

Review

Epithelial Cell Polarity Determinant CRB3 in Cancer Development

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Abstract

Cell polarity, which is defined as asymmetry in cell shape, organelle distribution and cell function, is essential in numerous biological processes, including cell growth, cell migration and invasion, molecular transport, and cell fate. Epithelial cell polarity is mainly regulated by three conserved polarity protein complexes, the Crumbs (CRB) complex, partitioning defective (PAR) complex and Scribble (SCRIB) complex. Research evidence has indicated that dysregulation of cell polarity proteins may play an important role in cancer development. Crumbs homolog 3 (CRB3), a member of the CRB complex, may act as a cancer suppressor in mouse kidney epithelium and mouse mammary epithelium. In this review, we focus on the current data available on the roles of CRB3 in cancer development.

Key words: apical-basal polarity, CRB3, EMT, cancer

Introduction

Cell polarity, a widespread feature in unicellular and multicellular organisms, is defined as asymmetry in cell shape, organelle distribution and cell function [1]. Delicate regulation of cell polarity signaling is required for development and maintenance of tissue integrity. Cell polarity is essential in numerous biological processes, including cell growth, cell migration and invasion, and molecular transport [2]. Deregulation of cell polarity signaling may lead to the change of cell fate. There are four modes of cell polarity: planar cell polarity, apical-basal polarity, front-rear polarity, and mitotic spindle polarity [3]. In this review, we focus on apical-basal polarity, which is a characteristic of the epithelium and may be involved in cancer development.

Apical-basal polarity maintenance mainly depends on cell polarity complexes and cell junction complexes. Apical-basal polarity formation starts with the establishment of various cell junction complexes [4, 5], including lateral adherens junctions (AJs), gap

junctions, desmosomes, basal lamina hemidesmosomes, and apical tight junctions (TJs). AJs and TJs can interact with cell polarity complexes. The TJ is mainly composed of transmembrane proteins, including junctional adhesion molecules (JAMs), claudin and occludin, which combine with the actin cytoskeleton via the intracellular molecule ZO1, ZO2 or ZO3. The AJ isolates the apical membrane from the basolateral membrane. The AJ is mainly composed of transmembrane proteins, nectin and cadherin, which combine with the actin cytoskeleton via afadin and β -catenin, respectively.

Apical-basal polarity is established and maintained by the cooperation and antagonism of cell polarity complexes, which lead to the distribution of cellular molecules into specific cellular areas [6-8]. Apical-basal polarity is mainly regulated by three evolutionarily conserved polarity protein complexes, namely, the Crumbs (CRB) complex, partitioning defective (PAR) complex, and Scribble (SCRIB) complex.

The SCRIB complex is composed of Scribble, Discs large (DLG) and Lethal giant larvae (LGL). In epithelia, the SCRIB complex is located basolaterally and is needed for maintenance of the basolateral membrane domain [9-12]. The PAR and CRB complexes have mutually antagonistic relationships with the SCRIB complex. The PAR complex consists of PAR3, PAR6 and atypical protein kinase C (aPKC). It is localized in the TJ of the epithelium and cooperates with the CRB complex in regulating the apical junction formation [13]. The CRB complex consists of the transmembrane protein CRB and the cytoplasmic proteins protein associated with Lin seven 1 (PALS1) and Pals1-associated tight junction protein (PATJ) [14]. Crb, a single-pass type membrane protein, was initially identified in *Drosophila* by Jürgens et al [24, 25]. To date, three vertebrate Crumbs orthologs CRB1, CRB2, and CRB3 have been identified [15]. CRB1 is related to a severe form of degenerative retinitis pigmentosa (RP12) and Leber congenital amaurosis. Diseases associated with CRB2 include Leber congenital amaurosis and cleft lip [16, 17]. CRB3 is considered a cancer suppressor [18]. Of the three CRB proteins, only CRB3 is found to be widely expressed in epithelial cells, which is consistent with the early anlystic result of *Drosophila* crb [19].

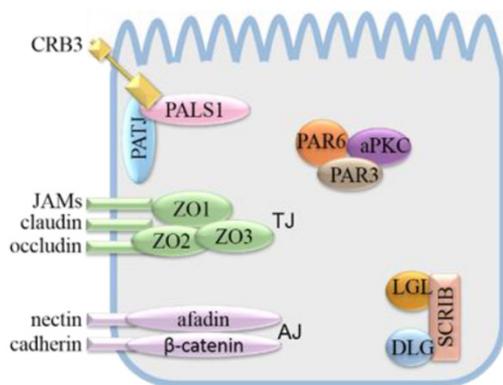


Fig.1 Schematic model of cell polarity complexes and cell junction complexes.

