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IGF-1 Mediates PTEN Suppression and Enhances Cell Invasion and Proliferation via Activation of the IGF-1/PI3K/Akt Signaling Pathway in Pancreatic Cancer Cells

Jiachi Ma, M.D., Hirozumi Sawai, M.D., Ph.D.,[1] Yoichi Matsuo, M.D., Ph.D., Nobuo Ochi, M.D., Ph.D., Akira Yasuda, M.D., Ph.D., Hiroki Takahashi, M.D., Ph.D., Takehiro Wakasugi, M.D., Ph.D., Hitoshi Funahashi, M.D., Ph.D., Mikinori Sato, M.D., Ph.D., and Hiromitsu Takeyama, M.D., Ph.D.

Department of Gastroenterological Surgery, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

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Background. **Type-1 insulin-like growth factor (IGF-1) up-regulates cell proliferation and invasiveness through activation of PI3K/Akt signaling pathway. IGF-1 also down-regulates the tumor suppressor chromosome 10 (PTEN). We investigated the mechanism by which IGF-1 affects cell proliferation and invasion by suppression of PTEN phosphorylation and interaction with PI3K/PTEN/Akt/NF-κB signaling pathway in pancreatic cancer.**

Materials and Methods. **The expression of IGF-1 receptor (IGF-1R) and PTEN in five pancreatic cancer cell lines was determined by RT-PCR and Western blot. Proliferation and invasion were investigated by WST-1 assay and Matrigel-double chamber assay. Pancreatic cancer cells were transfected with PTEN siRNA to investigate which signaling pathway correlates in regulation of cancer cell proliferation and invasion.**

Results. **Five pancreatic cancer cell lines expressed PTEN and IGF-1R in mRNA and protein levels. Suppression of PTEN phosphorylation strongly enhanced cell proliferation and invasion stimulated with IGF-1 via activation of PI3K/Akt/NF-κB signaling pathway. In addition, knockdown of PTEN by siRNA transfection also enhanced activation of PI3K/Akt/NF-κB pathway, subsequently up-regulating cell invasiveness and proliferation.**

Conclusions. **The IGF-1/PI3K/PTEN/Akt/NF-κB cascade may be a key pathway stimulating metastasis of pancreatic cancer cells. We suggest that interfering with the functions of IGF-1/PI3K/Akt/NF-κB might be a novel therapeutic approach to inhibit aggressive spread of pancreatic cancer.**

Key Words: **Akt; IGF-1; pancreatic cancer; PTEN.**

INTRODUCTION

Pancreatic cancer is one of the most malignant carcinomas, and prognosis is very poor. More than 37,600 pancreatic cancer patients are expected in the United States in 2008, and more than 250,000 persons succumb to it each year worldwide. It is still the fourth male and female leading cause of cancer-related death in the Western world [1, 2]. The 5-y survival rate of patients with pancreatic cancer receiving surgery and chemotherapy ranges from 1% to 2%.[3]. One of the reasons for this low survival rate is the insensitivity of pancreatic cancer to most antioncologic therapies such as chemotherapy, radiotherapy, and immunotherapy [4]. These data testify to the pressing need for improvements in the treatment of pancreatic cancer, such as genetic therapy.

Phosphatase with tensin homology, which is located on chromosome 10 (PTEN) is a potent tumor suppressor gene frequently mutated and deleted in human cancer. Mutations of PTEN are involved in a variety of human cancers, including brain, endometrium, prostate, and kidney. It dephosphorylates the lipid second messenger, phosphoinositol 3,4,5-trisphosphate [PI(3,4,5)P_3], a product of phosphatidylinositol 3' kinase (PI3K) activity, and negatively regulates the PI3K signaling

[1] To whom correspondence and reprint requests should be addressed at Department of Gastroenterological Surgery, Nagoya City University Graduate School of Medical Science, Kawasumi 1, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan. E-mail: sawai@med.nagoya-cu.ac.jp.

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pathway. The PI3K/Akt pathway is important for cell proliferation and survival, most notably in cells that are responding to growth factor receptor engagement survival signals from growth factors, cytokines, and hormones activate PI3K [5]. Subsequently, PI3K activates Akt/PKB that interferes with apoptotic machinery [6]. Activated Akt/PKB mediates cell survival via the regulation of numerous apoptotic relevant proteins such as the transcription factor NF-κB [7]. Prosurvival signaling by Akt is counteracted by PTEN, which antagonizes the actions of PI3K. PI3K and Akt are overexpressed in a variety of cancers [8, 9]. In addition, PTEN is frequently deleted in advanced tumors [10–12]. These alterations lead to a constitutively active prosurvival signaling pathway that enhances the insensitivity of tumor cells to apoptosis induction. By opposing the effects of PI3K activation, PTEN functions as a tumor suppressor. For this reason, the PI3K-PTEN signaling network functions as a crucial regulator of cell survival decisions. By reducing the levels of PI3P, PTEN inhibits the recruitment of Akt to the plasma membrane and prevents its activation. PTEN function is regulated by post-translational modification, cellular localization, and redox modulation at the active-site cysteine [13–15]. When PTEN is deleted, mutated, or otherwise inactivated, activation of Akt can occur in the absence of exogenous stimulus, and tumorigenesis can be initiated. Numerous types of tumors, both sporadic and those that arise as a component of a cancer predisposition syndrome, show alterations in PTEN [16].

