ACBD3, Its Cellular Interactors, and Its Role in Breast Cancer

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Abstract

ACBD3 breast cancer research to date reveals that overexpression at mRNA and protein level is near universal in breast tumour tissue and that high ACBD3 expression is associated with worse patient prognosis. ACBD3 has been shown to have an important role in specifying cell fate and maintaining stem cell pools in neurological development and deletion of ACBD3 in human cell lines prevents cell division. Combined with observations that β-catenin expression and activity is increased when ACBD3 is overexpressed it has been hypothesised that ACBD3 promotes breast cancer by increasing Wnt signalling. This may only be one aspect of ACBD3’s effects as its expression and localisation regulates steroidogenesis, calcium mediated redox stress and inflammation, glucose import and PI(4)P production which are all intrinsically linked to breast cancer dynamics. Given the wide scope for a role of ACBD3 in breast cancer, we explore its interactors and the implications of preventing these interactions.

Keywords: ACBD3, Breast cancer, Chromosome 1, Golgi, NUMB, PI4Kβ, Phosphatidylinositol, Protein kinase A, Steroidogenesis, Wnt signalling, 1q

Introduction

Although breast cancer incidence has increased in recent years, largely due to improved diagnostic techniques, greater awareness and the introduction of national screening programmes, mortality rates are declining as result of earlier detection and improved treatment regimes. Despite this, treating advanced disease remains difficult and there is a need to identify new therapeutic targets. Proteins encoded by the q-arm of chromosome 1 are of particular interest as regions of 1q are frequently amplified and over expressed in breast cancer leading to the hypothesis that 1q is important in disease development and progression [1-3]. The frequency at which regions of arm 1q were amplified was investigated and the 1q42.12 locus was found to be amplified in both breast cancer cell lines and primary tumours with a number of genes in or near this region also being over expressed [4]. Of emerging interest is ACBD3, a Golgi protein with multiple functions, only recently linked to breast cancer [5].

ACBD3 was discovered as an interactor of GOLGB1 and named GCP60, and independently discovered as an interactor of the mitochondrial translocator protein TSPO and protein kinase A and named PAP7 [6,7]. Having found that each of these names were describing one aspect of a diverse adapter protein it was renamed by the HUGO gene nomenclature committee in 2004 as AcetylCoA Binding Domain containing protein 3, or ACBD3, reflecting its functional groups and protein family rather than any particular role, of which there are many (www.genenames.org). In addition to the acyl CoA binding domain at its N-terminus, ACBD3 contains a Golgi dynamics (GOLD) and a glutamine rich Q domain as well as a proline rich region (Figure 1) [8]. The GOLD domain is found in Golgi and lipid trafficking proteins and makes up the C-terminus of ACBD3 (aa384-526). It is a β-strand rich domain and is responsible for ACBD3 localization to the Golgi via direct interaction with GOLGB1 [6]. ACBD3 is a largely unstructured or loosely structured protein, as

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Figure 1: Predicted 3D structure of human ACBD3. The structure is modelled by Phyre2 software using the primary amino acid sequence which agrees strongly with crystal structures of individual ACBD3 domains and related proteins [86]. From the N-terminus in blue to the C-terminus in red ACBD3 clearly contains 3 domains: the ACBP domain, the Q domain and the Golgi dynamics (GOLD domain) respectively connected by flexible linkers with an N terminal proline rich region. The hydrophilic surface of acbd3 has been superimposed on ACBD3 showing the electrostatic charge of the protein model with red depicting negative charge and blue depicting positive charge.

