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Restoration of Arpin suppresses aggressive phenotype of breast cancer cells

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ABSTRACT

Arpin, a negative regulator of the actin-related protein-2/3 (Arp2/3) complex, is downregulated and predicts poor prognosis in breast cancer patients. However, its biological relevance in breast cancer is still unclear. This study was conducted to investigate the roles of Arpin in breast cancer growth and invasion. We overexpressed Arpin expression in MCF-7 and MDA-MB-231 breast cancer cells and examined the effects of restoration of Arpin on cell proliferation, colony formation, cell cycle distribution, invasion *in vitro* and tumorigenesis *in vivo*. The related molecular mechanism(s) was determined. It was found that ectopic expression of Arpin significantly decreased cell proliferation, colony formation, and tumorigenicity. Flow cytometric analysis showed that overexpression of Arpin significantly increased the percentage of G0/G1-phase cells and decreased the percentage of S-phase cells. Moreover, restoration of Arpin impaired the invasiveness of breast cancer cells, as determined by Transwell invasion assays. Mechanistically, overexpression of Arpin inhibited the phosphorylation of Akt in breast cancer cells. Co-expression of a constitutively active form of Akt blunted the suppression of cell proliferation and invasion by Arpin. Taken together, we provide evidence that Arpin acts as a tumor suppressor in breast cancer, which is associated with inhibition of Akt signaling. Restoration of Arpin may represent a promising therapeutic strategy against breast cancer progression.

1. Introduction

Breast cancer is one of the most frequently diagnosed cancers in females worldwide [1]. Despite advances in therapeutic strategy, the prognosis of breast cancer patients, in particular those with advanced disease is still poor [2]. The phosphoinositide 3-kinase (PI3K)/Akt pathway is aberrantly activated in many types of cancers including breast cancer and represents a potential target for anticancer treatment [3]. The serine-threonine kinase Akt can modulate multiple downstream signaling nodes (e.g. GSK3, FoxO, mTORC1), thus affecting various cellular processes such as cell survival, proliferation, migration, and metabolism [4]. It was reported that activation of Akt signaling contributes to aggressive phenotype of breast cancer cells induced by very low-density lipoprotein (VLDL) and LDL [5]. However, the exact mechanisms governing breast cancer growth and progression are not completely understood.

Arpin is a negative regulator of the actin-related protein-2/3 (Arp2/3) complex, which mediates the assembly of actin filaments and regulates cell migration [6,7]. Arpin contains a homologous acidic motif to nucleation-promoting factors (NPFs) and can compete with NPFs for binding to the Arp2/3 complex [8]. Arpin participates in steering cell migration by interfering with the Arp2/3 complex-mediated signaling [9]. There is evidence for a link between the Arp2/3 complex and Akt activation [10]. Arp2/3-dependent actin branching is involved in Akt plasma membrane translocation and activation [10].

It has been reported that Arpin is underexpressed and correlates with more aggressive parameters in gastric cancer

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[11]. Similarly, Arpin mRNA and protein expression is down-regulated and predicts poor metastasis-free survival in breast cancer patients [12]. These studies encourage us to hypothesize that Arpin may serve as a repressor of breast cancer progression. To test this hypothesis, we restored the expression of Arpin and investigated its functional consequences on breast cancer cell proliferation, invasion, and tumorigenesis.

2. Materials and methods

2.1. Cell culture

Human breast cancer cells MCF-7, MDA-MB-231, Hs578T, and T-47D were purchased from Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China). Non-malignant MCF10A mammary epithelial cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS; Invitrogen, Carlsbad, CA, USA), penicillin (100 U/mL), and streptomycin (100 μg/mL) in a humidified incubator at 37 °C with 5% CO_2.

2.2. Plasmid construction and transfection

Full-length human *Arpin* cDNA (OriGene, Rockville, MD, USA) was amplified by PCR and subcloned into pcDNA3.1(+) vector (Invitrogen). The resulting plasmid (pcDNA3.1/Arpin) was confirmed by DNA sequencing. A plasmid encoding a constitutively active form of Akt [13] was obtained from Addgene (Cambridge, MA, USA).

Transfection of the plasmids was achieved using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Twenty-four hours after transient transfection, cells were tested for cell proliferation, cell cycle progression, and invasion. For generation of stable clones, cells transfected with pcDNA3.1/Arpin or vector were selected for 14 days in the presence of G418 (800 μg/mL; Sigma-Aldrich, St. Louis, MO, USA).

