Neoadjuvant chemotherapy for pancreatic cancer: Effects on cancer tissue and novel perspectives (Review)

HIDEHIRO TAJIMA, ISAMU MAKINO, YOSHINAO OHBATAKE, SHINICHI NAKANUMA,
HIRONORI HAYASHI, HISATOSHI NAKAGAWARA, TOMOHARU MIYASHITA,
HIROYUKI TAKAMURA and TETSUO OHTA

Department of Gastroenterological Surgery, Division of Cancer Medicine,
Graduate School of Medicine Science, Kanazawa University, Kanazawa 920-8641, Japan

Abstract. Chemotherapy for pancreatic cancer has diversified following the addition of more treatment regimens; however, in spite of this, pancreatic cancer remains a fatal disease. Preoperative (neoadjuvant) chemotherapy (NAC) or neoadjuvant chemoradiation therapy (NACRT) has been developed and implemented. For patients with borderline resectable pancreatic cancer (BRPC) and locally advanced pancreatic cancer (LAPC), a number of clinical trials have been conducted; NACRT was demonstrated to improve resectability, R0 resection rate, overall survival rate, disease-free survival rate and even an LAPC and BRPC survival advantage over NAC. However, from the knowledge obtained from resected specimens following preoperative treatment, residual pancreatic cancer tissues following NAC are rich in chemoresistant cancer stem-like cells and epithelial-mesenchymal transition (EMT) markers. Conversely, metformin, angiotensin receptor blocker, statins and low-dose paclitaxel are well-known as drugs that inhibit EMT, which is associated with cancer stem cell-like characteristics. Although clinical effectiveness is unlikely to be achieved using one of these as an anticancer agent, it is reasonable to use these drugs for patients with comorbidities in the treatment of pancreatic cancer. Furthermore, gemcitabine (GEM) affects antitumor immunity by stimulating the expression of major histocompatibility complex class I-related chain A on the surface of cancer cells to enhance the cytotoxicity of natural killer cells. Considering EMT and antitumor immunity, there is a possibility that GEM and nanoparticle albumin-bound paclitaxel therapy is the most suitable regimen for treating pancreatic cancer. However, even as preoperative treatment

progresses, R0 resection is the most important factor for the long-term survival of pancreatic cancer patients.

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1. Introduction

Pancreatic cancer is a fatal disease with an overall 5-year survival rate of <5% (1,2). For patients with localized disease, surgery is the only method of treatment that provides long-term benefits. Curative (R0) resection has been identified to be one of the key factors influencing the survival of patients with pancreatic cancer (3,4). Esposito *et al* (5) reported that the majority of pancreatic cancer resections include margin involvement (R1) resection and pathological reporting is important. Indeed, even in patients who undergo resection, the 5-year survival rate is poor at between 7 and 24%, whereas the median survival time is ~1 year in the majority of series, indicating that surgery alone is inadequate. A number of surgeons have attempted radical pancreatic resection, comprising wide lymphadenectomy and removal of the extrapancreatic nerve plexus, to improve outcomes (6-10). However, no improvement in the prognosis of pancreatic cancer has been achieved. Furthermore, Nimura *et al* (11) reported that extended lymphadenectomy had no effect on improving the prognosis in pancreatic head carcinoma. These disappointing results are possibly attributable to early vascular dissemination, because the majority of patients have metastases that are present at the time of diagnosis (12). This hypothesis underpins the investigation of adjuvant chemotherapy and chemoradio-therapy (CRT) following surgery. Adjuvant chemotherapy or CRT has been performed on the basis of 5-fluorouracil (5-FU)-containing regimens since the 1980 s, and its usefulness has been reported (13-18). More recently, Oettle *et al* (19) reported that adjuvant chemotherapy with gemcitabine (GEM) led to

a statistically significant improvement in the OS (overall survival) rate. Furthermore, results of the Japan Adjuvant Study Group of Pancreatic Cancer 01 study indicated that S-1, an oral fluoropyrimidine analogue, confers a significantly improved OS rate and recurrence-free survival rate following pancreatic cancer resection compared with GEM (20). A major drawback of adjuvant therapy for pancreatic cancer is marked and consistent failure of between 20 and 30% of patients to receive the designated therapy as a result of post-operative complications, delayed surgical recovery, patient refusal, comorbidity or early disease recurrence (16,21,22). These challenges can be overcome in certain cases by administering preoperative (neoadjuvant) therapy, so that an increased number of patients may receive potentially beneficial adjuvant treatment. Other theoretical advantages of this approach include the early treatment of micrometastases, delaying surgery, thereby sparing those patients who already have occult metastases from the morbidity and mortality of major surgery if disseminated disease becomes apparent at the time of reassessment, decreased risk of intraoperative tumor seeding, improved treatment tolerance compared with post-operative therapy, and decreased overall treatment time (23). Therefore, in recent years, neoadjuvant chemotherapy (NAC) and neoadjuvant chemoradiation therapy (NACRT) have been attempted, seeking further improvement in treatment results for pancreatic cancer. However, due to the lack of randomized studies, the optimal performance of neoadjuvant treatment remains a matter of debate. In the present review, the effectiveness of preoperative treatment and the impact of preoperative treatment on pancreatic cancer tissues are examined.

