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Measurement of copy number of *ACTN4* to optimize the therapeutic strategy for locally advanced pancreatic cancer

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ABSTRACT

The standard therapeutic strategy recommended for locally advanced pancreatic cancer (LAPC) is typically chemotherapy or chemoradiotherapy (CRT). Although the clinical benefit of chemotherapy alone versus CRT for LAPC has been compared in a number of clinical trials, the optimal therapy for LAPC remains unclear. Moreover, the clinical benefit derived from treatment in each clinical trial is a matter of controversy, and the superiority of one treatment over another has yet to be definitively demonstrated. The poor outcomes seen among patients with LAPC owe largely to the emergence of metastatic disease; therefore, accurately evaluating occult distant metastasis before choosing a therapeutic strategy could be expected to help stratify patients with LAPC into the most appropriate treatment regimen, namely local control or systemic therapy. In 1998, we identified the actinin-4 gene (*ACTN4*) as an actin-binding protein and showed its molecular mechanisms had clinical implications for cancer metastasis. We also identified *ACTN4* gene amplification in pancreatic, ovarian, and salivary gland cancer, and demonstrated its utility as a strong prognostic biomarker for stage I lung adenocarcinoma in patients who had never received chemotherapy. Moreover, we recently reported that *ACTN4* gene amplification could be a useful biomarker for predicting the efficacy of CRT for LAPC. In the present review, we summarize current knowledge regarding therapeutic strategies for LAPC and discuss the potential development of personalized medicine using *ACTN4* measurement for patients with LAPC.

1. Introduction

Globally, pancreatic cancer is the seventh most frequent cause of cancer-related death [1]. According to the Surveillance, Epidemiology and End Results (SEER) Stat Fact Sheets, 53% of patients with pancreatic cancer in the U.S. present with distant metastasis, 28% present with regional disease, 9% present with localized disease, and 10% present with an unknown status [2]. In Japan, approximately 27,000 patients are diagnosed with pancreatic cancer annually, with nearly the same number of pancreatic cancer-related deaths [3]. In fact, the current 5-year overall survival (OS) rate of patients with pancreatic cancer is 7.2% [2].

Surgical resection is a potentially curative treatment for non-metastatic pancreatic cancer, except for those found to have locally advanced unresectable disease at diagnosis, which accounts for about 30–40% of patients. In addition, patients with locally advanced pancreatic cancer (LAPC), for which there is currently no standard treatment, making effective management controversial, tend to have tumor invasion into adjacent critical structures such as the celiac and superior mesenteric arteries. Survival rates for patients with LAPC are generally low, even with modern therapeutic strategies such as chemotherapy and chemoradiotherapy (CRT) [4–9]. These poor outcomes owe largely to the emergence of metastatic disease. Therefore, the ability to accurately evaluate occult distant metastasis before choosing a therapeutic strategy would help stratify patients with LAPC into the appropriate treatment regimen, namely local control or systemic therapy. However, current imaging technologies cannot accurately detect micrometastatic lesions. Therefore, to help care providers choose the optimal therapeutic strategy, identifying biomarkers that could accurately evaluate metastatic potential from biopsy samples

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would be extremely beneficial for patients with LAPC.

Alpha-actinin is an actin cross-linking protein that is a member of the spectrin superfamily of proteins. The following four alpha-actinin isoforms have been identified: alpha-actinin-1 (*ACTN1*) [10], actinin-2 (*ACTN2*) [11], actinin-3 (*ACTN3*) [11], and actinin-4 (*ACTN4*) [12]. *ACTN2* and *ACTN3* are considered muscle isoforms, while *ACTN1* and *ACTN4* are considered non-muscle isoforms [13]. Muscle isoforms of actinin are expressed only in smooth and skeletal muscle, where they mediate actin filament bundling and interactions with the Z-disk. By contrast, non-muscle isoforms, which are associated with cell adhesion and cell migration, are expressed only in non-muscle cells, where, in addition to also mediating actin filament bundling, they interact with cell membranes. In 1998, we identified *ACTN4* as a metastasis-related gene in cancer [12]. Since that time, we have conducted detailed investigations into the biological mechanisms of actinin-4 in cancer metastasis and its clinical implications.

Recently, we recently published a report suggesting for the first time that *ACTN4* gene amplification in biopsy specimens of LAPC could be a biomarker for the metastatic ability of CRT in LAPC, as well as a predictor of its effectiveness [14]. In the present review, we describe the role of actinin-4 in cancer metastasis, focusing on the treatment of patients with LAPC.

2. Chemoradiotherapy (CRT) for locally advanced pancreatic cancer (LAPC)

2.1. Definitive CRT for LAPC

CRT has been compared with chemotherapy alone in a number of trials; these are summarized in Table 1. The results present conflicting data regarding the role of initial CRT in LAPC.

