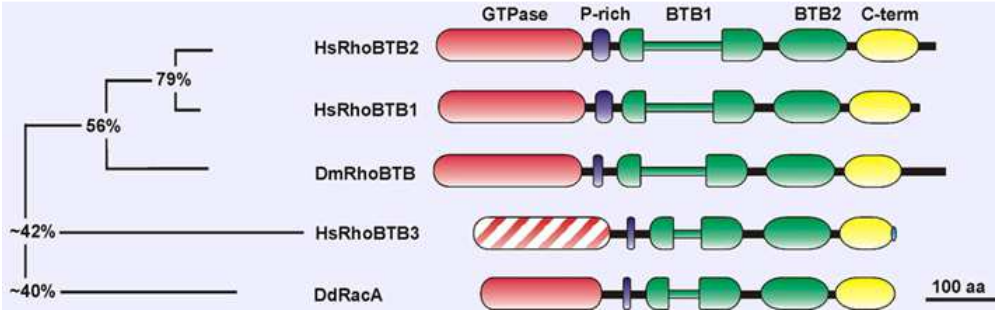
RhoBTB2 is 727 amino acids long. All RhoBTB proteins share the same domain architecture: a GTPase domain is followed by a proline-rich region, a tandem of two BTB domains and a C-terminal region (Figure 2).

The GTPase domain is Rho-related and contains a Rho insert that is longer than usual, two insertions and one deletion, as well as a few deviations from the GTPase consensus of most Rho GTPases. Consequently, RhoBTB2 would predictably display impaired enzyme activity and indeed it has been shown that in RhoBTB2 this domain does not bind GTP at all (Chang et al., 2006).

The proline-rich region links the GTPase to the first BTB domain. This region could act as a SH3 domain-binding site.

The BTB domain (broad complex, tramtract 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 cullin3-dependent ubiquitin ligase complexes. The first BTB domain is bipartite, being interrupted by an insertion of unknown function. 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. RhoBTB2 does not bear a CAAX motif that



The figure shows the three human (Hs) RhoBTB subfamily members as well as the Drosophila (Dm) and Dictyostelium (Dd) orthologues. The simplified phylogenetic tree on the left illustrates the relationship among the proteins (overall percentage similarity between branches). The different domains are indicated with colours.

is typical for classical Rho GTPases and serves for localization of the protein to membranes.

Expression

RHOBTB2 is weakly expressed, with relatively higher levels in neural and cardiac tissues. It is also expressed in fetal tissues (Ramos et al., 2002; Nagase et al., 1998). During mouse embryogenesis high and specific expression has been observed in the central and peripheral nervous system and comparatively weaker in the gut, but the mRNA becomes undetectable at embryonic day 18.5 (St-Pierre et al., 2004). One study has addressed the expression of RHOBTB2 during mammogenesis in the mouse and found that transcripts are expressed at low but constant levels. Attempts to study the spatial pattern of expression in the mammary gland using in situ hybridization were inconclusive because of undetectable mRNA levels (St-Pierre et al., 2004).

RhoBTB2 levels increase upon initiation of prophase and decrease at telophase. RhoBTB2 levels also increase during drug-induced apoptosis. Both effects depend on the E2F1 transcription factor (Freeman et al., 2007).

Expression of RHOBTB2 has been found decreased in cell lines derived from breast, lung and bladder tumours (Hamaguchi et al., 2002; Knowles et al., 2005) and in bladder cancer samples (Shi et al., 2008).

Localisation

The localisation of the endogenous RhoBTB2 protein has not been investigated extensively. In cells ectopicly expressing RhoBTB2 the protein tends to form aggregates in the cytoplasm (Aspenstrom et al., 2004; Berthold et al., 2008). When expressed at moderate levels it displays a vesicular pattern, frequently in the proximity of microtubules (Chang et al., 2006; Berthold et al., 2008).

Function

RHOBTB2 was initially described as a gene homozygously deleted in breast cancer samples and was proposed as a candidate tumour supressor gene (Hamaguchi et al., 2002). The mechanisms by which RhoBTB2 exerts this and other roles remain speculative. Following functions have been proposed for RhoBTB2:

1. RhoBTB2 as adaptor of cullin3-dependent ubiquitin ligases. The first BTB domain binds to the N-terminal region of cullin3, but not other cullins. RhoBTB2 is itself a substrate for the cullin3-based ubiquitin ligase complex (Wilkins et al., 2004). 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).

2. RhoBTB2, cell growth and apoptosis. Overexpression of RhoBTB2 in the breast cancer cell line T-47D (a cell line that lacks RHOBTB2 transcripts) effectively suppressed cell growth in vitro (Hamaguchi et al., 2002). Later it was shown that overexpression of RhoBTB2 leads to a short-term increase in cell cycle progression and proliferation, but long-term expression has a negative effect on proliferation (Freeman et al., 2007). The growth arrest effect has been explained by the downregulation of cyclin D1. Cyclin D1 is upstream of cyclin E, and the overexpression of any of both prevented the growth arrest effect of RhoBTB2 (Yoshihara et al., 2007). The effect on cyclin D1 is probably post-transcriptional, but only partially dependent on proteasomal degradation (Collado et al., 2007). RHOBTB2 has been identified as a target of the E2F1 transcription factor. RhoBTB2 levels also increase during drug-induced apoptosis in an E2F1-dependent manner, and the downregulation of RHOBTB2 delays the onset of apoptosis (Freeman et al., 2007).

3. RhoBTB2 and chemokine expression. Downregulation of RhoBTB2 by RNA interference in primary lung epithelial cells causes a decrease in CXCL14 mRNA expression. The same effect was observed in keratinocytes and is apparently independent of Cullin3-mediated protein degradation (McKinnon et al., 2008).