Type-1 insulin-like growth factor (IGF-1) is now established as a survival or proliferation factor in many *in vitro* systems. IGF-1 provides trophic support for multiple types of organ cultures explanted from various species, and delays the onset of apoptosis through the mitochondrial (intrinsic pathway) or by antagonizing activation of cytotoxic cytokine signaling (extrinsic pathway). In some instances, IGF-1 protects against other forms of death such as necrosis or autophagy. The effect of IGF-1 on cell survival appears to be context specific, being determined both by the organ (tissue specific) and the type of cellular stress. In many human cancers, there is a strong association with dysregulated IGF signaling, and this association has been extensively reviewed recently [17–21]. IGF-1 signaling modulates malignant cellular behavior and plays a central role in cellular invasiveness in a variety of human malignances [22–26]. Both IGF-1 and its tyrosine kinase receptor (IGF-1R) are overexpressed in human pancreatic tumors and IGF-1R signaling regulates proliferation, invasion, and angiogenic growth factor expression by pancreatic carcinoma cells [27].

In this study, we hypothesized (1) that IGF-1 induced suppression of PTEN phosphorylation and (2) that invasiveness of human pancreatic cancer cells is enhanced via the IGF-1/IGF-1R/PI3K/PTEN/Akt/NF-κB signaling pathway. We show here that the mechanism involves inactivation of the PTEN tumor suppressor. Dephosphorylation of PTEN by IGF-1 decreases its activity, elevates PI3P levels and increases signaling through Akt and its downstream targets. Furthermore, we inhibited PTEN function with PTEN siRNA and investigated the impact on cellular proliferation and invasion in pancreatic cancer cells. Thus, we demonstrate that IGF-1 not only enhanced proliferation and invasion but also suppressed PTEN activity in five pancreatic cancer cell lines.

MATERIALS AND METHODS

Cell Culture

BxPC-3, SW1990, AsPC-1, MIA PaCa-2, and PANC-1 cell lines were obtained from the American Type Culture Collection (Rockville, MD). The BxPC-3 and AsPC-1 lines were maintained in RPMI-1640 (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (FBS). SW1990, MIA PaCa-2, and PANC-1 were maintained in Dulbecco modified Eagle medium (Sigma Chemical Co.) supplemented with high glucose and 10% FBS. All cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Reagents and Antibodies

Recombinant human insulin-like growth factor I and anti-human IGF-I antibody were provided by R and D system Inc. (Minneapolis, MN). LY294002 (PI3-kinase inhibitor) was ordered from Cell Signaling Technology (Beverly, MA). Akt inhibitor was purchased from BioVision (Mountain View, CA). The monoclonal antibodies (mAb) included PTEN antibody, phospho-PTEN (ser380) antibody, IGF-1 receptor-β (111A9) rabbit mAb, Akt antibody, phospho-Akt (ser473), PI3-kinase p85 antibody, phospho-PI3-kinase p85 (Tyr 458)/p55 (Tyr199) antibody, NF-κ B p65 antibody, and phospho-NF-κ B p65 (Ser536) antibody were purchased from Cell Signaling Technology (Mountain View, CA).

RT-PCR Analysis

Total RNA was extracted from human pancreatic cancer cell lines using an Isogen Kit (Nippon Gene, Tokyo, Japan), and quantities determined spectrophotometrically. Total RNA aliquots (5 μg) were pretreated with random hexamers and dNTP mix, which were incubated at 65°C for 5 min, chilled on ice, and then reverse-transcribed into cDNA in a cDNA synthesis mix containing 10 × RT buffer, 25 mM $MgCl_2$, 0.1 mM DTT, RNaseOUT, and 200 U SuperScript III RT (Invitrogen, San Diego, CA) at 50°C for 50 min. The reaction was terminated at 85°C for 5 min. One μL reaction mixture aliquots were used as templates for PCR. The pairs of forward and reverse primer sets were designed using Primer 3 software. Primer sequences and PCR conditions were described in Table 1. Amplification reactions were performed using a DNA thermal cycler (Model TP300; Takara PCR Thermal Cycle MP). Amplified DNA fragments were resolved by electrophoresis on 1.5% agarose gels containing ethidium bromide.

Real-Time Quantitative RT-PCR

PCR was performed using a LightCycle apparatus. Freshly isolated RNA was converted to cDNA using the PrimeScrip TR Regent kit (Takara Bio, Shiga, Japan), and the PCR reaction was performed using a TaqMan Gene Expression Assay Kit (Applied Biosystems,