International Journal of Applied Research 2016; 2(2): 370-381

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(2): 370-381
www.allresearchjournal.com
Received: 16-12-2015
Accepted: 18-01-2016

Partha Majumder
Biomedical Scientist,
Former Head, Department of Applied Biotechnology & Bioinformatics, Sikkim Manipal University, CC: 1637, Kolkata, India.

Anjana Mazumdar
Professor,
Department of Oral Pathology, Dr. R. Ahmed Govt. Dental College, Kolkata, India.

Correspondence
Partha Majumder
Biomedical Scientist,
Former Head, Department of Applied Biotechnology & Bioinformatics, Sikkim Manipal University, CC: 1637, Kolkata, India.

Positive biofeedback of Crb3 protein favouring notch mediated tumour suppression in human body: A glimpse for better understanding of cancer biology and to identify potential therapeutic targets

Partha Majumder, Anjana Mazumdar

Abstract
Our study is a comprehensive approach of a biofeedback paying regards to homeostasis of human body. A biofeedback system involves a sensory organ and an appropriate stimulus. The stimulus is mediated through organs derived from specific biosensors. Homeostasis is the set of processes by which constant or static conditions are maintained within the internal environment of a subject and therefore a homeostat is a controller involved in maintaining homeostasis. Paying regards to extensive study on Cytology, we found that, Cell polarity is essential in many biological processes and required for development as well as maintenance of tissue integrity. Loss of polarity is considered both a hallmark and precondition for human cancer. According to our knowledge, three conserved polarity protein complexes regulate different modes of polarity that are conserved throughout numerous cell types and species. These complexes are the Crumbs, Par and Scribble complex. Defects in apical-basal polarity regulation are associated with tissue overgrowth and tumorigenesis. Recent studies performed on different vertebrates showing that apical polarity proteins of the Crumbs family act to repress tissue growth and epithelial to mesenchymal transition. Therefore, these proteins associated as potential tumor suppressors. Moreover, extensive analysis of the molecular function of Crumbs proteins reveals a function for these polarity regulators in junctional complexes stability and control of signaling pathways regulating proliferation and apoptosis. Thereby, aim of our in-depth study is to unwind a molecular basis explaining how regulation of epithelial polarity is coupled to tumorigenesis which will be really beneficial for Human Civilization.

Keywords: cell polarity, Crumbs, cancer, asymmetric cell division, EMT, Par, scribble, N-linked Glycosylation, Tight junction, AJC

Introduction
Epithelial polarity is organized by a complex network of evolutionarily conserved proteins, including the apical trans-membrane proteins Crumbs (Crb) [1, 4-6]. A protein with all the characteristics for a Crumbs homologue has been identified from patients suffering from retinitis pigmentosa group 12, but this protein (CRB1) is only expressed in retina and some parts of the brain, both in human and mouse. Here, we describe CRB3 (Open Reading Frame, ORF Names: UNQ588/PRO1158), another Crumbs homologue that is preferentially expressed in epithelial tissues and skeletal muscles in human. Preferentially expressed in epithelial tissues. Also, expressed at high levels in lung, kidney, retina, colon and mammary glands and expressed at moderate levels in liver, spleen, pancreas, placenta and prostate [15-17]. CRB3 shares the conserved cytoplasmic domain with other Crumbs but exhibits a very short extracellular domain without the EGF- and laminin A-like G repeats present in the other Crumbs. CRB3 is localized to the apical and subapical area of epithelial cells from the mouse and human intestine, suggesting that it could play a role in epithelial morphogenesis [5-8]. Mutual antagonism between Crb and basolateral polarity modules is crucial for segregation and size control of membrane domains in epithelial cells, thus impacting on tissue morphogenesis [9-13]. Indeed, expression of CRB3 or of a chimera containing the extracellular domain of the neurotrophin receptor p75NTR and the transmembrane and cytoplasmic domains of CRB3 led to a slower development of functional tight junctions in Madin-Darby canine kidney cells. This phenotype relied on the presence of CRB3 four last amino acids (ERLI) that are involved in a direct interaction with Par6 through an intermediate

PALS1, a membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5), a regulator of epithelial polarity and tight junction formation [14]. Thus, CRB3, through its cytoplasmic domain and its interactors, plays a role in apical membrane morphogenesis and tight junction regulation.

The importance of the polarized architecture of epithelial cells:

Usually, epithelial tissues cover the surface and line internal cavities of the human body. Epithelial cells require apical–basal plasma membrane polarity to carry out crucial vectorial transport functions and cytoplasmic polarity to generate different cell progenies for tissue morphogenesis. Extensive study shows that, establishment and maintenance of a polarized epithelial cell with apical, basolateral and ciliary surface domains is guided by an epithelial polarity programme (EPP) that is controlled by a network of protein and lipid regulators. The EPP is organized in response to extracellular cues and is executed through the establishment of an apical–basal axis, intercellular junctions, epithelial-specific cytoskeletal rearrangements and a polarized trafficking machinery. The apical domain faces the external environment or a lumen, the lateral domain spans across the plane of the epithelium and contacts neighboring cells, and the basal domain is attached to the basement membrane. The function of these proteins is conserved from worm to man, reflecting the significance of epithelial polarity. The importance of the polarized architecture of epithelial cells is further emphasized by the fact that numerous pathologies are associated with epithelial polarity defects, including most human cancers [2, 3].

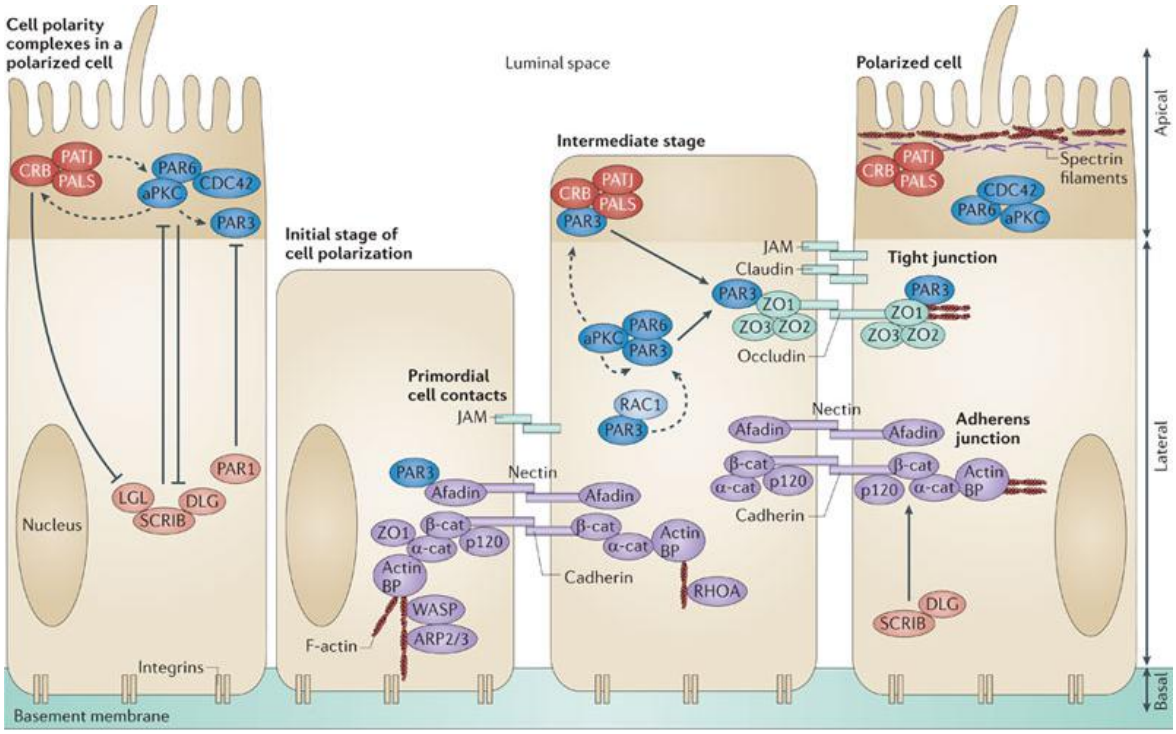


Fig 1A: demonstrates the organization of Epithelial polarity.