2.1.1. 5-fluorouracil-based CRT

In the 1980s, three trials were conducted using 5-fluorouracil (5-FU)-based chemotherapy [7,15,16]. The results demonstrated the superiority of chemotherapy (bolus 5-FU) with radiation versus radiation alone in patients with LAPC (median survival time [MST], 42 vs. 32 weeks, respectively, and 1-year survival, 41% vs. 19%, respectively) [7]. On the other hand, for cases involving resectable pancreatic cancer, the European Study Group for Pancreatic Cancer 1 Trial (ESPAC-1) failed to confirm any benefit of adjuvant 5-FU-based CRT over adjuvant chemotherapy [17].

2.1.2. Gemcitabine(GEM)-based CRT versus GEM alone

To our knowledge, two trials using GEM-based CRT have been conducted. The first of these, the French FFCD-SFRO trial, compared GEM-based CRT and GEM alone in 119 patients with LAPC [8]. Patients in the GEM-based CRT group received radiotherapy (RT) (60 Gy in 30 fractions) plus concomitant infusion of 5-FU (300 mg/m^2/day, days 1–5), followed by GEM (1000 mg/m^2 weekly, 3/4 weeks) until progression, while those in the chemotherapy alone group received GEM (1000 mg/m^2 weekly for 7 of the first 8 weeks, then for 3 of every 4 weeks) until progression. No survival benefit was seen for GEM-based CRT compared with GEM alone (8.6 vs. 13.0 months, respectively; $P = 0.03$). The lower survival observed in the CRT group was considered the result of the lower dose intensity of maintenance GEM.

The second study (ECOG 4201), which compared GEM plus RT (50.4 Gy in 28 fractions) with GEM alone [9], was terminated prematurely (with only 74 of 316 patients enrolled) because of poor accrual. Although the low accrual rate decreased the statistical power of the findings, the study showed that CRT led to a significantly longer survival time than chemotherapy alone (11.1 vs. 9.2 months, respectively; $P = 0.17$).

In addition, no survival benefits were observed for CRT compared with chemotherapy alone in two meta-analyses [18,19]. However, the sample sizes for those studies were small, and there was obvious heterogeneity in terms of the chemotherapy regimens and RT doses. Therefore, the effectiveness of CRT as a first-line treatment in patients with LAPC remains unclear.

2.2. Chemotherapy followed by CRT in LAPC

During initial treatment, up to 30% of patients with LAPC develop overt metastasis [20,21]; therefore, for patients without occult micrometastatic disease, several cycles of systemic chemotherapy followed by CRT is an option. The National Comprehensive Cancer Network guidelines [22] recommend this therapeutic sequence when it is highly unlikely that the disease will become resectable, when suspicious metastasis is found, or when the patient is unable to tolerate chemoradiation. For example, one retrospective study reported that first-line treatment with chemotherapy alone was an effective strategy for selecting patients with LAPC who were more likely to benefit from subsequent rounds of CRT [20]. However, an international prospective study, the LAP 07 trial, could not confirm this strategy [23]. The LAP 07 trial had a randomized 2 × 2 factorial design and enrolled 442 patients with LAPC. After GEM-based induction chemotherapy, 269 patients with disease control were randomized to receive additional chemotherapy or CRT (50.4 Gy) plus capecitabine. Median OS was longer in the chemotherapy group than in the CRT group (16.5 vs. 15.2

Table 1
Major trials directly comparing initial chemoradiotherapy versus chemotherapy alone for locally advanced pancreatic cancer.

Trial	Treatment arms	N	RT[5] dose	Median OS[4]	p-value
5-FU [1]-based chemotherapy					
Hazel et al. (1981) [15]	5-FU + methyl-CCNU	15		7.8 months	
	RT+5-FU	15	46 Gy	7.3 months	
Klaassen et al. (1985) [16]	5-FU	44		8.2 months	
	RT+5-FU	47	40 Gy	8.3 months	
GITSG [3] (1988) [7]	5-FU + streptozocin +mitomycin	21		32 weeks	
	RT+5-FU + streptozocin +mitomycin	22	54 Gy	42 weeks	p < 0.02
GEM [2]-based chemotherapy					
Chauffert et al. (2008) [8]	GEM	60		13.0 months	
	RT+5-FU + cisplatin	59	60 Gy	8.6 months	p = 0.03
Loehrer et al. (2011) [9]	GEM	37		9.2 months	
	RT + GEM	34	50.4 Gy	11.1 months	p = 0.17

5-FU [1]: 5-fluorouracil; GEM [2]: gemcitabine; GITSG [3]: Gastrointestinal Tumor Study Group; OS[4]: overall survival; RT[5]: radiation therapy.

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