Structure and distribution of CRB3

CRB3 is comprised of a short extracellular signal peptide and three large intracellular domains [an FERM-binding domain (FBD), a SH3-binding domain, and a carboxy-terminal PDZ-binding domain (PBD)] (Fig.1). FBD is involved in polarity formation due to its ability to bind to PALS1 and Epb4.115 [20, 21]. PBD interacts with the PDZ domains of PALS1 and PAR6 and is also important for polarity formation. Comparatively speaking, it is quite interesting to note that CRB3 is the only human CRB that has a PxxP motif

which can bind to the SH3 domain [22-24]. SH3 domain interacts with the transcription factor ZONAB (ZO1-associated nucleic acid-binding protein), a protein that is required for normal cell proliferation [25, 26].

CRB3 includes two splice variants, CRB3A and CRB3B. CRB3A is connected with PALS1 via the ERLI motif at the C terminus of CRB3A, while CRB3B is connected with PALS1 via the CLPI motif at the C terminus of CRB3B; thus the ERLI or CLPI motif is crucial for the formation of the CRB complex [14]. The two splice variants show differential functions in human epithelial cells. CRB3A is found at the plasma membrane and is important for formation of cell junctions and repression of human cancer cell growth. In contrast, CRB3B is localized in the nucleus and plays a specific role in the ciliogenesis and mitotic spindle construction via interacting and colocalizing with importin- β [23, 24, 27-30]. Carcinogenesis is either prevented or delayed when the nuclear localization of CRB3B is disrupted [31].

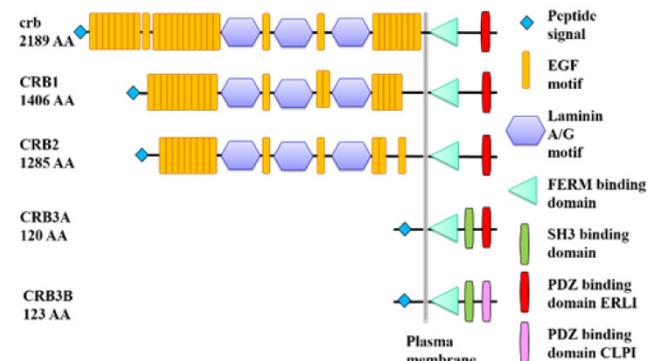


Fig 2. Protein domain structures of *Drosophila* crb and human CRB.

All CRB homolog proteins (crb, CRB1, CRB2, CRB3A and CRB3B) contain a signal peptide and a FERM binding domain (FBD). Extracellular parts of crb, CRB1 and CRB2 are composed of EGF and Laminin A/G domains. CRB3A and CRB3B have a SH3 binding domain. The PDZ binding domain of crb, CRB1, CRB2 or CRB3A is ended with ERLI, while the PDZ binding domain of CRB3B has a C-terminal sequence ending in CLPI.

CRB3 has been found to be expressed in humans, mice, rats, cows, and dogs, but not in *Drosophila* and *zebrafish* [27]. CRB3 is mainly localized above the TJ, a prime position at the apical membrane of the epithelium [32]. *Drosophila* crb has also been detected in the cytoplasm. The distribution of *Drosophila* crb is regulated by endocytosis [33], exocytosis [21], and the retromer. The retromer is a newly discovered retro-

grade complex which binds to the intracellular domain of crb via vacuolar protein sorting 35 (VPS35, a major component of the retromer) and recycles crb from endosomes to trans-Golgi network (TGN) in epithelia [34]. Unfortunately, little is known about CRB3 metabolism in mammals. The intracellular domains of crb and CRB3 are highly conserved. Therefore, it is reasonable to presume that CRB3 metabolism may be similar to that of *Drosophila* crb. Further studies are needed to validate this presumption.

CRB3 in apical-basal polarity maintenance

Eileen L Whiteman found that CRB3 knockout mice died shortly after birth and showed defects in epithelial morphogenesis (cystic kidneys, proteinaceous debris throughout the lungs, villus fusion and apical membrane blebs in intestines) [35]. The results suggest that CRB3 play crucial roles in growth and development of epithelial cells and the establishment and maintenance of apical-basal polarity.