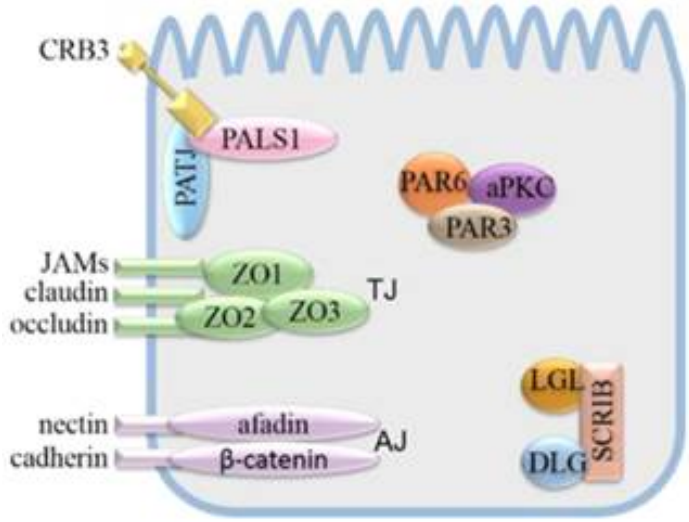


Fig 1B: Demonstrates the site of interaction of CRB3 and epithelial cell

Out of multivariate functions it has been found that each specialist function is achieved by the distinct structural organization of epithelial cells within different tissues. Consequently, the integrity of their architecture is crucial. The majority of human cancers are derived from epithelial tissues, and display loss of cell polarity and often, as a consequence, tissue disorganization [18]. However, it is emerging that epithelial cell polarity may exert a tumour suppressive function in mammals through its participation in the establishment and maintenance of the three dimensional organization of epithelial tissues as a whole. This theory is supported by the findings that polarity proteins are cellular targets of oncogenes, and an increasing list of tumour suppressors has been shown to regulate polarity pathways.

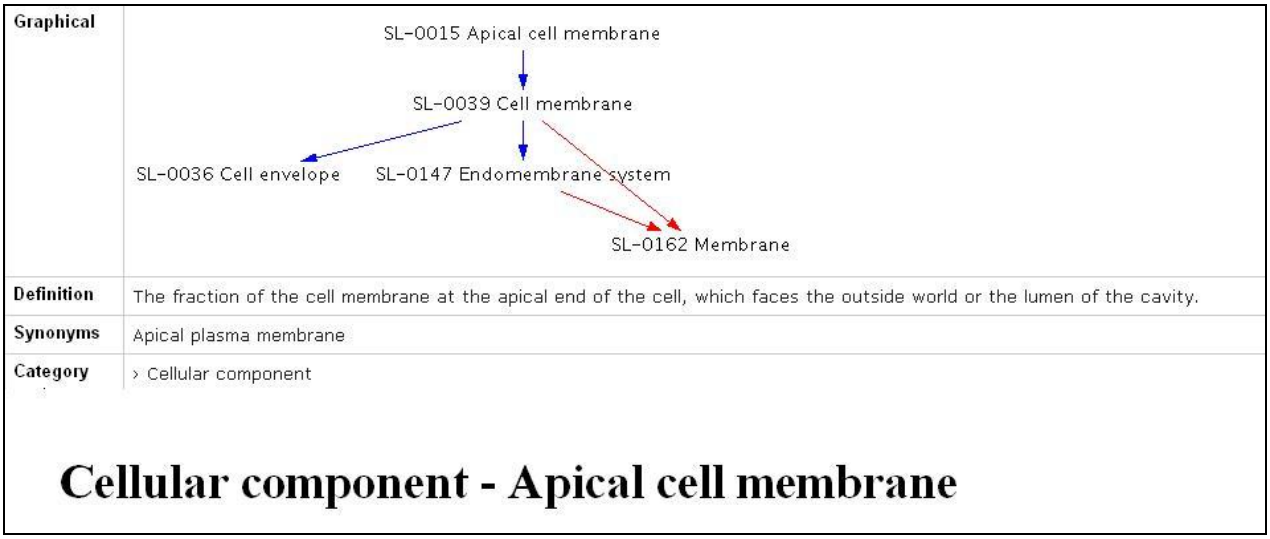


Fig 2: demonstrates the structure of human CRB3 at apical cell membrane

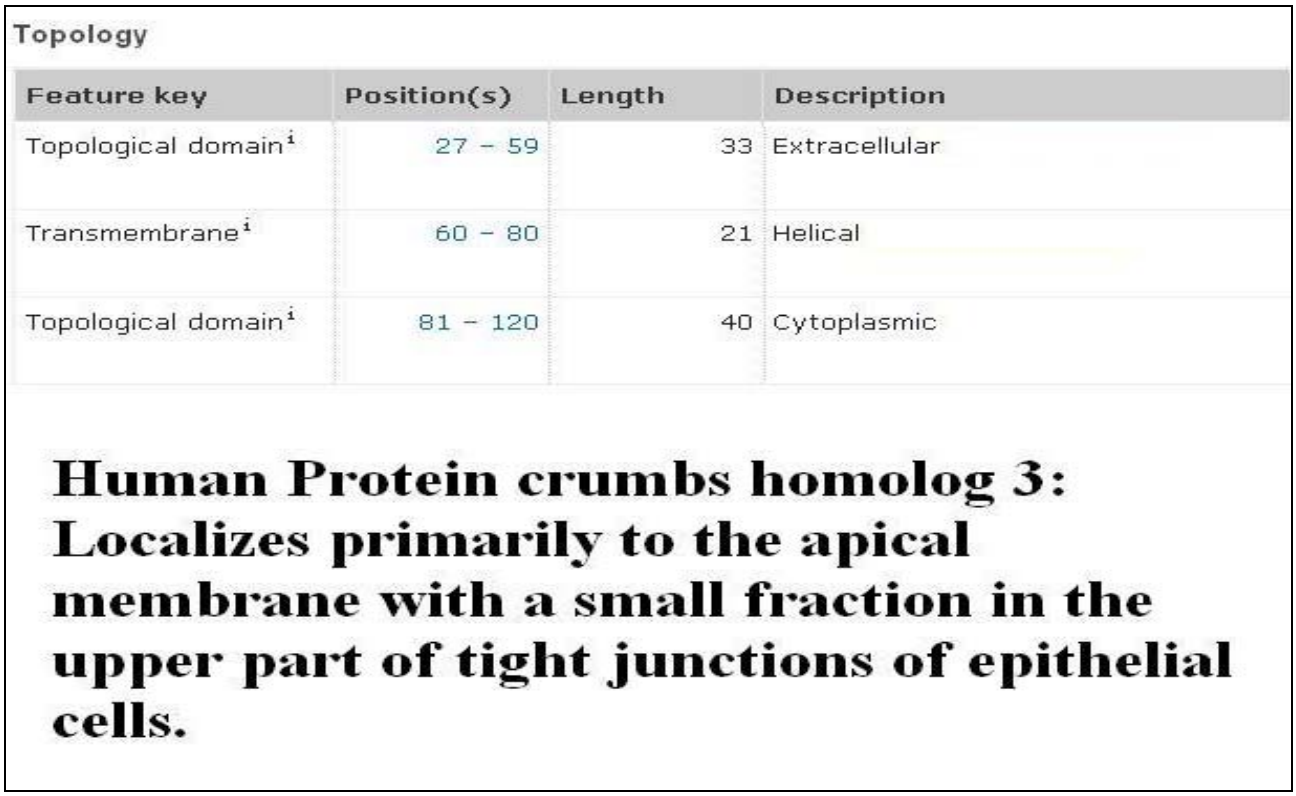


Fig 3: demonstrates the architecture of human CRB3 protein.