2.3. MTT assay

Cells were plated at 5×10^3 cells/well in 96-well culture plates and cultured for 48 or 72 h. Cell viability was assessed using 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide

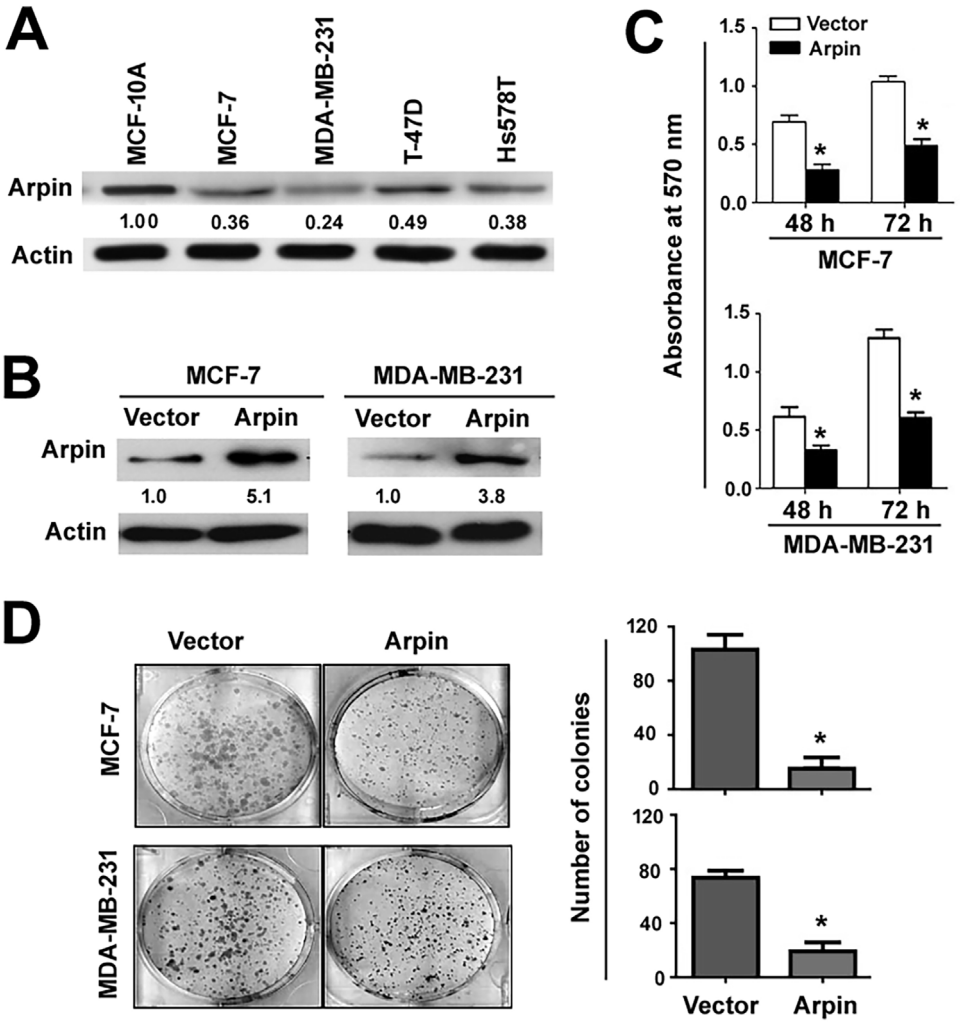

Fig. 1. Arpin impedes breast cancer cell proliferation and colony formation. (A) Western blot analysis of Arpin expression. The levels of Arpin protein were lower in breast cancer cells than MCF-10A non-malignant cells. Numbers below blots represent fold-change relative to the level in MCF-10A cells. (B) Western blot analysis of Arpin protein levels in cells after transfection with vector or Arpin-expressing plasmids. Numbers below blots represent fold-change relative to the level in vector-transfected cells. (C) Measurement of cell proliferation. Cells transfected with vector or Arpin-expressing plasmids were cultured for 48 or 72 h and viability was determined by MTT assays. (D) Colony formation assay. Cells stably transfected with vector or Arpin-expressing plasmids were seeded at a low density and cultured to allow to form colonies. $^{*}P < 0.05$ *vs.* vector-transfected cells.