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Overexpressed ACBD3 has prognostic value in human breast cancer and promotes the self-renewal potential of breast cancer cells by activating the Wnt/beta-catenin signaling pathway

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ABSTRACT

Acyl-CoA binding domain containing 3 (ACBD3) is involved in the maintenance of Golgi structure and function through its interaction with the integral membrane protein. However, the clinical significance and biological role of ACBD3 in breast cancer remain unclear. Herein, we found that the mRNA and protein levels of ACBD3 were markedly up-regulated in breast cancer cells and tissues. Immunohistochemical analysis of breast cancer tissues demonstrated that ACBD3 overexpression was significantly associated with advanced clinicopathological features. Univariate and multivariate analysis indicated that ACBD3 overexpression correlates with poor prognosis in breast cancer. Furthermore, overexpressing ACBD3 promoted, while silencing ACBD3 inhibited, self-renewal and tumorigenesis in breast cancer cells in vitro and in vivo respectively. Importantly, upregulating ACBD3 promoted the self-renewal and tumorigenesis of breast cancer cells via activating the Wnt/beta-catenin signaling, and the pro-self-renewal effect of ACBD3 in breast cancer was antagonized by the Wnt signaling inhibitor TCF4-siRNA and Lef1-siRNA.These findings indicate that ACBD3 may represent candidate therapeutic targets to enable the elimination of breast cancer stem cells, providing the preclinical proof-of-concept for the prevention and treatment of breast cancer.

1. Introduction

With roughly half a million deaths per year worldwide, breast cancer has become the second cause of tumor-related deaths in females [1]. The treatment and prognosis of breast cancer are complex and vary dramatically depending on the subtype of breast cancer [2]. At present, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (Her2) are the most common receptors in breast cancer cells and specifically determine therapeutic approaches and prognosis of breast cancer [3]. Quantities of investigations show that most of the patients died of tumor recurrence within 5–10 years after surgery or chemotherapy [4]. Adjuvant therapies have significantly reduced breast cancer recurrence rates, but a substantial proportion of breast cancer patients are faced with recurrence. It is believed that breast cancer stem cells (BCSCs) are probably the major source of breast cancer recurrence [5]. Because of the abilities of exhibiting self-renewal activity and long-term cancer-propagating capacity and developing acquired drug resistance, CSCs can produce more malignant subclones over time [6]. Moreover, the efflux chemotherapy drugs function of CSC are associated with the activation of the critical signaling pathways, such as epidermal growth factor receptor (EGFR), Notch pathway and Wnt/beta-catenin pathway [7–9]. Wnt signaling influences tissue homeostasis, cell renewal, and regeneration in a continuous way [10,11]. As a key nuclear effector of canonical Wnt

Abbreviations: ACBD3, Acyl-CoA binding domain containing 3; ER, estrogen receptor; PR, progesterone receptor; Her2, human epidermal growth factor receptor-2; CSC, cancer stem cell; BCSC, breast cancer stem cell; EGFR, epidermal growth factor receptor; ACBP, acyl-CoA-binding protein; PTEN, phosphatase and tensin homolog; NBEC, Normal human breast epithelial cell; IF, Immunofluorescent; NOD/SCID, nonobese diabetes/severe combined immunodeficiency disease; TCGA, The Cancer Genome Atlas; PKARIA, Protein Kinase-A Regulatory Subunit Iα; TSPO, translocator protein

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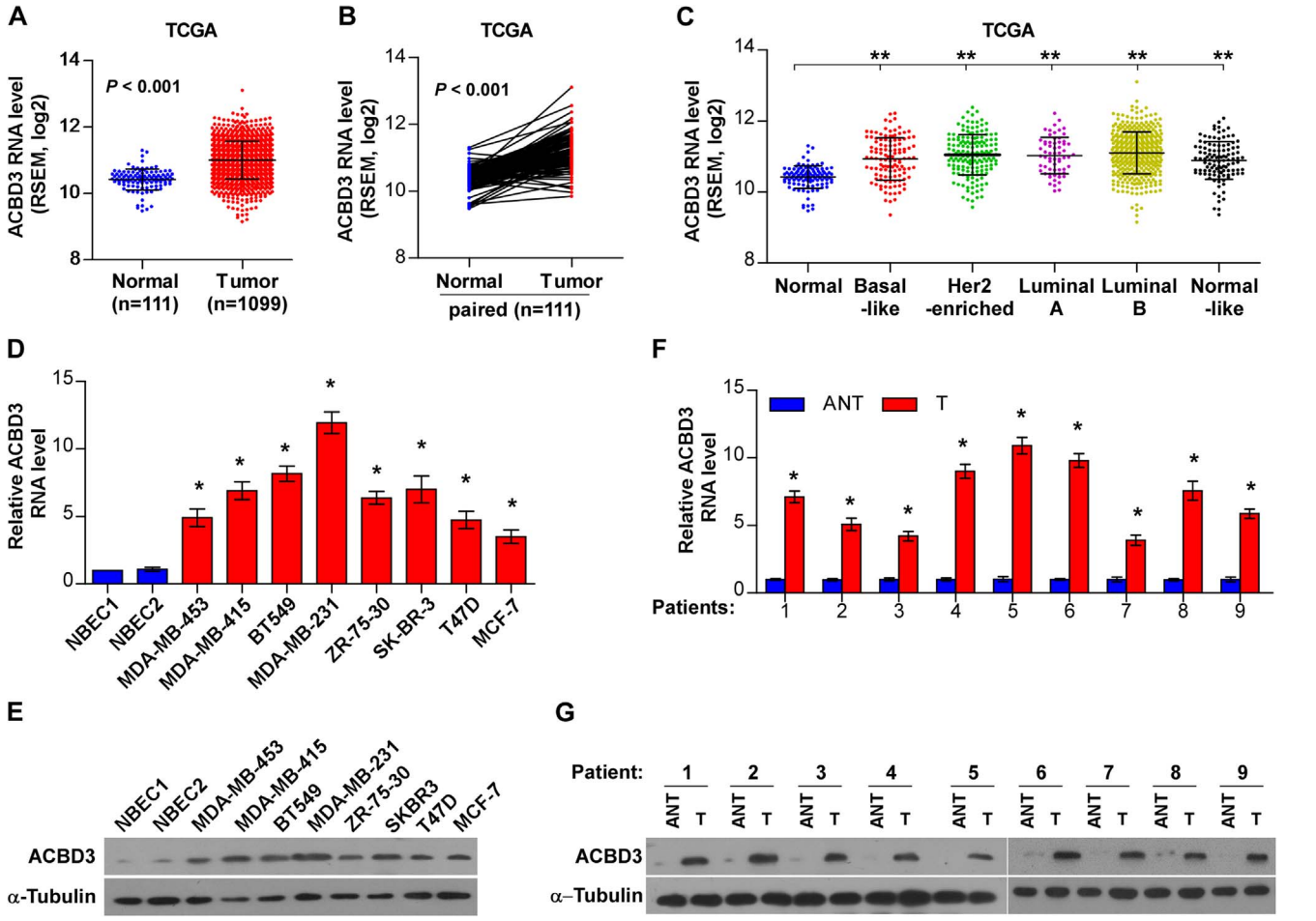