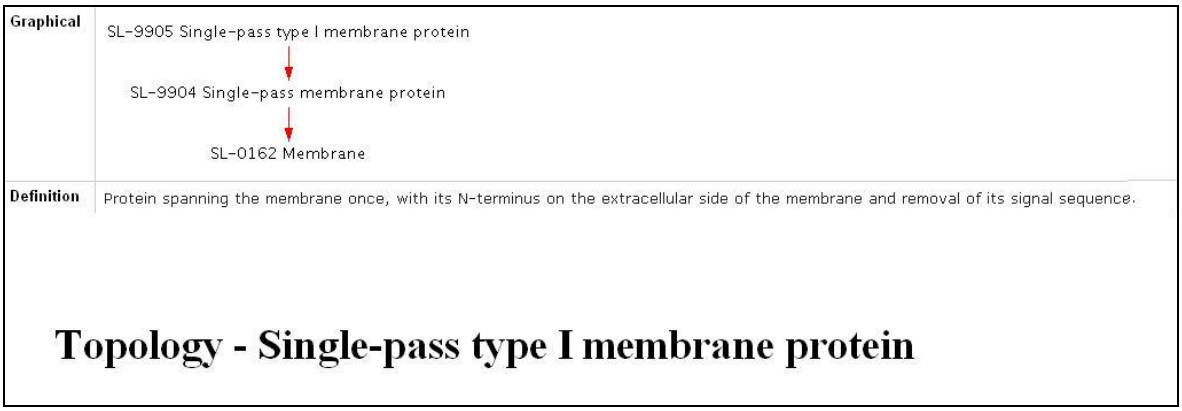


Fig 4: demonstrates special architecture of Human CRB3 protein.

Functionally, apical–basal polarity has two fundamental roles in epithelial cells that are intimately linked to tumour suppression: (1) the regulation of asymmetric cell division and (2) the maintenance of the apical junctional complex (AJC). In epithelial stem cells, polarity proteins control asymmetric cell division by regulating the polarized localization of cell fate determinants and the correct orientation of mitotic spindles. As a result, asymmetric cell division has a fundamental role in the control of progenitor or stem cell numbers and differentiation. This is of particular interest in the context of the cancer stem cell theory, as a shift from asymmetric division of epithelial stem cells or cancer-initiating cells towards symmetric divisions would result in dedifferentiation on one hand and an increase in cancer-initiating cells on the other. Thus, a defect in asymmetric division could contribute to the emergence of tumours. As a result, over the last few years, there has been increasing interest in the identification of core cell-polarity mechanisms that govern the asymmetric division of epithelial stem cells, and understanding how their disruption may contribute to the development of cancer.

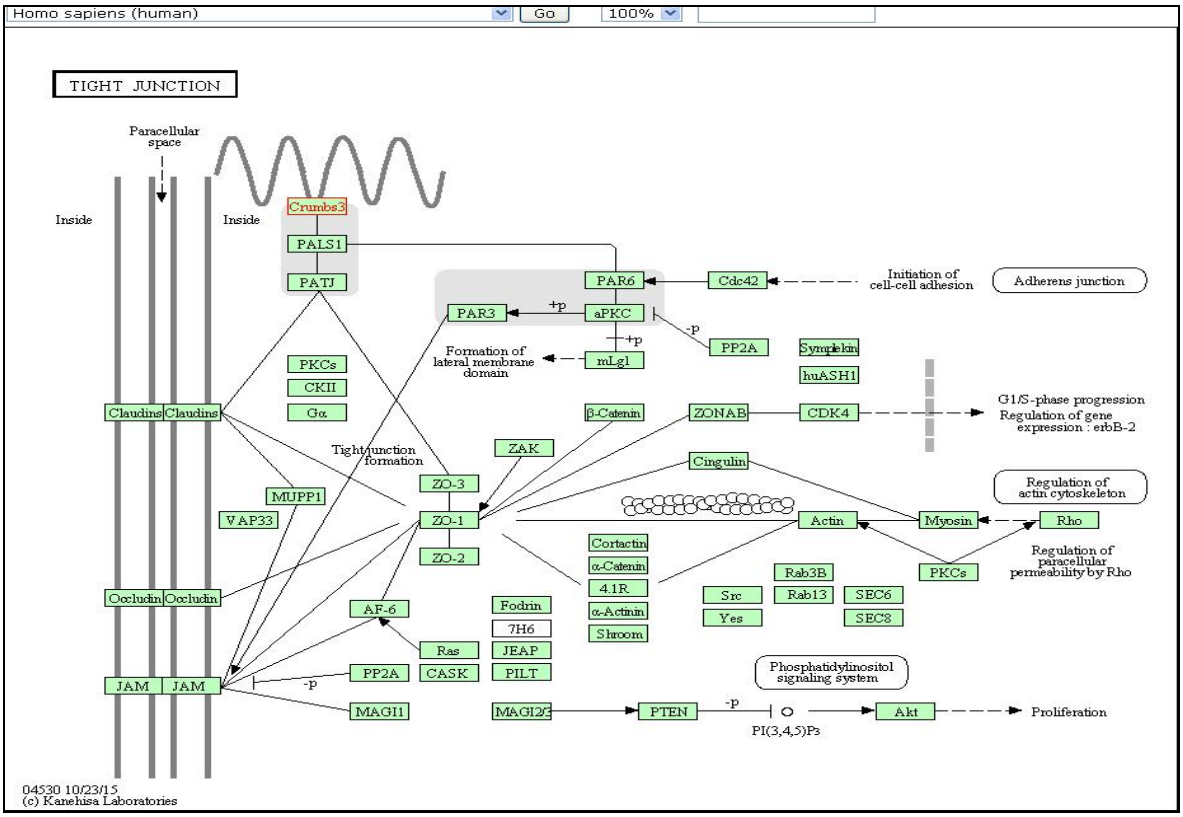


Fig 5: demonstrates the detail interactions of tight junction (KEGG Pathway)

In addition to their role in the prevention of tumour initiation, core epithelial cell polarity mechanisms may also constitute a barrier to tumour metastasis and malignancy through their close connection to the AJC. Maintenance of polarity in the epithelial cells also determines the entry of substances inside the cytoplasm The AJC encompasses tight and adherens junction complexes, and its structure is dependent on the integrity of the apical and basolateral polarity complexes. The loss of one of the key components of adherens junctions, E-cadherin, often occurs in later stages of tumourigenesis

and is thought to contribute to epithelial mesenchymal transition epithelial mesenchymal transition (EMT), which represents a crucial step in metastasis. The importance of the AJC in suppressing cancer malignancy is supported by cancer genome sequencing data, which show that a large number of AJC components are frequently mutated in human cancers. In this review, we will therefore discuss whether a loss or deregulation of epithelial cell polarity favours tumour initiation, or is responsible for later stages of tumour development and malignancy.

Challenging Factors in the Establishment and Maintenance of Epithelial Cell Polarity:

In order to understand how cells become disorganised in tumours it is vital to understand the key players and regulators that control and maintain cell polarity, which lead to epithelial tissue organisation. As expected, the molecular complexes involved in the establishment and maintenance of cell polarity are largely conserved throughout the metazoa, despite the wide range of epithelial tissue types and biological processes that require them. Three major complexes involved in the regulation of apical–basal cell polarity of epithelial cells have been described: The Crumbs-Pals1 (Stardust)-Patj-Lin-7 (Crumbs complex) and Par3 (Bazooka)-Par6-aPKC (Par complex) complexes, which are found apically, and the lethal giant larvae (Lgl)-Scribble (Scrib)-Disc large (Dlg) proteins (Scribble complex) that localise at the basolateral membrane [19, 20, 21]. Both the Par and Crumbs complexes promote apical membrane identity, whereas the Scribble complex promotes basolateral membrane identity by antagonising the other two (Figure:6).

