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Growth factor progranulin contributes to cervical cancer cell proliferation and transformation *in vivo* and *in vitro*

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HIGHLIGHTS

- Progranulin (PGRN) is overexpressed in cervical cancer cells and tissue.
- PGRN contributes to cervical cancer tumorigenesis *in vitro* and *in vivo*.
- Akt and extracellular signal-regulated kinase (Erk) signaling mediates PGRN-stimulated cell proliferation and transformation.

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ABSTRACT

Objective. The growth factor progranulin (PGRN) is overexpressed in a number of tumors. We aimed to investigate the expression and role of PGRN in cervical cancer tumorigenesis.

Methods. PGRN expression and secretion was assessed in cells and normal and cancerous cervical tissues by Western blot analysis, ELISA or immunohistochemistry. The role of PGRN in cervical carcinogenesis was explored by cell-proliferation, colony-formation and tumor-growth assays. We assessed the role of PGRN-mediated signaling in the cervical cell with specific inhibitors.

Results. PGRN expression was upregulated in cervical cancer cell lines and tissue. PGRN promoted the transformation of human cervical mucosa epithelial H8 cells *in vitro* and tumor formation *in vivo*. Knockdown of PGRN expression in cervical cancer cells *in vivo* decreased cell proliferation and slowed tumor growth. PGRN stimulated cervical cell proliferation, and transformation was mediated, at least in part, by Akt and Erk signaling.

Conclusions. PGRN is overexpressed in cervical cancer and promotes the malignant growth and transformation of cervical cells. Therefore, PGRN plays a critical role in carcinogenesis of cervical cancer and shows promise for therapeutic strategies for cervical cancer.

Introduction

Cervical cancer is the third most common neoplastic disease in women worldwide, responsible for an estimated incidence of 529,000 cases and 274,000 deaths in 2008 [1]. Human papillomavirus (HPV) infection is the most important cause of cervical cancer [2,3] and more than 99% of cervical carcinomas are positive for human HPV DNA [4]. However, cancer develops in only a small fraction of women infected by HPV, so other factors contribute to the progression to cervical cancer [5,6].

Growth factors and their receptor-mediated signaling cascades are the major regulators of tumor progression after tumor initiation [7]. Previous studies have shown increased expression of growth factors such as epidermal growth factor [8,9], insulin-like growth factor [10,11], and vascular endothelial growth factor [12,13] in cervical cancer. These growth factors in the extracellular space are involved in the development and progression of cervical cancer by binding to cell membrane receptors and stimulating a cascade of molecular events, which results in cell proliferation.

Progranulin (PGRN), also referred to as granulin–epithelin precursor, proepithelin, PC-cell-derived growth factor, or acrogranin, is a 593 amino-acid autocrine growth factor abundantly expressed in

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rapidly cycling epithelial cells, cells of the immune system, neurons, and chondrocytes [14]. PGRN plays a critical role in various physiologic and disease processes, including early embryogenesis, wound healing, inflammation, and host defense [14]. PGRN also functions as a neurotrophic factor [15], and mutations in *PGRN* resulting in partial loss of the PGRN protein cause frontotemporal dementia [16,17]. High levels of PGRN have been reported in human cancers, and PGRN is strongly believed to contribute to tumorigenesis [18,19]. However, the involvement of PGRN in the etiology and progression of cervical cancer is unknown.

In the present study, we investigated the role of PGRN in cell transformation and malignant growth in cervical cancer cells and clinical samples to provide the first evidence linking PGRN to cervical cancer.

