Human Pathology (2015) **46**, 884–893

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Human PATHOLOGY

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Original contribution

Pancreatic adenocarcinoma up-regulated factor expression is associated with disease-specific survival in cervical cancer patients☆,☆☆

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Received 7 January 2015; revised 23 February 2015; accepted 26 February 2015

Keywords:
PAUF;
Adenocarcinoma;
Prognosis;
Uterine cervical neoplasms;
Survival

Summary Pancreatic adenocarcinoma up-regulated factor (PAUF) is a novel soluble protein involved in tumor development and metastases. This study was to investigate the PAUF expression and its prognostic value in cervical cancer patients. The expression of PAUF was immunohistochemically determined in 345 formalin-fixed, paraffin-embedded cervical cancer tissues and 107 normal cervical epitheliums. Subsequently, its associations with clinicopathological characteristics and patient survival were assessed. PAUF protein was expressed both in cytoplasm and nucleus, and cytoplasmic expression was more frequent in cancers than normal tissues (32% versus 17%, $P = .002$), and the difference was prominent in glandular cells. Notably, the expression was more frequent in adenocarcinoma than in squamous cell carcinoma (57% versus 25%, respectively; $P < .001$), and the differential expression was also seen at the messenger RNA level ($P = .014$). Cox regression analysis showed that the cytoplasmic expression of PAUF protein was independently associated with poor disease-free (hazard ratio = 2.3; 95% confidence interval, 1.2-4.3; $P = .008$) and overall survival (hazard ratio = 2.9; 95% confidence interval, 1.2-7.5; $P = .020$). Detection of PAUF expression may aid current evaluation of prognosis in cervical adenocarcinoma.
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☆ Competing interests: There is no conflict of interest.

☆☆ This study was supported in part by a grant from the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Republic of Korea (2013R1A1A2013629) and Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research.

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http://dx.doi.org/10.1016/j.humpath.2015.02.016
0046-8177/Published by Elsevier Inc.

1. Introduction

Cervical cancer is the third most common type of cancer among women worldwide and is the most prevalent and lethal female malignancy in many developing countries [1,2]. Screening for premalignant stages and preventive vaccination are good options to prevent cervical carcinogenesis, but once invasive cancer develops, recurrence remains a major problem despite the improvement of treatment. Although clinical factors such as Federation of Gynecology and Obstetrics (FIGO) stage, lymph node metastasis, and tumor size may serve as markers for prognosis, they have limited utility in accurately predicting survival. In this context, new markers including molecular markers are needed to predict more accurately the prognosis of an individual patient.

Recently, we performed genetic profiling to predict recurrence of early cervical cancer, using the complementary DNA (cDNA)–mediated annealing, selection, extension, and ligation (DASL) assay [3]. We developed a gene set model using 12 selected genes and suggested that the genetic quantitative approach could be better in predicting recurrence than clinical prognostic model. The 12 genes selected were *DNM2*, *RERGL*, *CLUAP1*, *HTATSF1*, *PAPLN*, *APLP1*, *ZNF585B*, *OXCT1*, *SBF2*, *ABCB7*, *PAFAH1B2*, and *ARHGAP6*. Pancreatic adenocarcinoma up-regulated factor (PAUF; LOC124220) was one of the differentially expressed genes.

PAUF, the protein by *ZG16B*, was recently described to be highly expressed in pancreatic cancer tissues [4,5]. Several in vitro studies showed that it may have a role in cancer progression and metastasis through the activation of intracellular signaling molecules such as extracellular signal–regulated kinase, c-Jun N-terminal kinase, and AKT [4]; up-regulating CXCR4 expression [5]; and activation of focal adhesion kinase [6]. It is also known to have function in promoting angiogenesis and vascular permeability [7]. Data showing PAUF increases proliferation by up-regulating β-catenin, at least in pancreatic cancer, are important because this may be an avenue for pharmaceutical inhibition [8-10]. The aim of this study was to examine the clinical correlation and prognostic significance of PAUF protein expression by immunohistochemistry (IHC) in a large cohort.

2. Materials and methods

2.1. Patients and tumor samples

Between 2002 and 2009, a total of 440 patients with early stage cervical cancer underwent type I to III radical hysterectomy with or without pelvic/para-aortic lymph node dissection in the Department of Gynecologic Oncology, Samsung Medical Center, Sungkyunkwan University School of Medicine. Of them, patients with rare histology such as sarcoma, malignant melanoma, and neuroendocrine carcinoma and patients with limited availability of tissue block specimens were not included in the tissue microarray (TMA) construction. Therefore, we studied 345 cases of cervical cancer tissues, and as a control, 107 normal cervical epitheliums were obtained from the patients with no history of cervical cancer. Tissue samples were collected from patients who had signed informed consent form, which was approved by Institutional Review Board at Samsung Medical Center, Seoul, Korea. This study was additionally approved by the Office of Human Subjects Research at the National Institutes of Health.

All the patients were treated primarily with radical surgery, and they received adjuvant radiotherapy with or without concurrent chemotherapy if they had the following risk factors: positive pelvic lymph node, microscopic parametrial invasion, positive resection margins with tumor, stromal invasion of more than half of the cervix, lymphovascular space invasion, or a tumor larger than 4-cm diameter. Patients had follow-up examinations every 3 months for the first 2 years, every 6 months for the next 3 years, and every year thereafter. Disease-free survival (DFS) was assessed from the date of surgery to the date of recurrence or the date of the last follow-up visit. Overall survival (OS) was measured from the date of surgery to the time of death, or for the living patients, to the date of last contact. Data for patients who had not had an event were censored as of the date of the final observation.

2.2. TMA construction and IHC

TMAs were constructed from tissue blocks used for routine pathologic evaluation. Three 1.0-mm diameter tissues cores were retrieved from formalin-fixed, paraffin-embedded tissue blocks and arrayed on a 38 × 25 mm recipient paraffin block using a manual tissue arrayer MTA-1 (Beecher Instruments, Silver Spring, MD). Sections were cut at 4 μm with a microtome and placed on charged glass slides. The presence of tumor tissues on the sections was verified by hematoxylin and eosin staining.

TMA sections were cut at 4-μm thickness followed by deparaffinization through xylene and dehydration with graded ethanols. Heat-induced antigen retrieval was performed for 20 minutes in an antigen retrieval buffer of pH 9.0 (Dako, Carpinteria, CA) using a steam pressure cooker (Pascal; Dako). The endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 minutes. The sections were incubated with anti-PAUF mouse monoclonal antibody (clone no. 817310; R&D systems, Minneapolis, MN) at 1:100 for 120 minutes. The antigen-antibody reaction was detected with Dako EnVision+ Dual Link System-HRP (Dako) and DAB+ (3,3′-diaminobenzidine; Dako). Negative controls were processed by omitting the primary antibody, and TMAs included pancreatic adenocarcinoma-positive control tissues. Tissue sections were lightly counterstained with hematoxylin and then examined by light microscopy.