4. RhoBTB2 and vesicle transport. Knockdown of endogenous RhoBTB2 hindered the ER to Golgi apparatus transport of a VSVG-GFP reporter and resulted in the altered distribution of the fusion protein. Ectopic RhoBTB2 distributes in a vesicular pattern

occasionally adjacent to microtubules and an intact microtubule network seems to be required
for the mobility of RhoBTB2 (Chang el al., 2006).

5. RhoBTB2 and the actin filament system. RhoBTB2 displays only a moderate influence on the morphology and actin organisation of porcine aortic endothelial cells upon ectopic expression. It does not interact with the GTPase-binding domain of WASP, PAK1 or Rhotekin, which are well-known effectors of many typical Rho GTPases (Aspenstrom et al., 2004).

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

Note

Following table compiles mutations identified in RHOBTB2. Polymorphisms that could result in functional alterations are also included (marked with *). All mutations found in tumours are somatic. For mutations found in cell lines it has not been determined whether they are somatic or germinal.

Implicated in

Various cancers, including breast and lung

Note

RHOBTB2 was found homozygously deleted in 3.5% of 200 breast tumours. A mutation analysis revealed two somatic missense mutations in breast tumours (E5 G>A D299N and E9 C>A P647T) and two more missense mutations each in a breast (E5 A>C D368A) and a lung (E5 T>G Y284D) tumour cell line. In the same study expression of
RHOBTB2 appeared extinguished in about 42% of 19 breast and 50% of 14 lung cancer cell lines (Hamaguchi et al., 2002).

A more extensive mutation analysis of 100 sporadic breast cancers revealed some polymorphisms as well as two somatic mutations in the promoter (-238G>A, -121C>T) and 5'UTR (+48G>A) of RHOBTB2. The analysis of 17 familial breast tumours negative for BRCA1/BRCA2 mutations failed to reveal additional mutations in the coding region of RHOBTB2 (Ohadi et al., 2007).

Oncogenesis

RHOBTB2 was proposed as a candidate tumor suppressor gene based on the fact that its re-expression in T-47D (a breast cancer cell line that lacks RHOBTB2 transcripts) caused growth inhibition, whereas expression of the mutant D299N did not have the same effect (Hamaguchi et al., 2002). The Y284D mutant protein, but not the D299N and D368A mutants, presents abolished binding to cullin3 and has consequently a longer half life than the wild type protein. The Y284D mutation resides in the dimerisation interface of the first BTB domain and could prevent proper folding (Wilkins et al., 2004). The mutations found in the promoter and 5'UTR of RHOBTB2 in some breast tumors might affect regulation of gene expression (Ohadi et al., 2007).

Gastric cancer

Note

In a study on primary gastric cancers loss of heterozygosity was found in 29% of 95 tumours. Sequence analysis identified several polymorphisms and one missense somatic mutation (E5 C>T R275W) of unknown effect (Cho et al., 2008).

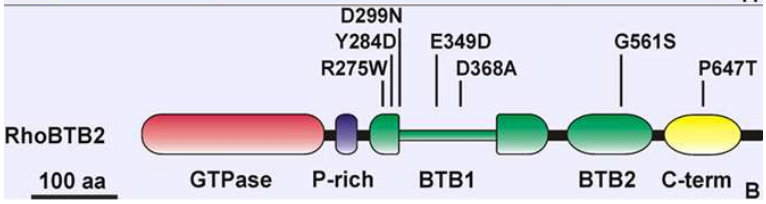
Bladder cancer

Note

A loss of heterozygosity (LOH) and mutation

Mutation	Effect	Tumour
Prom –238G>A*	Altered expression?	Breast
Prom –121C>T	Altered expression?	Breast
5'UTR +48G>A	Altered expression?	Breast
E5 C>T R275W	Unknown effect	Stomach
E5 T>G Y284D	Abolished binding to cullin3	Lung (cell line)
E5 G>A D299N	No growth inhibition when re-expressed	Breast
E5 G>C E349D	Unknown effect	Bladder
E5 A>C D368A	Unknown effect	Breast (cell line)
E7 G>A G561S*	Unknown effect	Bladder
E9 C>A P647T	Unknown effect	Breast

A



B

A: Table of mutations identified in RHOBTB2. B: Localisation of mutations found in the coding region of RHOBTB2 in tumours and cancer cell lines. Note that most missense mutations affect the first BTB domain of the protein and reside in exon 5.

analysis on 54 tumour samples and 32 cell lines of bladder cancer revealed LOH in the target region in 42% of informative tumours and 38% of cell lines. Sequence analysis revealed numerous polymorphisms and one missense somatic mutation (E5 G>C E349D) of unknown effect. One polymorphism (E7 G>A G561S) may have some functional effect. In addition, expression of RHOBTB2 was found reduced by 2 to 20-fold in 9 of 12 cell lines with predicted LOH in the region of interest (Knowles et al., 2005). Significantly higher RHOBTB2 promoter methylation correlated with decreased expression compared to normal tissue was reported in a study of 75 bladder cancer samples (Shi et al., 2008).

Head and neck squamous cell carcinoma

Note

Expression of RHOBTB2 was found reduced in four clonal keratinocyte cell lines derived from patients with HNSCC. This was accompanied by reduced expression of the chemokine CXCL14 (McKinnon et al., 2008).

Oncogenesis

RhoBTB2 seems to be required for expression of the chemokine CXCL14 (McKinnon et al., 2008). CXCL14 controls leukocyte migration and angiogenesis and its expression is frequently lost in diverse epithelial tumours, including most HNSCCs.

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