CRB3 links to actin-binding proteins

During polarization, the CRB complex forms the apical domain by recruiting actin cytoskeleton regulators Moesin and β H-spectrin to the cell membrane [36]. Partners of CRB3 (i.e., Epb4.115, PAR6 and PALS1) serve as adaptors linking the CRB3 to the actin cytoskeleton. Lee et al observed an apical redistribution of F-actin and the formation of ectopic F-actin foci in Epb4.115 knockout mice [37]. PAR6 interacts with cytoskeleton-related proteins 14.3.3 [38] and Cdc42 [39]. In PALS1-downregulated cells, the pattern of F-actin staining obtained is more diffuse [40]. Whiteman et al have observed a direct interaction between CRB3 and the membrane-cytoskeleton linker protein Ezrin, which indicates that CRB3 can link the apical membrane to the cytoskeleton [35]. In addition, recruitment and reorganization of cytoskeletal elements (such as F-actin) through these proteins may contribute to the cell junctions formation.

CRB3 induces junctions formation

CRB3 seems to be a crucial determinant of TJ and AJ formation. Immortal baby mouse kidney (iBMK) epithelial cells selected in vivo has been found to display dramatic repression of CRB3 expression, which is associated with disruption of TJ formation. CRB3 expression restores TJ formation in TDCL 5D, where CRB3 endogenous expression is undetectable. Importantly, expressed CRB3 localizes to the plasma membrane and substantially rescues TJ formation, which is accompanied by increased occluding and ZO1 at the junctions between cells [18]. Downregulation of CRB3 results in loss of TJ, while overexpression of CRB3 disrupts the cell polarity by enlarging the apical domain at the expense of the lateral domain

in MCF 10A cells [20]. In the normal *Drosophila* embryonic tissue, crb interacts with the Baz/PAR3 and forms the AJ in epithelial cells [41]. CRB3 can also form the CRB3/PAR6/aPKC complex and regulate the actin structure, endocytosis, and AJ remodeling [12, 42, 43].

CRB3 interacts with other polarity proteins

The CRB, PAR, and SCRIB complexes maintain apical-basal polarity by interacting with each other. For example, CRB3 determines the apical identity by recruiting and activating the downstream apical PAR complex and inhibiting the basolateral SCRIB complex. Crb competes with Par3 for binding to Par6 during TJ formation in *Drosophila* epithelium [23, 44]; Crb can also bind to Par3 and recruit it from the subapical domain during photoreceptor remodeling [45]. aPKC can regulate CRB3 activity *in vivo* by phosphorylating two conserved threonine residues in the cytoplasmic tail of CRB3 [14, 46]. Phosphorylation of proteins usually plays an important role in the process of cell signal transduction. However, the function of phosphorylated CRB3 in cell growth and development is still unknown.

CRB3 associates with epithelial-mesenchymal transition (EMT)

EMT is a biologic process that allows a polarized epithelium to undergo the change in cell cytoskeleton, lose the epithelial features of junctions, and acquire the mesenchymal characteristics such as migration and invasion, anti-apoptosis, and extracellular matrix degradation. EMT is initiated by transcriptional repressors, including ZEB1, ZEB2, Snail (SNAI1), Slug (SNAI2), FOXC2, TCF/LEF, TWIST and E47. These transcriptional repressors down-regulate the expressions of E-cadherin, EVA1, MAL2, P-cadherin and cytokeratin in epithelial cells and up-regulate mesenchymal cell markers N-cadherin and vimentin [47-51]. EMT and mesenchymal-epithelial transition (MET) are rapid and reversible phenomena occurring in the natural process of cell development, a variety of chronic diseases, and human cancer development and metastasis [52-55]. Migration and invasion are the leading causes of cancer-associated mortality [56]. Recent studies have suggested that EMT is a hallmark of development, migration and invasion of epithelial cancer cells. *Drosophila* crb can maintain the epithelial integrity by repressing the Rac1-PI3K positive-feedback loop embryos [57]. Rac1-PI3K up-regulates N-cadherin via JNK and induce EMT in mouse mammary epithelial cells [58].