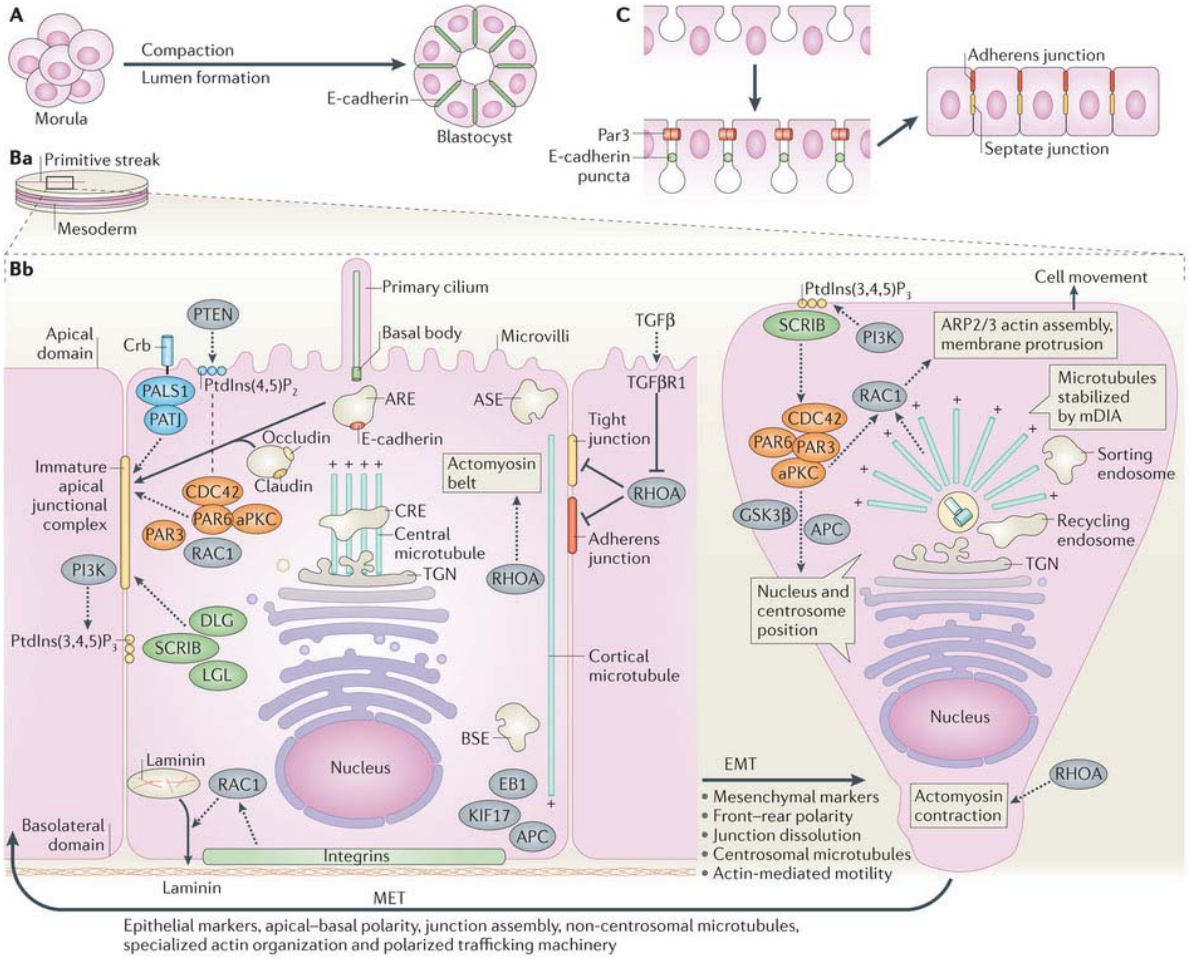


Fig 6: Diagram representing the core polarity components involved in the maintenance of apical–basal polarity in epithelial cells and the establishment of membrane domain identity. AJ, adherens junctions; ALJ, apical/lateral junction; TJ, tight junctions.

The activity of the Par complex is further regulated by the dynamic nature of Par3's association with the stable Par6–aPKC complex. The correlation with the observation that in many epithelial tissues, including in mammals, Par3 and the Par6–aPKC complex do not colocalise [23, 24, 25, 26]. However, in mammals, the apical/lateral domain is formed by tight junctions, which are more apical and distinct from the adherens junctions, thus Par3 is essentially localised at the level of tight junctions where it colocalises with zonula occludens-1 (ZO-1) [27, 28]. This model for Par3 exclusion from the apical domain involves both the Par6–aPKC complex and the Crumbs complex, in order to prevent the interaction between Par3 and the Par6–aPKC complex. On one hand, aPKC phosphorylates Par3 on Ser827 in mammalian Par3 to decrease their affinity for each other while, on the other hand, Crumbs and Stardust compete with Par3 to interact with the same domain of Par6 (Figure 6). This exclusion mechanism is crucial to restrict the extent of the apical/lateral junction and define the border between the apical and lateral domains in *Drosophila* epithelial cells. Further investigation is required, but the existing evidence

suggests that the observations outlined above may be generalised to epithelial tissues in mammals [22].

Cell Polarity, Asymmetric Cell Division and Cancer:

Loss of cell polarity is a typical hallmark of tumor progression in epithelial tissues. The polarized architecture of epithelial cells is compromised at early steps of epithelial to mesenchymal transition (EMT), a process also associated with loss of cell-cell adhesion and acquisition of migratory and invasive properties [29]. Thus, EMT is a critical step in carcinoma progression and metastasis. These observations predict that regulators of apical-basal polarity are fundamental to preserve epithelial homeostasis and to limit tumorigenesis. This hypothesis was first supported by studies in Drosophila showing that loss of any members of the lateral-promoting Scribble (Scrib) polarity module promotes epithelial tissue overgrowth and disorganization, resulting in a tumor-like phenotype [39]. The tumor suppressor function of Scrib is conserved in mice [30]. These data have inspired a number of scientists who demonstrated that the expression of many epithelial polarity regulators is altered in several cancers and that proteins required for epithelial polarization are important targets of viral oncoproteins [2, 3, 31]. Finally, mutations in the gene encoding the polarity protein Lkb1 cause a genetic syndrome associated with a high incidence of cancer [32]. Together, these recent discoveries clearly established that further characterization of proteins coordinating epithelial polarity will contribute to our understanding of cancer biology. Deciphering the molecular mechanisms by which polarity proteins act as tumor suppressors is a major issue yet to be solved in this field of research [Figure:7].

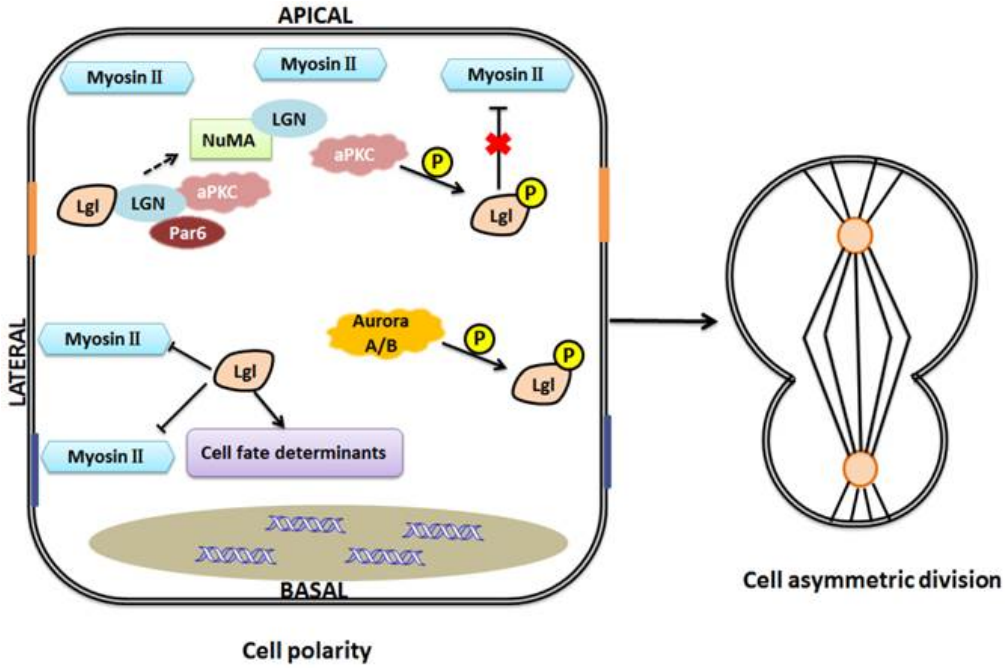


Fig 7: Demonstrates Epithelial polarity and asymmetric cell division.