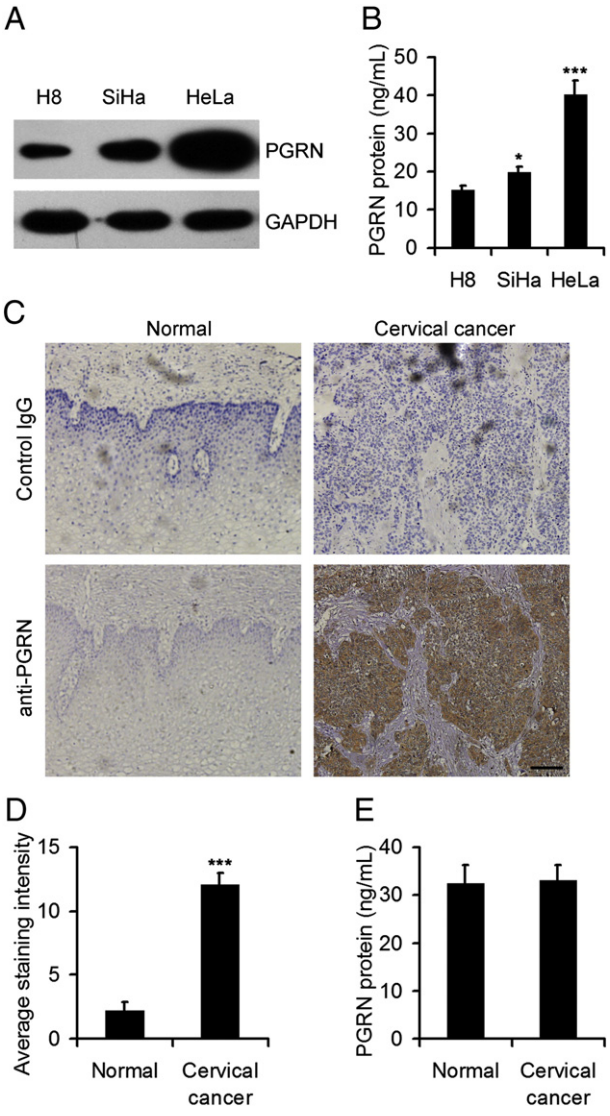

Fig. 1. Expression of PGRN is increased in cervical cancer. (A) Western blot assay of PGRN protein level in H8, SiHa and HeLa cells. GAPDH was a loading control. (B) ELISA of PGRN levels in the supernatant of cervical cells. Data are mean ± SD from 3 independent experiments. *, $P < 0.05$; ***, $P < 0.001$ compared with H8 cells. (C) Immunohistochemistry of PGRN in normal and cervical cancer tissues (scale bar, 100 μm) and (D) quantification. Data are mean ± SEM. ***, $P < 0.001$ compared with normal tissues. (E) PGRN levels in plasma from healthy volunteers and cervical cancer patients. Data are mean ± SEM.

Materials and methods

Patients and tissue samples

We obtained 58 paraffin-embedded tissue blocks (38 cervical cancer and 20 normal cervical tissues) and plasma samples from patients with cervical cancer (n = 21) and age-matched controls (n = 21) from Qilu Hospital for immunohistochemistry staining and ELISA respectively. We obtained patient consent for the use of these materials, and the study was approved by the Institutional Research Ethics Committee. Diagnoses followed the World Health Organization Classification of Tumors.

Cell lines

All cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% fetal calf serum (Invitrogen), 100 IU/mL penicillin, 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA), and 2 mmol/l L-glutamine. Cervical cancer cell lines SiHa, HeLa and murine TC-1 constitutively expressing HPV16 E6 and E7 were purchased from the American Type Culture Collection (Manassas, VA). The HPV16 immortalized human cervical mucosa epithelial H8 cell line was obtained from the Department of Biophysics, Institute of Preclinical Medicine, Peking Union Medical University.

Cell transfection

pcDNA3.1-PGRN and pSUPER-PGRN plasmids were a gift from Dr. CJ Liu (New York University School of Medicine, New York, USA). H8 cells were transfected with pcDNA3.1 or pcDNA3.1-PGRN and SiHa and HeLa cells were transfected with pSUPER (siRNA) or pSUPER-PGRN (siPGRN) by the Lipofectamine 2000 reagent method (Invitrogen) and were harvested and used for further experiments 48 h after transfection.

Immunohistochemical staining

Immunoreactivity of PGRN in cervical tissue was assessed by immunohistochemistry as described [20] with control IgG and human PGRN specific antibody (1:100, Abcam, UK). Semi-quantitative analysis was performed as described [21].

ELISA of PGRN secretion

H8, SiHa and HeLa cells were seeded onto 6-well culture plates at 4×10^5 cells/well; conditioned medium was collected 24 h after incubation. The amount of PGRN in plasma samples and conditioned medium was assayed by the use of an ELISA kit (R&D Systems, Minneapolis, MN, USA).

Western blot analysis

Total protein was extracted from cells by the use of NP-40 lysis buffer (Beyotime, China). Western blot assay was performed as described [22] with the primary antibody for PGRN (1:1000, Abcam) and GAPDH antibody as a control (1:2000, Hangzhou Goodhere Biotech, China). For signaling pathway analysis, H8 cells were stimulated with 500 ng/mL recombinant human PGRN (rhPGRN, generated and purified as reported previously [23]) for the indicated times after serum-free treatment for 18 h. H8, SiHa and HeLa cells transfected with indicated plasmids were incubated for 48 h after transfection. Then total protein was extracted from cells by the use of NP-40 lysis buffer (Beyotime, China). Western blot assay was performed as described [22] with primary antibodies for phospho-Akt (Ser473, 1:2000), total Akt (1:1000), phospho-extracellular signal-regulated kinase (phospho-Erk1/2; Thr202/Tyr204, 1:2000) and total-Erk1/2 (1:1000), phospho-forkhead box protein O1 (phospho-FoxO1; Ser256, 1:1000) and total-FoxO1 (1:1000, all Cell Signaling Technology).