many linkers are, and of all the recognisable domains only the GOLD domain structure has been solved by X-ray crystallography with the rest of ACBD3 being modelled by NMR and predictive modelling software (Figure 1). The Q domain is a glutamine rich region (aa241-308) which forms a long loop made of alpha helices [8]. The N-terminal ACB domain binds Acyl CoA and Palmitoyl CoA in other ACBD family proteins but the function of this domain in ACBD3 is unclear. To the N-terminus of the ACB domain is a proline rich region (aa21-60), which is indicative of protein-protein interaction sites and may complement the ACB domain which is often found paired with protein-protein interaction domains such as the Pleckstrin homology domain (PH) and the Src homology domain in other proteins. ACBD3 makes essential interactions with an unusually high number of protein partners in cellular processes as diverse as Golgi structure, steroid synthesis and glucose import; other functions not reviewed here include iron transport and a causal role in Huntington's disease progression [7,9-12]. ACBD3 is essential for neural development and human cell lines do not divide when ACBD3 is excised by CRISPR-CAS9 [13].

ACBD3 in Breast Cancer

The q arm of Chromosome 1 contains many genes important in cancer progression or tumour suppression: NRAS, JUN, MYCL, ESRRG, ARFI and RAB25 are amongst the best known. There are however many more 1q genes that are amplified in breast cancer with deletions strikingly rare despite common deletions in the p arm. Some of these genes (PI4Kδ, PIP5K1A and HIST2H2BE) have more recently been recognised as oncogenic with ACBD3 being the latest 1q gene observed to affect breast cancer [14]. ACBD3 mRNA is reported to be upregulated in breast tumour tissue matched against adjacent normal tissue in all subtypes [5]. Protein levels of ACBD3 were upregulated in 8 breast cancer cell lines (MDA-MB453, MDA-MB-415, BT549, MDA-MB-231, ZR-75-30, SKBR3, T47D and MCF7) compared to 2 normal breast epithelial cell samples (NBEC1 and NBEC2). In a cohort of Chinese breast cancer patients ACBD3 protein expression increased as cancer stage became more advanced. Kaplan-Meier survival curves were plotted and it was found that high levels of ACBD3 mRNA in breast tumour tissue predicted lower rates of patient survival and that this made a large difference in stage III and IV cancers with 60% probability of survival at 120 months when ACBD3 expression is low but less than 30% probability of survival when ACBD3 expression is high.

The ACBD3 containing 1q42.12 locus is seen to be amplified in an additional 6 breast cell lines (BRCAMZ0, BT20, HCC2218, MDAMB436, SUM149, ZR751) and 6 out of 25 primary breast tumours in a breast cancer 1q amplification study [4]. Loss of region 1q42.12 was seen in only 1 cell line (UACC812) where the terminal ~38 megabases of arm 1q were deleted and loss of 1q42.12 was not observed in any primary tumour samples. RNA expression levels revealed that 1q42.12 is located in the middle of a region of gain coined G7, the largest region of gain (in bases) on chromosome 1 in breast cancers. Overexpression of ACBD3 in cell cultures caused increased side populations of stem like cancer cells and inhibition of ACBD3 by siRNA significantly reduced these populations [5]. GSEA analysis found that CTNNB1- and TCF4-activated gene signatures both positively correlated with ACBD3 expression [5]. CTNNB1 encodes the β-catenin protein which, in response to Wnt signalling, accumulates in the cytoplasm and then translocates to the nucleus where it propagates the Wnt signal. ACBD3 overexpression led to an increase of β-catenin in the cytoplasm and nucleus compared to when ACBD3 expression was low (65% versus 20% nuclear and cytoplasmic localisation) [5]. TCF4 is a transcription factor for genes that code proteins in the Wnt signalling pathway. When TCF4 was knocked down, the self-renewal ability of ACBD3-expressing cells was abolished suggesting that ACBD3 may promote cancer stem cell propagation via the Wnt/β-catenin signalling pathway in breast cancer. All of this provides strong evidence that ACBD3 overexpression affects breast cancer but the ACBD3 protein has many binding partners, in disparate cellular pathways and cells appear to have few redundancies for the essential roles of ACBD3.