Human CRB3 and Tumor Growth:

The role of the polarity protein CRB3 in cancer was not thoroughly studied yet, but increasing evidence suggests that this protein could restrict tumor progression. Gene expression profiling revealed that repression of CRB3 expression correlates with increased tumorigenic potential in mouse kidney epithelial cells. Re-expression of CRB3 restored cell-cell junctions integrity and cell polarization, while limiting cell motility and metastasis [33]. This suggests that loss of CRB3 in tumor cells is not coincidental, but plays an active role in tumorigenesis. A mechanism leading to the loss of CRB3 expression was recently elucidated. Indeed, CRB3 expression is repressed by two factors promoting EMT, namely, the transcription regulators Snail and ZEB1 [34–36]. Expression of these proteins alters cell-cell adhesion, while increasing migration, invasion, and metastasis [45, 37, 38]. Snail and ZEB1 directly bind to and repress CRB3 promoter [44, 46]. Importantly, expression of exogenous CRB3 in Snail-expressing cells partially restores the formation of cell-cell junctions and the epithelial phenotype, suggesting that CRB3 gene is a functional target of Snail and that its repression contributes to EMT [46]. Expression of Snail and ZEB1 is increased in many human tumors, and it correlates with dedifferentiation and invasion [47–49]. This suggests that CRB3 expression is reduced in human cancers allowing for tumor progression, but a former demonstration of this hypothesis remains awaited. Collectively, these findings establish that it is of great interest to study CRB3 in tumor of epithelial origin, which accounts for the vast majority of human cancers.

"In silico" analysis of Human CRB3 and its positive role in suppression of Tumorigenesis:

The current pace of high-throughput genome sequencing programs coupled with high-throughput functional genomic screens has provided researchers with a bewildering array of sequence and biological data to contend with. Identification of proteins of interest from a particular biological study requires the application of bioinformatics tools to process and prioritize the data. From a protein function standpoint, transfer of annotation from known proteins to a novel target is currently the only practical way to convert vast quantities of raw sequence data into meaningful information. New bioinformatics tools now provide more sophisticated

methods to transfer functional annotation, integrating sequence, family profile and structural search methodology. The importance of these approaches to medical research is increasing as we move to annotate the proteome through functional and structural genomic efforts [39-49].

Detail review of Human CRB3 protein:

Tight Junction Interactions:

Tight junctions (TJs) are the most apical component of the epithelial junctional complex forming a belt-like structure at the cellular junction. When visualized by freeze-fracture electron microscopy they appear as a branched network of intramembrane strands that correspond to the sites of direct membrane contacts and that are composed of the integral membrane claudin proteins. The TJs act as a primary barrier to the diffusion of solutes through the paracellular space (barrier function) (Tsukita *et al*., 2001). They also prevent the intermixing of intramembrane proteins and lipids and thus create a boundary between the apical and the basolateral membrane domains of polarized epithelial cells (fence function) (Tsukita *et al*., 2001). Interestingly, the fence function seems not to depend on TJ strands (Umeda *et al*., 2006). Recents evidence indicates that the TJs also participate in signal transduction mechanisms which regulate cell proliferation and morphogenesis (Matter and Balda, 2003; Matter and Balda, 2007). This module describes the major molecular interactions responsible for the formation of TJ strands and for the recruitment of the PAR-3-PKC-PAR-6 and CRB3-Pals1-PATJ complexes that function in tight junction formation (Ebnet, 2008).

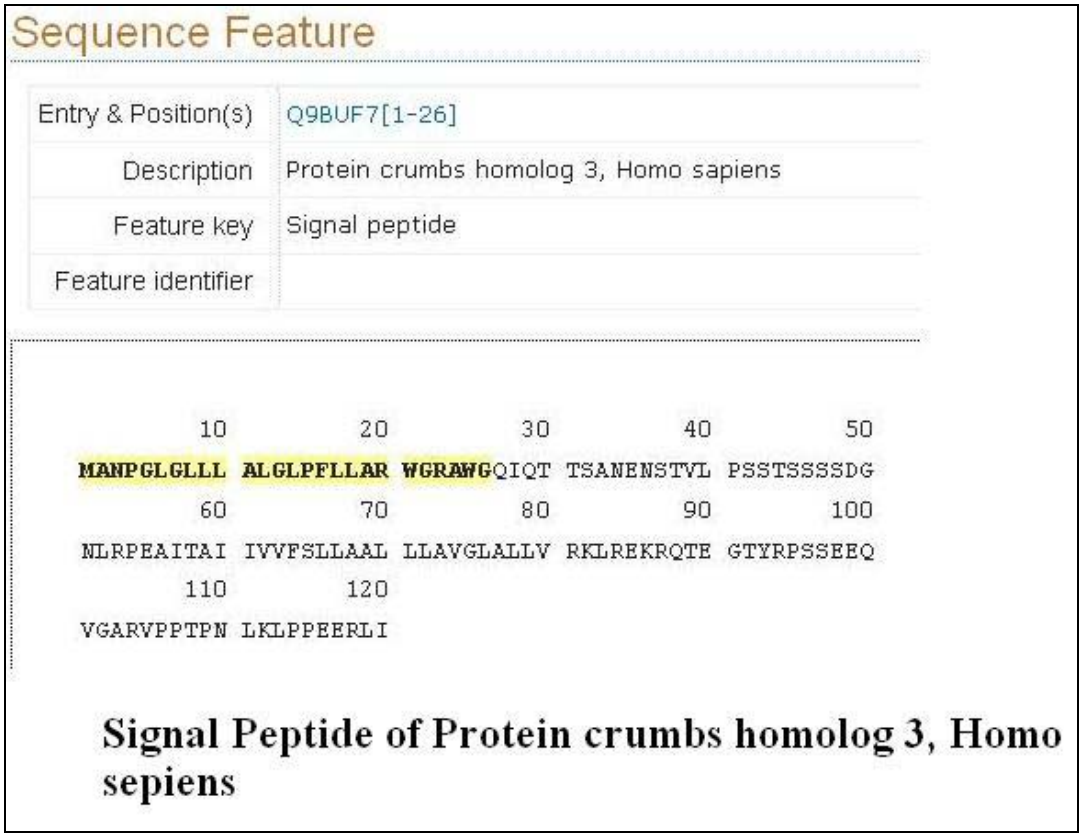


Fig 8: demonstrates the entire peptide sequence of Human crumbs 3 and also the sequence of 'signal peptide' (1-26, shown in color).

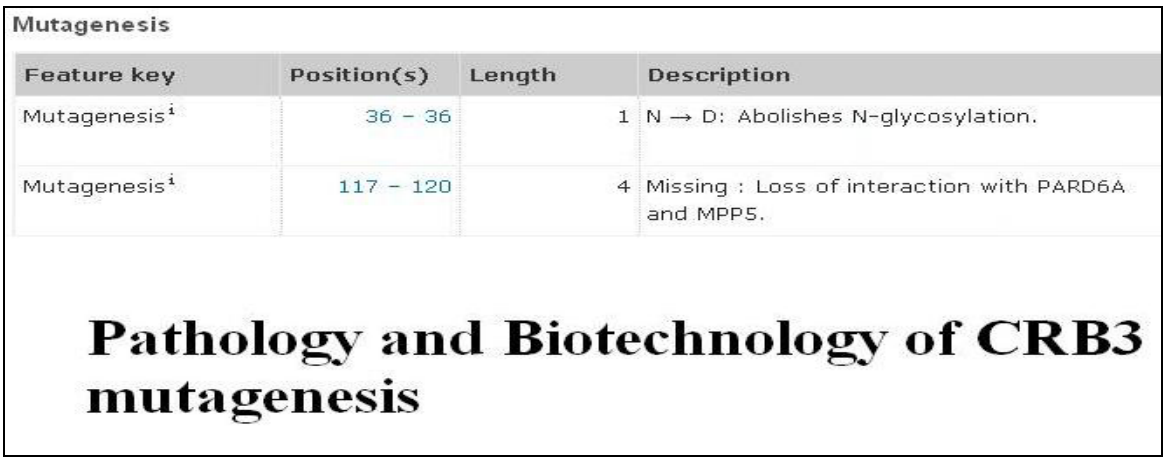
Muitation in CRB3: Abolition of N-linked glycosylation

N-linked glycosylation is the most important form of post-translational modification for proteins synthesized and folded in the Endoplasmic Reticulum (Stanley *et al*. 2009). An early study in 1999 revealed that about 50% of the proteins in the Swiss-Prot database at the time were N-glycosylated (Apweiler *et al*. 1999). It is now established that the majority of the proteins in the secretory pathway require glycosylation in order to achieve proper folding.