Fig. 1. ACBD3 is up-regulated in breast cancer. (A) Expression of ACBD3 was up-regulated in 1099 breast cancer tissues compared with 111 normal breast tissue samples in the TCGA profile. **(B)** ACBD3 expression was markedly elevated in 111 paired breast cancer tissues compared with patient-matched adjacent normal tissues in the TCGA profile. **(C)** ACBD3 expression was markedly increased among different subtypes of breast cancer. **(D)** Real-time PCR analysis of ACBD3 expression in NBEC1, NBEC2 and breast cancer cell lines. **(E)** Western blotting of ACBD3 expression in NBEC1, NBEC2 and breast cancer cell lines. α-Tubulin was detected as a loading control in the western blotting. **(F)** ACBD3 mRNA expression level in nine paired breast cancer tissues. The average ACBD3 mRNA expression level was normalized to the expression of GDPDH. **(G)** ACBD3 protein expression level in nine paired breast cancer tissues.

signaling, beta-catenin plays a role in triggering transcription of Wnt-specific genes responsible for the control of cell fate decisions in many cells and tissues [12]. Besides, beta-catenin-mediated regulation of c-Myc and p21 may help balance the cell death and proliferation in breast cancer [13]. All these observations point to the need of developing new BCSC-eliminating treatment strategies through which cure rates and survival can be improved

The acyl-CoA-binding protein (ACBP), including ACBD1, ACBD2, ACBD23,ACBD4, ACBD5, ACBD6, and ACBD7, possesses a conserved ACBP domain at the N-terminal end [14]. Functionally, these proteins are released to the cytosol, interacting with other signaling molecules to regulate various cellular processes [15]. By shuttling acyl-CoA between the mitochondria and ER (microsomes), ACBD1 plays a role in protecting the long chain fatty acyl-CoAs from microsomal acyl-CoA hydrolase activity [16,17]. It was reported that ACBD2 has also been linked to hepatocellular carcinoma-associated antigen 64, indicating its role in human hepatocellular carcinoma [18]. ACBD3 was reported to be located in cytoplasm and membrane in eukaryotic cell, especially in the cells, tissues and systems of active metabolism, such as live and kidney [19]. ACBD4 is up-regulated in a panel of cancer cell lines treated with the histone deacetylase inhibitor valproic acid [20]. ACBD5 is up-regulated in phosphatase and tensin homolog (PTEN) positive neural stem cells in vitro, compared with PTEN null neurosphere cultures, indicating a potential function of tumor formation regulation [21]. ACBD6 is expressed in circulating CD34 $^{+}$ progenitors, and embryonic-like stem cells that are derived from placenta. It is assumed that ACBD6 functions in tumorigenesis [22]. ACBD7 is expressed in spleen, thymus and brain [23]. Considering the roles of these proteins, we speculate that ACBPs may be linked to cell differentiation and metabolism. ACBD3 was first observed to be intracellularly localized in the Golgi body, and is released from the Golgi body into the cytosol [24,25]. ACBD3 has not only been reported to be involved in iron homeostasis via its interaction with the divalent metal transporter 1 [26], but also involved in apoptosis through interaction with golgin-160 caspase cleavage fragments [24,27,28]. Moreover, microarray data has mentioned that ACBD3 is involved in cell cycle control [29]. In Gefitinib-non-responders non-small cell cycle control, it is 3.8 fold higher and it also plays a role in cellular asymmetric division in neural progenitor cell-fate specification [30]. As has been reported, ACBD3 plays a role in cellular asymmetric division in neural progenitor cell-fate specification [31]. Evidence showed that standard chemotherapy can be resisted by CSCs which have similar features to normal tissue stem cells. And it is those cancer stem cells that do the work of driving tumor regrowth [32]. Strong evidence supported that CSCs with similar features to normal tissue stem cells are resistant to standard chemotherapy and drive tumor regrowth. These findings indicate that ACBD3 plays an important role in tumorigenesis; however, it remains unclear whether ACBD3 can regulate tumorigenesis. Since asymmetric cell division and self-renewal is also characteristic of CSCs, it would be of interest to make a further investigation about the role of ACBD3 in