ACBD3 and Steroidogenesis

Although often considered a resident Golgi protein due to its structural role and interactions with other structural components, ACBD3 can also be found at other membranes including the cytosolic cell membrane and at the outer mitochondrial membrane (OMM). ACBD3 interacts with translocator protein TSPO (previously the peripheral-type benzodiazepine receptor) on the cytosolic OMM and stimulates cholesterol transport from the OMM to the IMM (inner mitochondrial membrane) (Figure 2) [7,15]. P450sc (CYP11A1) makes direct contact with the IMM and converts cholesterol to pregnenolone, the precursor to mammalian steroids, by side chain cleavage [16,17]. TSPO is anchored to the voltage dependent anion
channel VDAC1 and makes up approximately 2% of OMM proteins. TSPO tethers cytosolic ACBD3 at the OMM and ACBD3 subsequently recruits protein kinase A (PKA) via the PKARRα subunit. This brings PKA into proximity with one of its substrates, the steroidogenic acute regulatory (StAR) protein which is phosphorylated on residues S57 and S195 by PKA [18]. StAR then facilitates cholesterol import from the OMM to the IMM, the rate limiting step in steroidogenesis. ACBD3 overexpression increases choric gonadotropin-induced steroid production; increased steroid production has obvious implications for cancer progression by enabling self-sufficiency in growth signals, a hallmark of cancer [19,20].

PKAŘα is a tumour suppressor gene and is important in primary pigmented nodular adrenocortical disease (PPNAD) nodule formation and tumorigenesis in mice and humans. Mutation of PKAŘα leads to hypercortisolemia that drives tumorigenesis, and high ACBD3 expression in steroidogenic tissues (of which the adrenal cortex is one) may contribute to the overexpression/over activity of the mutant PKAŘα [21]. PPNADs are characterised by a resistance to apoptosis which in itself contributes to cancer occurrence and is another hallmark of cancer [20]. The first publication to suggest any link between ACBD3 and cancer demonstrated that ACBD3 follows the same expression profile as PKAŘα in PPNAD tissue and speculated that, in tumorigenesis, this could lead to deregulation of steroid synthesis [21]. More recent studies have shown that PKA activation may instead have a suppressive effect on cancer [22,23], whilst others show PKAŘα is upregulated in cancer cell lines [24,25].

**ACBD3 in Redox Stress**

In a separate process, glutamate induces expression of TSPO and increased TSPO recruits ACBD3 and PKA to the mitochondria. Glutamate is a signalling molecule that is known to cause acute neurotoxicity [26-28]. PKA phosphorylates the calcium channel protein VDAC1, preventing Ca2+ import into the mitochondria (Figure 2). This causes Ca2+ accumulation in the cytosol which signals redox stress via the calcium sensing CamKII and its effector NADPH oxidase (NOX5) leading to inflammation by increased reactive oxygen species stress via the calcium sensing CamKII and its effector NADPH oxidase (NOX5) leading to inflammation by increased reactive oxygen species. Glutamate mediated redox stress is particularly important in neuro-inflammation where TSPO is not expressed in healthy brain tissue but can accumulate in age related degenerative disease or after traumatic stress, leading to increased ACBD3 mitochondrial recruitment and subsequent VDAC1 phosphorylation by PKA which may contribute to neurodegeneration [29]. VDAC1 is important in Ca2+ homeostasis, especially mitochondrial Ca2+ homeostasis which controls the metabolism of mitochondria and therefore energy availability in the cell [30]. Dysregulation of cellular energetics is a hallmark of cancer and an inflammatory environment can be tumour promoting when chronic and over time [20].