The addition of an N-glycan to a protein can have several roles (Shental-Bechor & Levy 2009). First, glycans enhance the solubility and stability of the proteins in the ER, the golgi and on the outside of the cell membrane, where the composition of the medium is strongly hydrophilic and where proteins, that are mostly hydrophobic, have difficulty folding properly. Second, N-glycans are used as signal molecules during the folding and transport process of the protein: they have the role of labels to determine when a protein must interact with a chaperon, be transported to the golgi, or targeted for degradation in case of major folding defects. Third, and most importantly, N-glycans on completely folded proteins are involved in a wide range of processes: they help determine the specificity of membrane receptors in innate immunity or in cell-to-cell interactions, they can change the properties of hormones and secreted proteins, or of the proteins in the vesicular system inside the cell. All N-linked glycans are derived from a common 14-sugar oligosaccharide synthesized in the ER, which is attached co-translationally to a protein while this is being translated inside the reticulum. The process of the synthesis of this glycan, known as Synthesis of the N-glycan precursor or LLO, constitutes one of the most conserved pathways in eukaryotes, and has been also observed in some eubacteria. The attachment usually happens on an asparagine residue

within the consensus sequence asparagine-X-threonine by an complex called oligosaccharyl transferase (OST). After being attached to an unfolded protein, the glycan is used as a label molecule in the folding process (also known as Calnexin/Calreticulin cycle) (Lederkremer 2009). The majority of the glycoproteins in the ER require at least one glycosylated residue in order to achieve proper folding, even if it has been shown that a smaller portion of the proteins in the ER can be folded without this modification. Once the glycoprotein has achieved proper folding, it is transported via the cis-Golgi through all the Golgi compartments, where the glycan is further modified according to the properties of the glycoprotein. This process involves relatively few enzymes but due to its combinatorial nature, can lead to several millions of different possible modifications. The exact topography of this network of reactions has not been established yet, representing one of the major challenges after the sequencing of the human genome (Hossler *et al*. 2006). Since N-glycosylation is involved in an great number of different processes, from cell-cell interaction to folding control, mutations in one of the genes involved in glycan assembly and/or modification can lead to severe development problems.

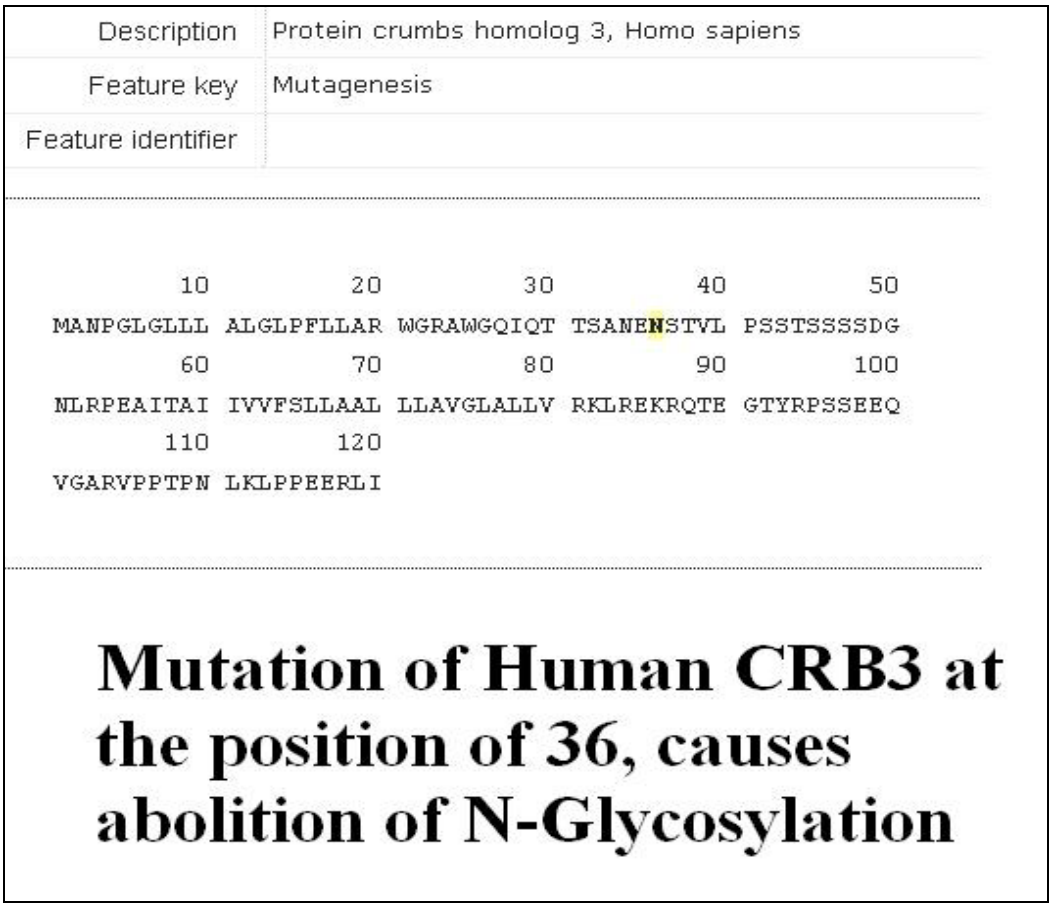
Mutagenesis:



Mutagenesis

Feature key	Position(s)	Length	Description
Mutagenesis[i]	36 - 36	1	N → D: Abolishes N-glycosylation.
Mutagenesis[i]	117 - 120	4	Missing : Loss of interaction with PARD6A and MPP5.

Fig 9: Demonstrates mutagenesis of Human CRB3 homologue in 36th position.



Description	Protein crumbs homolog 3, Homo sapiens
Feature key	Mutagenesis
Feature identifier	

```
        10         20         30         40         50
MANPGLGLLL ALGLPFLLAR WGRAWGQIQT TSANENSTVL PSSTSSSSDG
        60         70         80         90        100
NLRPEAITAI IVVFSLLAAL LLAVGLALLV RKLREKRQTE GTYRPSSEEQ
       110        120
VGARVPPTPN LKLPPEERLI
```


Fig 10: Demonstrates the mutation at 36th residue that causes abolition of N-linked Glycosylation.

Mutation of CRB3 in the position of four last amino acids (ERLI) that are involved in a direct interaction with Par6 through an intermediate PALS1, a membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5), a regulator of epithelial polarity and tight junction formation [14]. Thus, CRB3, through its cytoplasmic domain and its interactors, plays a role in apical membrane morphogenesis and tight junction regulation.

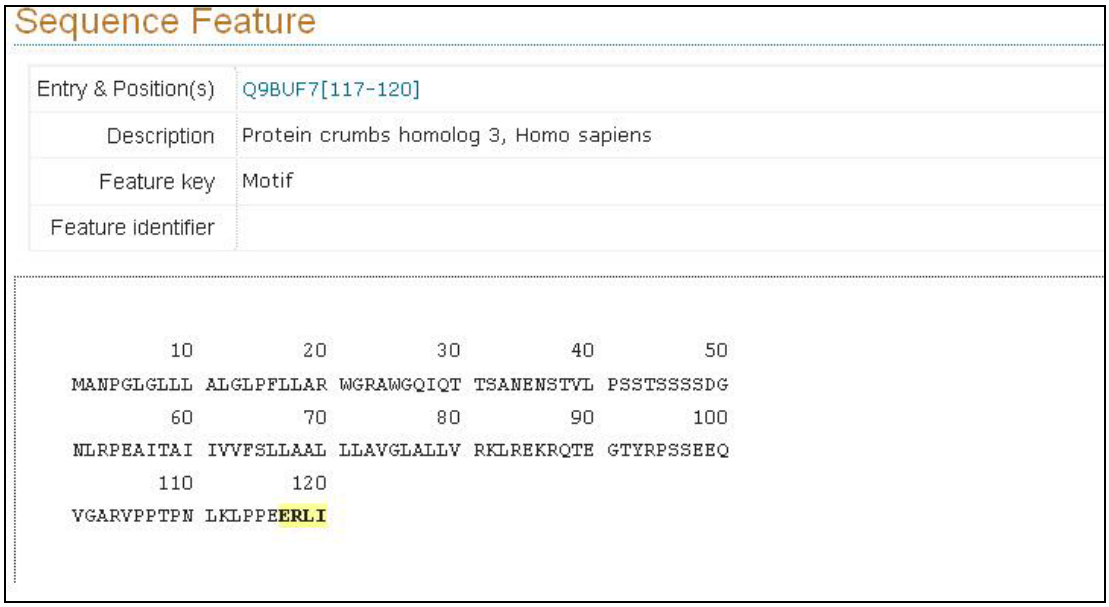


Fig 11: Demonstrates the mutation in last four residues favoring suitable environment for tight junction.

