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Research paper

PRAME is critical for breast cancer growth and metastasis

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ABSTRACT

Breast cancer is the most common cause of cancer death in women and ranks second among cancer deaths. Metastasis is the main cause of death in breast cancer patients. However, the mechanisms underlying the invasion and metastasis of breast cancer cells remain largely elusive. Here we report that the protein PRAME, a tumor-associated antigen isolated from a melanoma, plays a role in preventing the proliferation and metastasis of breast cancer cells. Knocking down of PRAME promotes breast cancer cell proliferation and inhibits apoptosis. In addition, inhibition of PRAME promotes the invasion of breast cancer cells. To further examine the role of PRAME in vivo, we utilized mouse model and found the volume and the weight of tumors was markedly increased after PRAME was knocked down. This study demonstrates that PRAME functions as a tumor suppressor in breast cancer.

1. Introduction

Breast cancer is the most frequently diagnosed cancer in women worldwide with nearly 1.7 million new cases diagnosed in 2012, accounting for 25% of all new cancer cases in women. An estimated 521,900 breast cancer deaths occurred in women in 2012 (Torre et al., 2015). Although once primarily considered a disease of Western women, more than half of new breast cancer cases and deaths occur in economically developing countries. In developed countries, many breast cancers are caught early and prognosis is often very good. By contrast, in economically developing countries, breast cancers are often diagnosed after the disease has progressed and survival is poorer (Ferlay et al., 2015; Torre et al., 2015).

Metastasis is the spread of tumor cells to tissues and the metastatic cascade is composed of three main processes: invasion, intravasation and extravasation. A large number of molecular and cell-biological events are involved in each of these processes (Valastyan and Weinberg, 2011). Epithelial-mesenchymal transition (EMT) has been the favored explanation of distant metastases for epithelial cancers including breast cancer (Kalluri and Weinberg, 2009). Loss of E-cadherin decreases the strength of cellular adhesion and cellular polarity of epithelial cells and promotes the cell migration (Onder et al., 2008). The expression of E-cadherin is under the control of a variety of signaling molecules including transforming growth factor-β (TGF-β) and epidermal growth factor (EGF). The pathologic evidence of EMT in human cancer tissue samples has not yet been well established. It has been commonly believed that EMT may theoretically contribute to breast tumor metastasis (Armstrong et al., 2011; Yu et al., 2013). Similar studies also detected EMT markers such as Twist and Vimentin in circulating tumor cells from early and metastatic breast cancer patients (Kallergi et al., 2011; Papadaki et al., 2014).

The protein PRAME was initially detected as a tumor-associated antigen in cells isolated from a melanoma, and high PRAME expression has been detected in 88–95% of primary melanomas (Ikeda et al., 1997).

PRAME encodes a putative protein of 509 amino acids, and its function is still unknown. PRAME has found to be an absent or low expression in most normal tissues tested, such as CD34 + sorted bone marrow cells or sorted B and T lymphocytes (Abdelmalak et al., 2014; Mitsuhashi et al., 2014). It was reported that PRAME was expressed not only in solid tumors but also in leukemia cells (Hermes et al., 2016). High levels of PRAME are found in malignant cells, such as primary and metastatic melanomas (Haqq et al., 2005), Hodgkin's lymphoma (Ercolak et al., 2015), and neck squamous cell carcinomas (Atanackovic et al., 2006). However, it was reported that PRAME's expression was associated with reduced proliferation of KG-1 leukemic cells (Tajeddine et al., 2005). PRAME promotes in vitro leukemia cell ls death by regulating

Abbreviations: EMT, epithelial-mesenchymal transition; TGF-β, transforming growth factor-β; EGF, epidermal growth factor; PRAME, melanoma antigen preferentially expressed in tumors; E-cadherin, epithelial cadherin.

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S100A4/p53 signaling recently (Xu et al., 2016). Therefore, PRAME functions depending on the different genetic or epigenetic mechanisms. Although some reports have linked PRAME gene expression data to clinical information (Epping et al., 2008), the function and mechanism of PRAME in breast cancer is still elusive.

In this study, we demonstrated that knockdown of PRAME decreased the expression of E-cadherin and promoted the proliferation of breast cancer cells. Subsequently, the migration and invasion of breast cancer cells were enhanced after PRAME inhibition. Knocking down PRAME downregulated E-cadherin, suggesting that it serves as a tumor suppressor of breast cancer.

2. Materials and methods

2.1. Cell culture and RNA interference

MCF-7 and MDA-MB-231 cells were purchased from ATCC. They were cultured in DMEM and MEM in a humidified atmosphere of 95% air and 5% CO2 at 37 °C. The MDA-MB-231 stable cell lines were generated by integration of retroviral shRNA vectors specific for PRAME or a control gene.

The transfection reagent TransExcellent-siRNA was purchased from Cenji Biotech. (Shanghai, China) 2 μg of total RNA was transcribed into cDNA with M-MLV reverse transcriptase (Invitrogen) following the manufacturer's instruction. The cDNA was amplified by PCR using specific primer pairs for PRAME. We used two independent hairpins(1#/2#). The siRNA against PRAME(1#) shown as follows was described previously (Tajeddine et al., 2005). Si-PRAME: 5′-TTCATCACGTGCCTGAGCAAC-3′. The siRNA against PRAME(2#) was 5′-TTGAGAAAGTGAAGCGAAA-3′.

