Human Pathology (2014) **45**, 817–825

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Presence of primary cilia in cancer cells correlates with prognosis of pancreatic ductal adenocarcinoma☆,☆☆

Katsura Emoto MD[a], Yohei Masugi MD, PhD[a], Ken Yamazaki PhD[a], Kathryn Effendi MD, PhD[a], Hanako Tsujikawa MD[a], Minoru Tanabe MD, PhD[b], Yuko Kitagawa MD, PhD[b], Michiie Sakamoto MD, PhD[a,*]

[a]*Department of Pathology, School of Medicine, Keio University, Tokyo 160-8582, Japan*
[b]*Department of Surgery, School of Medicine, Keio University, Tokyo 160-8582, Japan*

Received 5 September 2013; revised 18 November 2013; accepted 27 November 2013

Keywords:
Primary cilia;
Pancreatic cancer;
Survival factor;
Adenocarcinoma;
Prognosis

Summary Primary cilia are microtubule-based organelles that protrude from basal bodies and are involved in cell differentiation, sensory functions, and planar cell polarity. Although there are many studies examining the roles of primary cilia in the fields of embryology and physiology, few such studies have been carried out in the field of oncology, and the role of primary cilia in cancer cells is poorly understood. In this study, we identified primary cilia by immunofluorescence analysis in which primary cilia were visualized as green rods labeled with anti-acetylated α-tubulin adjacent to basal bodies detected as red dots labeled with anti–γ-tubulin. Primary cilia were found in human pancreatic cancer cell lines and in cancer cells in 25 of 100 pancreatic ductal carcinoma patients. In the clinical samples, most primary cilia in cancer tissue were observed in areas showing well-differentiated glandular structures. Patients whose cancers were primary cilia positive had a higher frequency of lymph node metastasis than those whose cancers were primary cilia negative ($P = .016$). Univariate analysis demonstrated that tumor size ($P = .009$), tumor grade ($P = .001$), lymph node metastasis ($P = .008$), and the presence of primary cilia ($P = .002$) correlated with overall survival. Multivariate analysis found that tumor grade ($P < .001$) and the presence of primary cilia ($P = .001$) were independent prognostic indicators. In conclusion, we showed that pancreatic cancer cells can form primary cilia and that the presence of primary cilia is significantly associated with the prognosis of pancreatic ductal adenocarcinoma.

1. Introduction

Cilia are cellular organelles and can be classified as primary cilia or motile cilia [1-4]. Primary cilia can be observed as nonmotile projections in most types of quiescent vertebrate cells. Primary cilia are microtubule-based organelles that protrude from basal bodies (centrioles) and consist of 9 peripheral microtubule doublets, known as the "9 + 0" structure, whereas motile cilia have 9 peripheral microtubule doublets with a central pair of microtubules, known as the

☆ This work was supported by a grant-in-aid for the Third Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan and JSPS KAKENHI grant number 24790362.

☆☆ Disclosures: There are no relevant financial or other disclosures, and we declare no conflicts of interest.

* Corresponding author. Department of Pathology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo, Japan 160-8582.
E-mail address: msakamot@z5.keio.jp (M. Sakamoto).

0046-8177/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2013.11.017

"9 + 2" structure. Usually, only 1 primary cilium is observed per cell, and it disappears during the mitotic phase. Current knowledge indicates that primary cilia are involved in cell differentiation, sensory functions, planar cell polarity, and signal transduction pathways including Sonic hedgehog (SHH), Wnt, and platelet-derived growth factor pathways [1-3,5,6]. Studies using mouse models showed that the presence or absence of primary cilia is important for tumorigenesis mediated by the SHH signal in basal cell carcinoma of the skin and medulloblastoma of the brain [7,8]. However, few studies have been carried out on the importance of primary cilia in tumor biology; in vivo and human studies in this field are particularly rare [1,9]. As a result, the potential for clinical applications based on the role of primary cilia in cancer remains unexplored.