Positive -biofeedback of mutagenesis in N-glycosylation site of Human Protein crumbs homolog 3:

Extensive research work has demonstrated that human CRB1 can form a complex with mammalian orthologues of Stardust and Discs Lost, known as protein associated with Lin-7 (Pals1) and Pals1 associated tight junction (PATJ), respectively. In the recent scientific report it has been found from the cloning of a full length cDNA for a human paralogue of CRB1 called Crumbs3 (CRB3). In contrast to Drosophila Crumbs and CRB1, CRB3 has a very short extracellular domain but like these proteins it has a conserved intracellular domain that allows it to complex with Pals1 and PATJ. Mouse and human CRB3 have identical intracellular domains but divergent extracellular domains except for a conserved N-glycosylation site. CRB3 is localized to the apical surface and tight junctions but the conserved N linked glycosylation site does not appear to be necessary for CRB3 apical targeting. CRB3 is a specialized isoform of the Crumbs protein family that is expressed in epithelia and can tie the apical membrane to the tight junction [50].

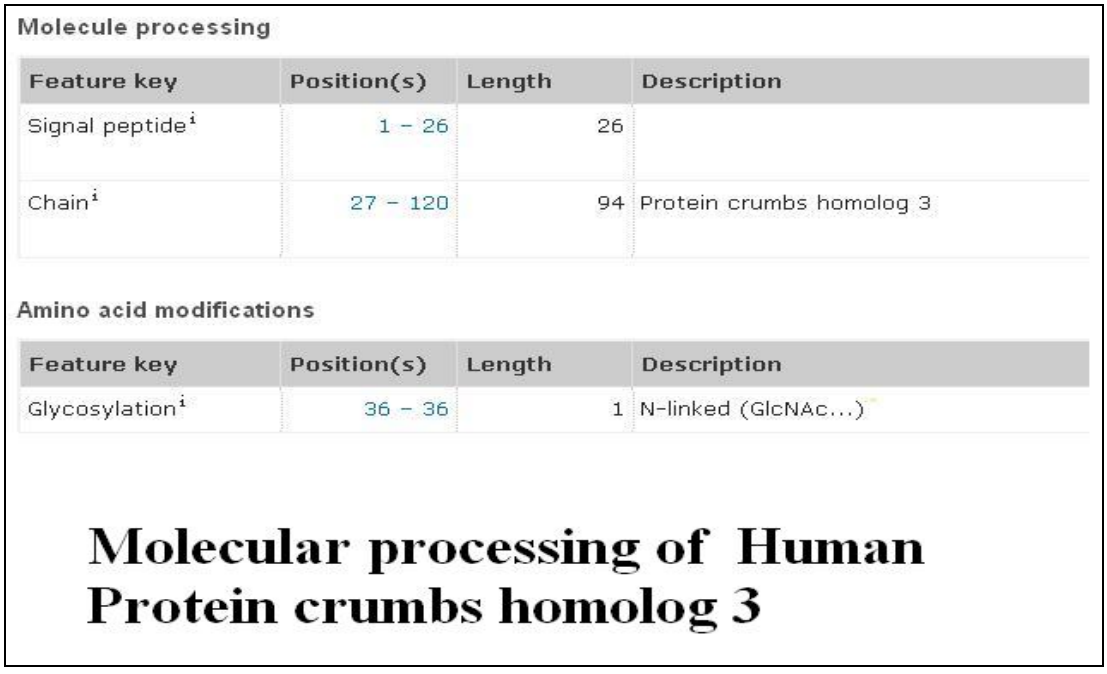


Fig 12: Demonstrates the molecular processing of Human Protein crumbs homolog 3

Control of Notch Signaling by Crb

Notch proteins are evolutionarily conserved transmembrane receptors, which are activated by transmembrane ligands expressed at the surface of adjacent cells [51]. Notch-mediated short-range intercellular communication fulfills crucial roles in embryonic development and tissue renewal. Notch activation influences cell fate specification, proliferation, apoptosis, and differentiation. It is therefore not surprising that deregulation of Notch signaling has profound effects on tissue homeostasis and results in human pathologies, including many human cancers [52]. Following ligand binding, Notch intracellular domain (NICD) is released by proteolysis. Full proteolytic processing of NICD requires the multimeric γ-secretase complex. Processed NICD reaches the nucleus where it partakes in a transcription complex allowing for expression of Notch pathway target genes [51]. The Notch signaling mediates TGF-β-induced EMT through the induction of Snail [52]. The Notch function changes during the development of cell differentiation and can be either oncogenic or tumour-suppressive, depending on cellular context [53]. 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 lager extracellular structure; this may also negatively affect the epithelial tissue growth [54, 55]. However, overexpression of human CRB3 does not have this effect on Notch signaling in mammalian cells [55] *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.

Conclusions

Recent studies have established that Crb/CRB proteins regulate epithelial tissue growth by acting as transmembrane proteins controlling intracellular signaling important for proliferation and apoptosis. Crb/CRB proteins are linked to many pathways through different domains, showing a complex function for these proteins in relaying growth-control signals. These studies therefore provide mechanistic insights linking a cell polarity regulator to restriction of tissue hyperplasia. One outstanding question is whether CRB3 acts as a receptor transmitting extracellular cues inside the cell to maintain epithelial homeostasis. Although the extracellular domain of CRB3 is short, it may be involved in protein-protein interaction or may bind to lectin proteins, as it is glycosylated. Identification of binding partners for the extracellular domain of CRB3 would help in better understanding in favour of a concrete resolution in order to establish that CRB3 is a tumor suppressor in humans, *but deciphering the molecular mechanisms acting downstream of CRB3 seems a promising avenue to better understand cancer biology and to identify potential therapeutic targets*.

Authors' contributions

Dr. Anjana Mazumder, (M.D.S) is the Professor of the Department of Oral Pathology in Dr. R. Ahmed Govt. Dental College, Kolkata, India. She furnished the innovative idea in the present paper and provided comprehensive guidance to the total research work in favour of this hypothesis in order to achieve a positive output. Partha Majumder is Gold Medalist in Human Physiology, having area of cultivation in recent Biomedical research and former Head of the Department of Applied Biotechnology and Bioinformatics, Sikkim Manipal University, CC:1637, Kolkata, India.

Acknowledgement

Both of authors achieved extreme guidance favoring the in depth cultivation with a positive output from Dr. D.N. Tibarewala, Professor, School of Bio-Medical Engineering , Jadavpur University, Kolkata, India. Dr. D.N. Tibarewala contributed a pioneer role to the design of the study, data analysis, and revision of the manuscript. And for that, both of the authors are grateful for his extreme support to make the endeavor successful.

References

1. Laprise P, Tepass U. Novel insights into epithelial polarity proteins in Drosophila, Trends in Cell Biology. 2011; 21(7): 401–408.
2. Dow LE, Humbert PO. Polarity regulators and the control of epithelial architecture, cell migration, and tumorigenesis, International Review of Cytology. 2007; 262:253–302.
3. Coradini D, Casarsa C, Oriana S. Epithelial cell polarity and tumorigenesis: new perspectives for cancer detection and treatment, Acta Pharmacologica Sinica. 2011; 32(5):552–564.
4. Bazellieres E, Assemat E, Arsanto JP, Le Bivic A, Massey-Harroche D. Crumbs proteins in epithelial morphogenesis, Frontiers in Bioscience. 2009; 14:2149–2169.
5. Bulgakova NA, Knust E. The Crumbs complex: from epithelial-cell polarity to retinal degeneration, Journal of Cell Science. 2009; 122(15):2587–2596.
6. Pieczynski J, Margolis B. Protein complexes that control renal epithelial polarity, The American Journal of Physiology—Renal Physiology, 2011; 300(3):F589–F601.
7. Tepass U, Theres C, Knust E. Crumbs encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia, Cell, 1990; 61(5):787–799.
8. Wodarz A, Hinz U, Engelbert M, Knust E. Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of Drosophila, Cell, 1995; 82(1):67–76.
9. Tanentzapf G, Tepass U. Interactions between the crumbs, lethal giant larvae and bazooka pathways in epithelial polarization," Nature Cell Biology. 2003; 5(1):46–52.
10. Bilder D, Schober M, Perrimon N. Integrated activity of PDZ protein complexes regulates epithelial polarity, Nature Cell Biology, 2003; 5(1):53–58.
11. Laprise P, Beronja S, Silva-Gagliardi NF. The FERM protein Yurt is a negative regulatory component of the Crumbs complex that controls epithelial polarity and apical membrane size, Developmental Cell, 2006; 11(3):363–374.
12. Laprise P, Lau KM, Harris KP. Yurt, Coracle, Neurexin IV and the Na(+), K(+)-ATPase form a novel group of epithelial polarity proteins," Nature, 2009; 459:7250, 1141–1145.
13. Laprise P, Paul SM, Boulanger J, Robbins RM, Beitel GJ, Tepass U. Epithelial polarity proteins regulate Drosophila tracheal tube size in parallel to the luminal matrix pathway, Current Biology, 2010; 20(1):55–61.