Recent reports have provided evidence that members of the CRB complex are direct targets of the EMT transcriptional repressors, and CRB3 in turn

affects on the expression of EMT transcriptional repressors. It has been found that CRB3, PATJ and PALS1 are up-regulated after the E-cadherin repressor ZEB1 is silenced in colorectal carcinoma cells SW480 and undifferentiated breast tumor cells MDA-MB-231 [59, 60]. The transcriptional repressor Snail alters epithelial cell polarity by repressing the transcription of CRB3 and abolishing the localization of Par and CRB complexes at cell-cell junctions in MDCK cells [61]. ZEB1 or Snail/Slug represses the mRNA expressions of CRB3 by directly binding to their promoter elements in normal and transformed epithelial cell lines [62]. CRB3 knockdown increases the expression of Snail in TGF- β -treated Eph4 cells grown at high density [63]. These findings suggest that CRB3 may inhibit the process of EMT via transcriptional repressors such as ZEB1 or Snail/Slug.

CRB3 in cancer

Cancer development is a multistep process caused by initial benign overproliferation and inhibition of apoptosis. The process by which benign cells are transformed to malignant cancer cells includes altered cell-matrix junctions and migration and metastasis to distal parts. Mammalian organs mainly consist of epithelial cells and epithelial cells are the source of approximately 90% of human cancers. Changes in multiple polarity proteins have been found in cancer tissues. For example, PAR6 is up-regulated in breast cancer cells and it induces cell proliferation by interacting with aPKC and cdc42 [64, 65]. Depletion of SCRIB in *Drosophila* cells results in activation of downstream oncogenes such as c-myc, destroying cell polarity, blocking 3D morphogenesis of cells, inhibiting apoptosis, enhancing proliferation, and promoting carcinogenesis [66].

Loss of CRB3 occurs concomitantly with increased expression of vimentin and reduced expression of E-cadherin, two important hallmarks of EMT. In addition, the expression level of CRB3 negatively correlates with migration and invasion of the mouse kidney epithelial cells. Reduced expression of the murine CRB3 gene also can promote carcinogenesis of kidney epithelia [18]. Loss of CRB3 enhances TGF- β signaling and predisposes cells to TGF- β -mediated EMT in mouse mammary epithelia [63]. The above studies show that loss of CRB3 may favor EMT and migration and invasion of cancer cells. Presently, studies have been mainly focused on the regulatory role of CRB3 in Hippo and Notch signaling.

CRB3 regulates Hippo signaling

The molecular mechanism by which CRB3 exerts its cancer suppressive function may be related to its interaction with the Hippo signaling [9, 10, 67]. The

Hippo signaling is a critical regulator of cell cycle, proliferation, apoptosis, differentiation and contact inhibition [68]. CRB3 regulates proliferation and apoptosis via mediation of the Hippo signaling. Crb suppresses cancer development in *Drosophila* by regulating the Hippo signaling via the FERM-domain protein Expanded [9, 10, 67]. However, the mechanism underlying the interaction between CRB3 and Hippo signaling in human is unclear. It has been suggested that FRMD6/Willin is a homologue of Expanded [69], and it also has an FERM domain. Whether CRB3 regulates the Hippo signaling in human needs to be further verified [70]. FRMD6/Willin may be regulated by CRB3 and forms a complex with KIBRA and MER. The complex ultimately phosphorylates the transcription co-activator Yes-associated protein (YAP)/the transcriptional co-activator with PDZ-binding domain (TAZ, also known as WWTR1). The phosphorylated YAP/TAZ binds to the 14-3-3 protein and is retained in cytoplasm. Cytoplasmic YAP/TAZ suppresses TGF- β signaling, which in turn prevents the nuclear accumulation of the SMAD complex and inhibits the Wnt/ β -catenin signaling [71-74]. Inhibition of the Wnt/ β -catenin signaling downregulates TJ markers ZO1 and E-cadherin, induces EMT [63], and ultimately triggers cancer metastasis [70, 75, 76].

Direct biochemical interactions between proteins are indicated by solid lines. Dashed lines indicate genetic interactions for which no direct interactions have been reported. Arrowed and blunted ends indicate activation and inhibition, respectively.