**ACBD3 and Insulin Mediated Glucose Import**

GLUT4 (glucose transporter type 4) allows the facilitated diffusion of glucose from the surroundings into cells, and is sequestered into storage vesicles (GSVs) that are tethered to Golgi membranes by TUG (Tether containing UBX domain for GLUT4). Golgin-160 ACBD3 and when insulin is absent (Figure 3) [31]. ACBD3-bound TUG can be acetylated on lysine 549 which has a higher binding affinity with Golgin-160 than with ACBD3 [10]. In response to insulin receptor activation the cytoplasmic effector of insulin PIST (PDZ interacting specifically with TC10) binds Golgin-160 and catalyses the cleavage of acetylated TUG. This releases GSVs allowing them to fuse with the plasma membrane where GLUT4 forms a channel for glucose import [32]. GLUT4 is continuously cycled away from plasma membranes back into GSVs to increase the on/off response of insulin sensitive cells when insulin is not present. GLUT4 exocytosis is regulated by tankyrase 1 as are several other ACBD3 related processes including Golgin-45 expression and the promotion of β-catenin transcription in the Wnt signalling pathway [33,34].

The hormone 17β-oestradiol has a central role in breast cancer progression, it has been found to upregulate GLUT4 expression and translocation to the membrane in breast cancer cell lines and was associated with increased glucose uptake [35-37]. GLUT4 is being investigated as a target for breast cancer therapy as part of an informed approach to target the Warburg effect. Downregulation of GLUT4 by siRNA impairs viability of MDA-MB-231 and MCF7 breast cancer cell lines and increases mitochondrial oxidation of pyruvate [38]. The EGFR/HER2-targeted drug lapatinib has been shown to downregulate GLUT4 in Er-/HER2+ HMEC cell lines, and GLUT4 downregulation by siRNA in these cell lines led to the formation of normal acini structures in 3D culture [39]. The insulin receptor (IR) is upregulated in breast cancer and is a potential target for breast cancer therapy as it has been demonstrated that knock down of IR by shRNA and inhibition by peptide drugs inhibits breast cancer cell growth [40-43].
ACBD3, PI4Kβ and Phosphatidylinositol 3-kinase I (PI4Kβ)

Phosphatidylinositol 4 Kinase III beta (PI4Kβ) is a lipid kinase that converts phosphatidylinositol 4-phosphate (PI(4)P) into phosphatidylinositol 4-phosphate (PI(4)P) [44]. PI4Kβ is localised to the Golgi by extension of an amphipathic helix at the N-terminus of PI4Kβ (aa44-64) through the Q domain alpha helices loop of ACBD3 (aa241-308) [8]. The small GTPase Rab11 binds PI4Kβ to support this interaction whilst ACBD3 also interacts GOLGB1 on the Golgi surface bringing PI4Kβ in close and constant contact with its PI substrate embedded in the lipid bilayer. ACBD3 does not affect the enzymatic activity of PI4Kβ directly by this interaction but, by tethering it to the Golgi membrane, PI4Kβ is proximal to the PI substrate and does not rely on diffusion through the cytoplasm for the phosphorylation of substrate. PI4Kβ is heavily implicated in breast cancers with 20% of primary tumours showing over expression of PI4Kβ at the protein level [45,46]. PI4Kβ is a chromosome 1q gene (at 1q21.3) and is reported to have increased gene copy number in 62% of 939 patient breast tumour samples [14]. Evidence of PI4Kβ upregulation at the protein level in breast ductal carcinoma samples from the human protein atlas was also found by Waugh. Independent of its lipid kinase function, PI4KIIIβ also mediates indirect phosphorylation and activation of AKT (Protein kinase B), an important kinase in breast cancer signalling [46,47]. AKT dysregulation drives many breast cancers by promoting cell cycle progression and suppressing apoptosis, it is commonly overexpressed or constitutively active [47].

Both PI and PI(4)P are cellular signalling molecules and docking sites on the membrane for other proteins including ARF1 (ADP-riboseylation Factor 1), which is essential for the formation of COPI vesicles and Golgi function including localisation of Golgin-160 to the Golgi. ARF1 is encoded by a gene adjacent to ACBD3 on chromosome 1 (1q22.13) [48-50]. PI4Kβ is also positioned on chromosome 1q, where amplification is common in breast cancers and its substrates localise ARF1 to membranes. ACBD3 is hijacked by some picornavirus viral proteins to form replication organelles and recruits PI4Kβ to these sites to enrich them for PI(4)P [51,52]. This is another example of how the role of ACBD3 is contextual and dependent on its cellular location, cell cycle position and binding partners. PI4Kβ has been found to be a target in malaria and drugs to inhibit PI4Kβ have already been developed [53]. There have so far been no publications on PI4Kβ drug inhibition in cancer.