14. KEGG Pathway, Homo sapiens (human): 64398.
15. CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells. Mol. Biol. Cell, 2004; 15:1324-1333 [Full text:10.1091/mbc.E03-04-0235] [PubMed: 14718572.
16. Mammalian Crumbs3 is a small transmembrane protein linked to protein associated with Lin-7 (Pals1). Makarova O., Roh M.H., Liu C.J., Laurinec S., Margolis B. Gene, 2003; 302:21-29. PubMed: 12527193.
17. The Crumbs3-Pals1 complex participates in the establishment of polarity in mammalian epithelial cells. Roh M.H., Fan S., Liu C.-J., Margolis B. J. Cell Sci. 2003; 116:2895-2906. [PubMed].
18. Epithelial Cell polarity: A major gatekeeper against cancer. Cell Death Differ. 2011; 18(9):1470–1477.
19. Humbert PO, Grzeschik NA, Brumby AM, Galea R, Elsum I, Richardson HE. Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. Oncogene. 2008; 27:6888–6907. [PubMed].
20. Lee M, Vasioukhin V. Cell polarity and cancer--cell and tissue polarity as a non-canonical tumor suppressor. J Cell Sci. 2008; 121 (Part 8:1141–1150. [PubMed].
21. Medina E, Lemmers C, Lane-Guermonprez L, Le Bivic A. Role of the Crumbs complex in the regulation of junction formation in Drosophila and mammalian epithelial cells. Biol Cell/Under Aus Eu Cell Biol Organization. 2002; 94:305–313. [PubMed].
22. Morais-de-Sa E, Mirouse V, St Johnston D. aPKC phosphorylation of Bazooka defines the apical/lateral border in drosophila epithelial cells. Cell. 2010; 141:509–523.
23. Harris TJ, Peifer M. The positioning and segregation of apical cues during epithelial polarity establishment in Drosophila. J Cell Biol. 2005; 170:813–823.
24. Martin-Belmonte F, Gassama A, Datta A, Yu W, Rescher U, Gerke V, *et al*. PTEN-mediated apical segregation of phosphoinositides controls epithelial morphogenesis through Cdc42. Cell. 2007; 128:383-397.
25. Nam SC, Choi KW. Interaction of Par-6 and crumbs complexes is essential for photoreceptor morphogenesis in drosophila. Development. 2003; 130:4363–4372.
26. Vogelmann R, Nelson WJ. Fractionation of the epithelial apical junctional complex: reassessment of protein distributions in different substructures. Mol Biol Cell. 2005; 16:701–716.
27. Izumi Y, Hirose T, Tamai Y, Hirai S, Nagashima Y, Fujimoto T, *et al*. An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of Caenorhabditis elegans polarity protein PAR-3. J Cell Biol. 1998; 143:95–106.
28. Joberty G, Petersen C, Gao L, Macara IG. The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nat Cell Biol. 2000; 2:531–539.
29. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease, Cell, 2009; 139(5):871–890.
30. Zhan L, Rosenberg A, Bergami KC. Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma, Cell, 2008; 135(5):865–878.
31. Huang L, Muthuswamy SK. Polarity protein alterations in carcinoma: A focus on emerging roles for polarity regulators, Current Opinion in Genetics and Development, 2010; 20(1):41–50.
32. Herrmann JL, Byekova Y, Elmets CA, Athar M. Liver Kinase B1 (LKB1) in the pathogenesis of epithelial cancers, Cancer Letters, 2011; 306(1):1–9.
33. Karp CM, Ting TT, Mathew R. Role of the polarity determinant crumbs in suppressing mammalian epithelial tumor progression, Cancer Research, 2008; 68(11):4105–4115.
34. Aigner K, Dampier B, Descovich L. The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity, Oncogene, 2007; 26(49):6979–6988.
35. Spaderna S, Schmalhofer O, Wahlbuhl M *et al*., The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer, Cancer Research, 2008; 68(2):537–544.
36. Whiteman EL, Liu CJ, Fearon ER, Margolis B. The transcription factor snail represses Crumbs3 expression and disrupts apico-basal polarity complexes, Oncogene, 2008; 27(27):3875–3879.
37. Browne G, Sayan AE, Tulchinsky E. ZEB proteins link cell motility with cell cycle control and cell survival in cancer, Cell Cycle, 2010; 9(5):886–891.
38. Wu Y, Zhou BP. Snail: more than EMT, Cell Adhesion and Migration, 2010; 4(2):199–203.
39. Braun P, Gingras AC. History of protein-protein interactions: from egg-white to complex networks, Proteomics, 2012; 12(10):1478–1498.
40. Ofran Y, Rost B. Analysing six types of protein-protein interfaces, Journal of Molecular Biology. 2003; 325(2):377–387.
41. Nooren MA, Thornton JM. Diversity of protein-protein interactions, The EMBO Journal. 2003; 22(14):3486–3492.
42. Zhang A. Protein Interaction Networks-Computational Analysis, Cambridge University Press, New York, NY, USA, 2009.
43. Yanagida M. Functional proteomics; current achievements, Journal of Chromatography B. 2002; 771(1-2):89–106.
44. Berggård, S. Linse, and James P. Methods for the detection and analysis of protein-protein interactions, Proteomics, 2007; 7(16):2833–2842.
45. Von Mering C, Krause R, Snel B. Comparative assessment of large-scale data sets of protein-protein interactions," Nature, 2002; 417(6887):399–403.
46. Phizicky EM, Fields S. Protein-protein interactions: methods for detection and analysis, Microbiological Reviews, 1995; 59(1):94–123.
47. Pedamallu CS, Posfai J. Open source tool for prediction of genome wide protein-protein interaction network based on ortholog information, Source Code for Biology and Medicine, 2010; 5:8.
48. Dunker AK, Cortese MS, Romero P, Iakoucheva LM, Uversky VN. Flexible nets: the roles of intrinsic disorder in protein interaction networks, FEBS Journal. 2005; 272(20):5129–5148.
49. Sarmady M, Dampier W, Tozeren A. HIV protein sequence hotspots for crosstalk with host hub proteins, PLoS ONE, 2011; 6(8), Article ID e23293,.

50. Mammalian Crumbs3 is a small transmembrane protein linked to protein associated with Lin-7 (Pals1). Gene. 2003; 302(1-2):21-9.
51. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism, Cell, 2009; 137(2):216–233.
52. Guo S, Liu M, Gonzalez-Perez RR. Role of Notch and its oncogenic signaling crosstalk in breast cancer, Biochimica et Biophysica Acta-Reviews on Cancer, 2011; 1815(2):197–213.
53. Matsuno Y, Coelho AL, Jarai G, Westwick J, Hogaboam CM. Notch signaling mediates TGF-beta1-induced epithelial-mesenchymal transition through the induction of Snai1. The international journal of biochemistry & cell biology. 2012; 44:776–89.
54. Aster JC. In brief: Notch signalling in health and disease. The Journal of pathology. 2014; 232:1–3.
55. Herranz H, Stamataki E, Feiguin F, Milán M. Self-refinement of Notch activity through the transmembrane protein Crumbs: modulation of γ-Secretase activity. EMBO reports. 2006; 7:297–302.