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Research paper

PRDM14 inhibits 293T cell proliferation by influencing the G1/S phase transition

Yanan Lu, Zhiyi Wan, Xueqing Zhang, Xiaorong Zhong, Lei Rui, Zandong Li *

State key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing 100193, China

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ABSTRACT

PRDM14 (PRDI-BF1 and RIZ domain-containing 14), a transcription factor, plays important roles in primordial germ cell specification and embryonic stem cell pluripotency, and supports the maintenance of self-renewal by promoting the expression of stem cell markers while also repressing the expression of differentiation factors. As a proto-oncogene, the ectopic expression of PRDM14 can enhance breast cell growth and reduce breast cell sensitivity to chemotherapeutic drugs. Conversely, knockdown of PRDM14 expression induces apoptosis in breast cancer cells and restores their sensitivity to chemotherapeutic drugs. Here, we sought to identify the role of PRDM14 in 293T cells. PRDM14-infected 293T cells exhibited an abnormal morphology, and we found that ectopic expression of PRDM14 inhibits colony formation, cell proliferation and metastasis. In addition, our data indicated that PRDM14 influences the G1/S phase transition of 293T cells by inducing the expression of cell cycle regulators. In conclusion, these results showed that PRDM14 inhibits 293T cell proliferation by influencing the G1/S phase transition and impacts cell migration by regulating the level of MMP/TIMP expression, thus mediating extracellular matrix degradation.

1. Introduction

The PRDM (PRDI-BF1 and RIZ homologous domain-containing proteins) family contains novel transcriptional regulators that share a characteristic molecular structure, with C-terminal zinc finger repeats and an N-terminal PR domain, that shares homology with the SET domain (Jenuwein et al., 1998). The member of this family are known to be key regulators of cell differentiation, such as primordial germ cell specification and embryonic stem cell pluripotency (Bikoff and Robertson, 2008; Fog et al., 2012; Kurimoto et al., 2008; Tsuneyoshi et al., 2008), and can act as both tumor suppressors and oncogenes (Fog et al., 2012). However, as novel transcriptional regulators, little is known about the mechanisms by which PRDMs function or their roles in regulating gene transcription.

Abundant reports have shown that PRDMs play important roles in cell differentiation, cell proliferation, cell migration, development, and various types of diseases, including solid cancers and hematological malignancies (Bi et al., 2015; Carofino et al., 2013; Nishikawa et al., 2007; Shapiro-Shelef et al., 2003; Zhang et al., 2013). For instance, the *PRDM1* gene encodes a c-Myc transcriptional repressor and promotes B-lymphocyte maturation, induces growth arrest and exhibits proapoptotic activities (Lin et al., 1997; Turner et al., 1994). *PRDM2*, the first tumor suppressor identified in the PRDM family, is silenced through CpG island promoter DNA methylation in different cancer types, including breast, ovarian, and liver cancers (Du et al., 2001), and *PRDM5* exhibits a similar behavior (Deng and Huang, 2004). *PRDM4* has been mapped to chromosome region 12q23-q24 (Yang and Huang, 1999), and its overexpression inhibits DNA synthesis (Chittka and Chao, 1999).

In addition, overexpression of *PRDM14* has been reported in different cancers (Carofino et al., 2013; Moelans et al., 2010; Nishikawa et al., 2007), and it has been identified as a susceptibility locus for cancer (Ruark et al., 2013). *PRDM14* is located at a tumor suppressor locus on 8q13, a region where gene amplification has frequently been detected in multiple human tumors (Baykara et al., 2015). Liu and Zhang reported that PRDM14 was strongly expressed in non-small cell lung cancer (NSCLC) by immunohistochemistry and western blotting and found a correlation between the PRDM14 expression level and differentiation (Liu et al., 2010; Zhang et al., 2013). The location of *prdm14* on chromosome and the high expression level in multiple cancers indicate that PRDM14 may positively regulate certain cancer diseases. Moreover, methylation-mediated silencing of PRDM14 has been shown to contribute to apoptosis evasion in HPV-positive oral and cervical cancer cell lines (Snellenberg et al., 2014), that is, PRDM14 could promote cell apoptosis.

Abbreviations: PRDM, PRDI-BF1 and RIZ homologous domain-containing proteins; NSCLC, non-small cell lung cancer; PCNA, Proliferating Cell Nuclear Antigen; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

* Corresponding author.
E-mail addresses: nan0914@126.com (Y. Lu), gxmwzy@126.com (Z. Wan), xueqing_0117@126.com (X. Zhang), xrong96@163.cm (X. Zhong), ruilei@cau.edu.cn (L. Rui), lzdws@cau.edu.cn (Z. Li).

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Considering the functional diversity of PRDM14 and the known roles of PRDM14 in regulating multiple cancer cells, we hypothesized that PRDM14 plays a key role in the regulation of cell proliferation and cell cycle. In the present study, we examined the effect of PRDM14 on cell proliferation and cell cycle by forced expression of PRDM14 in the 293T cell line. Our results showed that ectopic expression of PRDM14 inhibited 293T cell proliferation, changed clonal formation ability and cell migration ability. We further found that PRDM14 overexpression influenced the G1/S phase transition of 293T cells through multiple parallel mechanisms including cell cycle proteins and p27 pathways.