Pancreatic cancer is one of the leading causes of cancer-related mortality worldwide [10]. In the United States, pancreatic cancer is the fourth leading cause of cancer-related death, and the 5-year survival rate for pancreatic cancer patients is approximately 5% [11]. Resectability is considered the most significant prognostic factor [12,13]; however, tumors in only approximately 20% of patients with pancreatic cancer are surgically resectable because of distant metastases or major vessel involvement [14]. Even after curative surgery, the 5-year survival rate is still as low as 10% to 25%, mainly because of the high rate of local recurrence, peritoneal dissemination, liver metastases, and lymph node recurrence [15]. Consequently, the mechanisms of pancreatic cancer tumorigenesis and malignant transformation are key areas of current research.

In this study, we identified primary cilia in pancreatic ductal adenocarcinoma (PDAC) by using an immunofluorescence (IF) method, and we investigated the clinicopathological significance of primary cilia.

2. Materials and methods

2.1. Cancer cell lines and cell block preparation

Human pancreatic cancer cell lines PANC-1 and CFPAC-1 were obtained from the American Type Culture Collection (Manassas, VA). These cells were grown at 37°C with 5% CO_2 in RPMI-1640 containing 10% fetal bovine serum; every 3 months, cell lines were reinitiated from cryopreserved stocks. Two 10-cm dishes of cells were prepared: one was routinely maintained at 100% confluency, whereas the other was subjected to serum starvation for 48 hours with RPMI-1640 containing 0.5% fetal bovine serum after 100% confluency. The cells were scraped off from the dishes and fixed with 4% paraformaldehyde phosphate buffer solution for more than 24 hours. Subsequently, the cells were washed several times with phosphate-buffered saline, encased within agar gel, and finally embedded in paraffin.

2.2. Surgical specimens and patient characteristics

From our database of patients who underwent pancreatectomy at Keio University Hospital between 1999 and 2010, we analyzed the data from 100 consecutive patients with PDAC who received no therapy before their initial surgery. The mean patient age was 66 years (range, 36-81 years); 62 patients were male, and 38 were female. Patients with special types of pancreatic malignancies (eg, mucinous cystadenocarcinoma, intraductal papillary mucinous carcinoma, acinar cell adenocarcinoma, endocrine tumor, adenosquamous carcinoma, mucinous carcinoma, and anaplastic carcinoma) were excluded from the study. Tumors were classified according to the World Health Organization (WHO) classification system [16], the Classification of Pancreatic Carcinoma of the Japan Pancreas Society, and the TNM staging system approved by the International Union Against Cancer and the American Joint Committee on Cancer [17-19]. This study was conducted with the approval of the Ethics Committee of the Keio University School of Medicine.

2.3. Survival and outcome analysis

Of the 100 patients, 6 were excluded from survival analysis because 5 patients died in the hospital and 1 had synchronous intraductal papillary mucinous carcinoma. Thus, 94 patients were ultimately analyzed for survival and outcome. The follow-up period was 3.6 to 125.5 months (mean, 32.2 months).

2.4. Histologic examination

Resected specimens of cancer tissue were fixed in 10% formalin, and the size and gross appearance of the tumors were recorded. The size of each tumor was confirmed by comparison with the tumor area measured on the histologic slides. All resected specimens were cut stepwise at 4- to 6-mm intervals, and all sections containing pancreatic tissue were routinely embedded in paraffin and cut into sections 3-μm thick. These sections were stained with hematoxylin and eosin (HE), and all the sections obtained from each patient were observed microscopically. All histologic examinations, including stain evaluation, were confirmed by 2 pathologists.

Tumor grade was evaluated according to the tumor grading system of the WHO classification system [16]. The WHO grading system, which is based on the criteria proposed by Klöppel et al and Lüttges et al [20,21], entails the combined assessment of glandular differentiation, mucin production, nuclear atypia, and mitotic activity. In this grading system, higher grades are assigned when intratumor heterogeneity is observed.

2.5. IF staining

IF staining was performed on paraffin-embedded cell block sections from the PANC-1 and CFPAC-1 cell lines and