CRB3 regulates Notch signaling

The Notch signaling is involved in regulating many processes (proliferation, differentiation, cell death, and cell fate) during development and renewal of adult tissues [77]. The Notch signaling mediates TGF- β -induced EMT through the induction of Snail [78]. The Notch function changes during the development of cell differentiation and can be either oncogenic or tumour-suppressive, depending on cellular context [79]. Crb has been shown to prevent endocytosis of the Notch receptor and/or its ligand Delta in *Drosophila* and *zebrafish* by directly interacting with these proteins via its larger extracellular structure; this may also negatively affect the epithelial tissue growth [11, 80, 81]. However, overexpression of human CRB3 does not have this effect on Notch signaling in mammalian cells [80]. *Drosophila* crb and human CRB3 have different roles in the Notch signaling because crb interacts with the Notch signaling mainly via the extracellular section of crb, whereas the extracellular CRB3 contains only signal peptide.

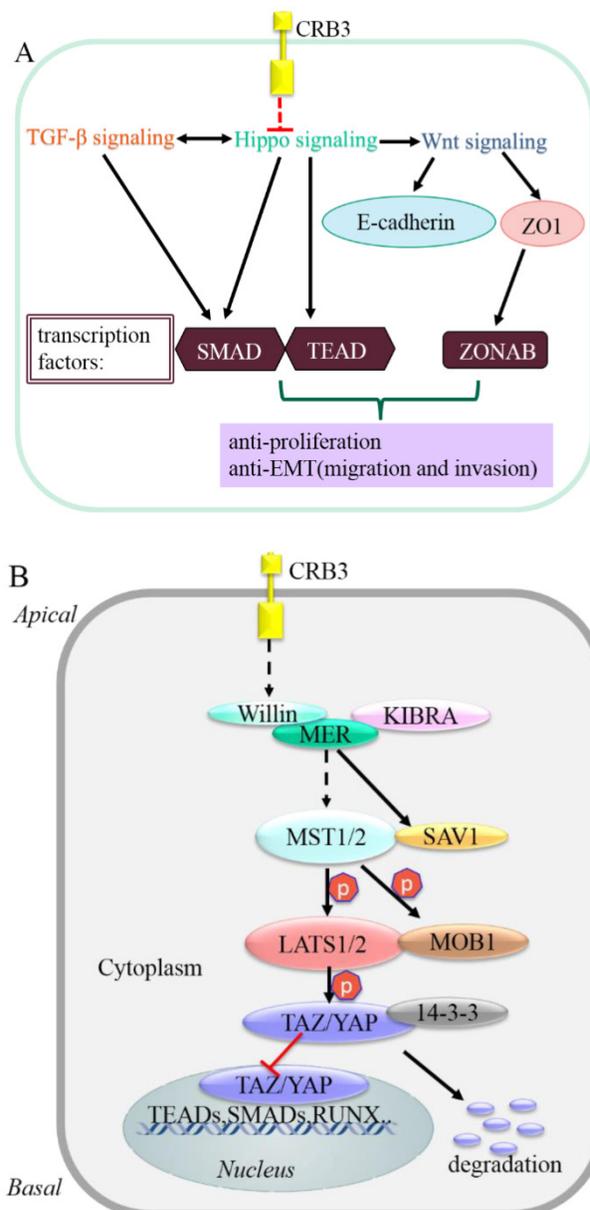


Fig 3. Schematic model of CRB3 in carcinogenesis and Hippo signaling cascade in mammals.

Conclusion

CRB3 is an essential determinant of epithelial apical identity in epithelia. CRB3 maintains apical-basal polarity by ensuring the delivery and stabilization of junctional proteins and interacting with other polarity proteins such as ZO1 and PAR6. CRB3 is mainly deposited in the plasma membrane; therefore, it may mediate the extracellular signal transduction in cancer development via intracellular signal, such as Hippo signal. However, little is known about its mechanisms. Currently, most CRB studies are done on *Drosophila*. *Drosophila crb* greatly differs in the structure from mammalian CRB3; therefore, studies

on mammalian CRB3 are needed. Based on the above review, new questions arise: Does CRB3 inhibit the Hippo signal by directly affecting Willin? Does CRB3 regulate activation of the Notch and other signals and how? In-depth research is needed to reveal the role of CRB3 in tumorigenesis.

Together, CRB3 plays important roles in epithelial polarity maintenance and may relate to cancer development. Understanding of CRB3-related molecular signaling pathways and their interactions with CRB3 will be of great help in exploitation of possible therapeutic interventions for cancers.

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Competing Interests

The authors have declared that no competing interest exists.

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