2. Materials and methods

2.1. Cell culture and plasmid transfection

The 293T cell line was obtained from the American Type Culture Collection (Manassas, VA, USA) and was cultured in basic DMEM (Gibco, CA, USA) supplemented with 10% fetal bovine serum and incubated under a 5% CO_2 atmosphere at 37 °C. Cells (2×10^5/well) were seeded in 6-well plates and transfected with 2 μg of phPRDM14-EGFP or pEGFP-N1 empty vector using Lipofectamine™ 2000. The morphological changes were analyzed by optical microscopy 24 hour post-transfection.

2.2. Cell proliferation assay

Cells (5×10^4/well) expressing hPRDM14-EGFP or EGFP-N1 were seeded in 24-well plates for growth analyses. Cell proliferation was evaluated by counting the number of cells every day. The cells were trypsinized and counted at the indicated time points, and all of the experiments were performed in triplicate.

2.3. Assessment of cell viability using the MTT assay

Twenty-four hours after transfection, cells were harvested and seeded in 96-well plates (6×10^3/well). Each condition was tested in quadruplicate. After 24, 48, and 72 h, the culture medium was supplemented with 1 mg/mL MTT for 4 h at 37 °C. The medium was then removed, and the cells were solubilized with DMSO. The absorbance was then measured at 490 nm using a microplate reader.

2.4. Colony formation assay

Twenty-four hours after transfection, cells were harvested and plated in 60-mm culture dishes (5×10^3/dish). The medium was changed every 3 days for 10 to 14 days until visible colonies formed. The colonies were washed with PBS, fixed with methyl alcohol for 10 min, stained with Giemsa, and manually counted. Only the colonies containing 50 cells were counted. The number of colony forming units was expressed as a percentage of the controls. Each result was the average of at least 3 independent experiments.

2.5. Cell cycle analysis by flow cytometry

At 24 h after transfection, the attached and detached cells were harvested and washed once in PBS. Cells were then fixed in ice-cold 70% alcohol and stored overnight at 4 °C. After washing twice with cold PBS, the cells were collected, resuspended in PBS containing 250 μg/mL RNase A, and incubated at 37 °C for 30 min. The cells were then stained with propidium iodide for 30 min, and cell cycle analysis was performed using flow cytometry with a FACScan instrument.

2.6. Quantitative RT-PCR

Total RNA was extracted with TRIzol. Then, 1 μg of RNA was reversed transcribed into cDNAs utilizing the GoScript reverse transcription system in a 20-μL reaction system. cDNAs were subjected to qRT-PCR with SYBR Green Master I. The primers specific for human cyclin B1, cyclin D1, cyclin E1, CDK4, CDK6, P27 and PCNA (Proliferating Cell Nuclear Antigen) are shown in Table 1. qRT-PCR was conducted using the following cycling conditions: 95 °C for 10 min for predenaturation, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The last step was conducted at 95 °C for 15 s, 60 °C for 15 s and 95 °C for 15 s, which is necessary to acquire a melting curve for the PCR products to confirm the specificity of amplification. The relative mRNA abundances were analyzed utilizing the $2^{-\Delta\Delta Ct}$ method, with GAPDH as a reference, and plotted as fold changes compared with the control samples.

2.7. Western blot assay

The cells were lysed, and equal amounts of the sample proteins were separated by SDS-PAGE and then transferred to a polyvinylidene difluoride membrane. The following primary antibodies (1:500, 1:200 and 1:4000 dilution) were incubated with the membrane: anti-PCNA, anti-P27 and anti-tubulin antibodies. Secondary antibodies conjugated to HRP were added and incubated for color development.

2.8. Scratch wound healing assay

293T cells were grown in basic DMEM supplemented with 10% fetal bovine serum. Cells overexpressing hPRDM14-EGFP or EGFP-N1 were seeded in 24-well plates at a density of 1×10^5 cells/mL. After 24 h, the monolayer was scratched with a fresh 1-mL pipette tip across the center of the well. The extent of cell migration was photographed after an additional 24 h and measured using image analysis software. Each experiment was performed in triplicate.

2.9. Statistical analysis

All data were analyzed with the *SPSS 16.0* software. GraphPad Prism 5.0 was used to create figures. p-Values ≤0.05 were considered significant.

3. Results

3.1. hPRDM14-EGFP is located in the cell nucleus

293T cells were transfected with the hPRDM14-EGFP plasmid or the EGFP-N1 control vector using Lipofectamine™ 2000. Fluorescence microscopy analysis indicated that the hPRDM14-EGFP protein was located in the cell nucleus, which is consistent with the fact that PRDM14 has a PR domain and plays an important role in gene regulation. However, pEGFP-N1 was distributed throughout the cell, including the cytoplasm and nucleus (Fig. 1A).

Table 1
Primer sequences used for qRT-PCR.

Gene	Primer sequence (5′ → 3′)	Fragment size
Cyclin B1	F: GGAGAGGTTGATGTCGAGCAA R: CTGCAATTTGAGAAGGAGGAAAG	120 bp
Cyclin D1	F: CCCTGACGGCCGAGAAG R: AGGTTCCACTTGAGCTTGTTCAC	122 bp
Cyclin E1	F: TGCTTCGGCCTTGTATCATTT R: TGGAACCATCCACTTGACACA	119 bp
CDK4	F: GGCGACTGGAGGCTTTTGA R: TCAAACACCAGGGTTACCTTGA	120 bp
CDK6	F: AGGAAAAATCTTGGACGTGATTG R: TGGTTGGGCAGATTTTGAATG	120 bp
P27	F: GACTTGGAGAAGCACTGCAGAGA R: GCCACTCGTACTTGCCCTCTA	120 bp
PCNA	F: ACTAAAATGCGCCGGCAAT R: AACTTTCTCCTGGTTTGGTGCTT	120 bp

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