ACBD3 Cell Signalling in Neurogenesis

Mammalian NUMB, an endocytic adapter protein, is involved in cytosolic signalling and is segregated asymmetrically into one daughter cell during the mitosis of neural progenitor cells and inhibits NOTCH [54]. This asymmetric distribution of NUMB results in 1 identical pluripotent daughter cell (high NUMB protein level) to maintain the population of neuronal precursors and 1 differentiated neuron cell (low NUMB protein level). This balances the need to create neurons through NOTCH signalling and maintain the pool of precursor cells in embryonic neurogenesis by NOTCH inhibition (Figure 4) [55,56]. ACBD3 was identified as a NUMB binding partner after observations that ACBD3 cytosolic release during mitosis was paired with NUMB mediated cell fate [57]. The ACBD3 interacting region on NUMB is essential for NUMB activity and interaction with ACBD3 increases NUMB activity [57]. The C-terminus of ACBD3 binds with the N-terminus of NUMB, and NOTCH also binds the N-terminus of NUMB [58]. Cytosolic ACBD3 expression leads to inhibition of NOTCH, suggesting that NOTCH inhibition by NUMB is conserved from drosophila to mammals indicating that ACBD3 and NUMB are both required to specify cell fate in neural progenitors. ACBD3 is bound to Golgi/mitochondrial membranes through most of the cell cycle and can only bind NUMB during mitosis when the breakdown
of the Golgi releases ACBD3 into the cytosol (Figure 4). Constitutively cytosolic mutant ACBD3 inhibits neurogenesis in mouse embryos resulting in fewer neurons. This indicates that permanently cytosolic ACBD3 is preventing differentiation in otherwise neuronal fated cells and it achieves this by binding NUMB outside of mitosis [57].

Krüppel-like factor 9 (KL9) is a tumour suppressor and is significantly down regulated in invasive breast cancers, endometrial carcinoma, glioblastoma and colorectal cancer, and its expression can inhibit growth of tumour xenografts from glioblastoma neurospheres [59,60]. ACBD3 and NOTCH1 expression are suppressed by KL9 in endometrial carcinoma cells and both proteins promote breast cancer progression, specifically in cancer stem cell maintenance [5,61,62]. KL9 suppresses glioblastoma derived neurosphere formation by 60% in controls but only by 33% when NOTCH1 expression is constitutively active strongly suggesting that KL9 must suppress other proteins relevant to glioblastoma progression and this could include ACBD3 [59]. NOTCH1, NOTCH3 and JAG1 expression is associated with poor survival in breast cancer patients with high NOTCH1 expression conferring a 66% chance of mortality and 74% chance of relapse at 10 years [63]. NOTCH receptors have been found to have activating mutations in triple negative breast cancers and result in the upregulation of NOTCH controlled genes [64]. NOTCH overexpression was able to transform the MCF10A breast cell line and reduce its sensitivity to apoptotic drugs such as staurosporine, melphalan, or mitoxantrone, and overexpression of NUMB reverted the transformation [61]. High levels of NOTCH in breast tumours are significantly associated with nuclear phospho-Erk 1 and 2 and conferring an association between NOTCH and Ras-MAPK expression [65]. Inhibition of NOTCH and NOTCH-related proteins have therefore become a target for therapy [66]. ACBD3 overexpression prevents NOTCH signalling in neurogenesis but this is reliant on NUMB expression and only during mitosis when the Golgi is fragmented. The NOTCH suppressors NUMB and its paralogue Numb-L are predictably down regulated in breast cancers and their overexpression reduces epithelial to mesenchymal transition in triple negative breast cancer cell lines [67-69]. NUMB-deficient breast cancer cells have an increased ability to form cancer stem cell pools and NUMB downregulation causes inactivation of p53 [70,71].