2.2. MTT assay

MCF-7 and MDA-MB-231 cells were seeded in 96-well microplates with 2000 cells for 24 h. Then the cells were treated control or PRAME siRNA for 48 h. MTT (5 g/l) was added to the cells and incubated for 4 h. The absorbance was measured at 570 nm by an ELISA reader.

2.3. Western blot

Total protein from each sample were prepared and separated using 10% SDS-PAGE gel. Then blots were loaded and analyzed using primary antibodies specific for PRAME protein (1:1000), E-Cadherin (1: 1000) and actin (1: 5000), respectively. After incubation with a fluorescent-labelled secondary antibody (1:5000 dilutions), specific signals for proteins were visualized by a LI-COR Odyssey Infrared Imaging System.

2.4. Invasion assay

Cell invasion assay was measured by using the Matrigel-coated transwell culture chambers as described previously (Liu et al., 2011). Briefly, MCF-7 and MDA-MB-231 cells were transfected with control or PRAME siRNAs and plated in the upper chamber of transwell chambers. Cells were incubated for 24 h at 37 °C in a humidified atmosphere. The invasive cells penetrated through the Matrigel in the lower chamber were fixed and photographed using a light microscope for quantification.

2.5. Xenograft animal model

All experiments were performed according to guidelines of animal ethical committee of Changzheng Hospital. The female BALB/c nude mice at the age of 5 weeks were anesthetized and the MDA-MB-231 stable cell lines (shN/shPRAME) were implanted into the dorsal flanking sites of nude mice at 2×10^6 cells in 100 μl per spot. Four weeks after injection, mice bearing tumors were killed for the assessment of tumor size and immunohistological examination.

2.6. Statistical analysis

Data are presented as mean ± s.e.m. of three independent experiments. Significance of means between two groups is determined by student's *t*-test. Difference in cell growth between control and PRAME siRNA-treated groups was evaluated by repeated measures analysis of variance (ANOVA). A P-value of <0.05 was considered significantly different.

3. Results

3.1. Knockdown of PRAME promotes breast cancer cell proliferation and inhibits apoptosis

To investigate the role of PRAME in breast cancer cells, we tested the effect of PRAME knockdown on breast cancer proliferation. As shown in Fig. 1A, 3 days after the PRAME siRNA transfection with two independent hairpins(1#/2#), the PRAME expression was significantly reduced in MCF-7 cells compared with that in control cells along with an increased level of PCNA which was as a proliferation marker. Next, we chosen one PRAME siRNA(1#) for additional experiment. The proliferation was significantly increased by MTT assay (Fig. 1B), suggesting that PRAME inhibits breast cancer cell proliferation. The increased cell proliferation was also observed in MDA-MB-231 cells (Fig. 1C, D). To further examine the effect of PRAME on cell growth, crystal violet staining assay suggested that PRAME negatively regulated cell proliferation in MDA-MB-231 and MCF-7 cells (Fig. 2A, B) Moreover, we analyzed cell apoptosis using flow cytometry. It indicated that knocking down PRAME in MCF-7 cells led to a significant decrease of the percentage of annexin V-positive fractions. Consistent with the MCF-7 cells (Fig. 2C, D), a decreased level of the percentage of annexin V-positive fractions was also detected in MDA-MB-231 cells (Fig. 2E, F).

3.2. Inhibition of PRAME promotes the invasion of breast cancer cells

Previous study displayed that PRAME suppressed tumor growth. Next, we wanted to test whether PRAME deficiency also promotes breast cancer invasion and metastasis. The epithelial cell marker E-cadherin decreased as PRAME expression reduction in MCF-7 and MDA-MB-231 cells (Fig. 3A, C). In addition, cell invasion assay were used and the number of cells penetrated through the Matrigel was dramatically increased in both MCF-7 and MDA-MB-231 cells when transfected with PRAME siRNA (Fig. 3B, D).

3.3. Suppression of PRAME increases tumor initiation

To examine the role of PRAME in metastatic potential of breast cancer cells in vivo, we utilized mouse model by injecting MDA-MB-231 cells (shN/shPRAME) into the dorsal flanking sites of nude mice. As shown in Fig. 4A, the volume and the weight of tumors in shN cells was markedly reduced in comparison with that in shPRAME cells (Fig. 4A, B), suggesting that PRAME functions as an tumor suppressor in MDA-MB-231 cells. As mentioned above, E-Cadherin plays important roles in cell-cell adhesion and reduced expression of E-Cadherin serves as the hallmark of invasive carcinomas (Onder et al., 2008). Our histological examinations revealed that PRAME shared the similar trend with that of E-Cadherin in tumors. After the knockdown of PRAME, the level of E-Cadherin was dramatically decreased (Fig. 4C).

4. Discussion

In the present study, we analyzed the potential role of PRAME in breast cancer using in vitro and in vivo model. We demonstrate that