Discussion and Future Perspectives

Throughout this review an argument is presented that ACBD3 may do more than promote Wnt signalling in the context of breast cancer. Dysregulation of cellular energetics, sustaining proliferative signalling, replicative immortality and tumour-promoting inflammation are all hallmarks of cancer and overexpression of ACBD3 could conceivably support any or all of these [10,18,21,27,57]. Other factors including the position of ACBD3 on chromosome 1 in close proximity to other oncogenes, and the number of its binding partners and pathways already being targeted for cancer therapies leave ACBD3 nothing short of overlooked. ARF1 and RAB4 are located close to ACBD3, both at 1q42.13, within Orsetti's (4) region of gain G6 and were both found to be significantly overexpressed at the mRNA level in breast cancer. RAB4 in conjunction with RAB5 promotes and drives metastasis by facilitating the formation of invadosomes containing membrane type 1 matrix metalloprotease (MT1-MMP) and β3 integrin which together degrade the extracellular matrix, a process vital for cancer invasion and metastasis [72]. RAB4 is overexpressed in breast cancers and unsurprisingly associated with increased cell motility, it is one of many RAS related proteins that has clinical significance in cancer [73]. ARF1 is the most amplified gene of the ADP-ribosylation factor family in breast cancers and its amplification is associated with increased gene transcription and worse prognosis for patients [74]. ARF1 inhibition prevents metastasis of tumour xenografts in immuno-deficient mice and is replicable in zebrafish models of breast cancer metastasis. ACBD3 proximity to ARF1, RAB4 and other 1q oncogenes may confer a huge selective advantage to breast cancer cells with amplifications of these loci providing these cells with both survival and invasive advantages.

Tankyrase 1 regulates GLUT4 exocytosis and β-catenin transcription, and ACBD3 interacts with proteins in both pathways [75-78]. Tankyrase 1 controls the expression of Golgin45 which is a direct binding partner of ACBD3 [79]. Tankyrase also targets Axin for degradation leading to increased Wnt signalling, known to be aberrant in breast cancers and is reported to be effected by ACBD3 [5,76,80]. Tankyrase 1 and 2 are currently being targeted as cancer therapeutics because of their interactions in many carcinogenic pathways [77,81-83]. PIHK1 expression in breast cancer correlates with poor patient outcomes and its locus (1q21.3) is a biomarker for breast cancer [46,84]. It is most associated as an ACBD3 binding partner and the ACBD3 interaction has a solved X-ray crystal structure [8]. PIHK1 mutants that do not bind ACBD3 have been engineered and drugs that inhibit PIHK1 are available which aids its study [53,85]. ACBD3 deletion is embryonic lethal and may be invaluable for normal cell division [12,57]. As it does not have an enzymatic function an inhibitor for ACBD3 may not be lethal and may be invaluable for normal cell division [12,57]. As it does not have an enzymatic function an inhibitor for ACBD3 may not be lethal and may be invaluable for normal cell division [12,57].

References


13. Lyoo H, van der Schaar, Hilde M, Dorobantu CM, Rabouw HH, et al. (2019) ACBD3 is an Essential Pan-enterovirus Host Factor That Mediates the Interaction between Viral 3A Protein and Cellular Protein PI(4)Kb. mBio 10:e02742-18. [CrossRef]


55. Uemura T, Shepherd S, Ackerman L, Jan LY, Jan YN (1989) numb, a gene required in determination of cell fate during sensory organ formation in Drosophila embryos. Cell 58: 349-360. [Crossref]
63. Mittal S, Subramaniam D, Dey D, Kumar RV, Rangarajan A (2009) Cooperation of Notch and Ras/MAPK signaling pathways in human breast carcinogenesis. Mol Cancer 8: 124,598-8-128. [Crossref]