2. Neoadjuvant treatment

The National Comprehensive Cancer Network (NCCN) guidelines endorsed by the American Hepatopancreatobiliary Association/Society of Surgical Oncology/Society for Surgery of the Alimentary Tract system (24) have been used for the treatment of pancreatic cancer worldwide. According to the NCCN guidelines, only ~20% of patients are diagnosed with resectable pancreatic cancer, 40% of patients have metastatic disease, and the remaining 40% have locally advanced pancreatic cancer in the form of either borderline resectable pancreatic cancer (BRPC) or locally advanced pancreatic cancer (LAPC) (25). Neoadjuvant treatment is often considered separately from resectable pancreatic cancer and BRPC and/or LAPC. Furthermore, patients with BRPC and LAPC have ~50% chance of curative resection compared with patients with resectable pancreatic cancer (26-29).

Recent meta-analyses have provided evidence of BRPC and LAPC being advantageous over neoadjuvant strategies (30): i) Neoadjuvant treatment may avoid handicapping postoperative surgical complications (31); ii) neoadjuvant treatment may assist in avoiding unnecessary major abdominal surgery during treatment; iii) chemotherapeutic agents have an improved effect owing to increased vascularization and subsequent drug delivery to neoplastic tissues without surgical trauma (32); iv) for BRPC and LAPC patients, neoadjuvant therapy leads to down-staging of the disease and increasing the rate of R0 resections (26,33-39); v) a number of studies identified a decreased incidence of anastomotic fistulas following neoadjuvant

treatment (40-43); and xi) analyses of the costs of various treatments for pancreatic cancer identified an economic advantage for neoadjuvant treatment regimens (40,44). As the most significant factor predicting long-term survival in pancreatic cancer patients is an R0 resection, the most important factor of neoadjuvant treatment for LAPC and BRPC patients is increasing the rate of R0 resections. The results of a number of clinical trials of neoadjuvant treatment for LAPC and BRPC have been reported. For instance, Lee *et al* (43) reported 43 patients with LAPC and BRPC treated with a combination of GEM and capecitabine. In the LAPC group, 24% underwent surgical resection with 83.3% having R0 resections. In the BRPC group, 61% underwent surgical resection with 81.8% having R0 resections. Sahora *et al* (44) reported the results of a Phase II study of NAC for LAPC and BRPC using GEM plus docetaxel; the overall resection rate was 32% with 87.5% having R0 resections and the median survival time of resected case was 16 months.

Conroy *et al* (45) reported the results of a Phase III study on the efficacy of 5-FU, leucovorin, irinotecan and oxaliplatin (FOLFIRINOX) chemotherapy for LAPC and metastatic pancreatic cancer, and demonstrated the significant superiority of FOLFIRINOX over GEM alone with respect to the OS rate, progression-free survival (PFS) rate and overall response rate. On the basis of the results of this Actions Concertées dans les Cancers Colo-Rectaux et Digestifs (ACCORD) trial, neoadjuvant therapy with FOLFIRINOX is currently available and leads to the most consistent results for the treatment of metastatic pancreatic cancer and LAPC (45). There are a number of retrospective studies of NAC using FOLFIRINOX for LAPC and BRPC (34,46-49). Hosein *et al* (34) performed a retrospective study of NAC using FOLFIRINOX for LAPC with a 62.5% R0 resection rate, 83% 1-year PFS rate and 100% 1-year OS rate. Ferrone *et al* (49) reported that, in spite of post-FOLFIRINOX imaging suggesting continued unresectability, 92% of patients underwent R0 resection. Furthermore, compared with no NAC, FOLFIRINOX resulted in a significantly longer operation time (393 vs. 300 min) and blood loss (600 vs. 400 ml), but significantly decreased operative morbidity (36 vs. 63%) and no postoperative pancreatic fistulas (49). From these results, FOLFIRINOX as a NAC is safe and effective for LAPC and BRPC; however, the long-term results remain unclear. Randomized control trials are warranted.

Nanoparticle albumin-bound (nab-)paclitaxel in combination with GEM has emerged as a novel treatment option for patients with metastatic pancreatic cancer on the basis of its superiority over GEM. The phase III Metastatic Pancreatic Adenocarcinoma Clinical Trial demonstrated the superior efficacy of nab-Paclitaxel and GEM compared with GEM alone for all trial endpoints, including the primary endpoint of overall survival in patients with metastatic pancreatic cancer treated to disease progression (50). Nab-paclitaxel and GEM treatment has also been used in a neoadjuvant setting, and effectiveness has been reported (51,52).

Studies on CRT for LAPC with 5-FU-based regimens have been published since the 1980s and survival prolongation has been demonstrated compared with radiation alone (53). The theoretical hypothesis on which CRT is based is that, whereas chemotherapy enables management of microdisseminated disease and also acts as a radiation sensitizer, radiotherapy

may have a marked impact on local disease management. However, ~30% of patients with LAPC develop distant metastases during the early cycles of treatment and radiotherapy may increase distant metastases (30,54). Therefore, the combination of chemotherapy and radiotherapy is essential. Evidence to support the use of NACRT for LAPC is accumulating (30,35). These data demonstrate that NACRT improves resectability, R0 resection rate, OS rate and disease-free survival (DFS) rate, and reveal a survival advantage to patients with LAPC and BRPC compared with NAC.

NAC strategies have also been considered in association with resectable pancreatic cancer. For instance, Heinrich *et al* (55,56) published Phase II and III trials of NAC with GEM vs. GEM and oxaliplatin treatment for resectable pancreatic cancer. A number of small trials have reported the use of NAC or NACRT for treating resectable pancreatic cancer (23,57,58); however, no data on OS and DFS have been reported. Therefore, only surgical resection with negative margins may offer a chance of long-term survival, and NAC or NACRT will be necessary for treating LAPC and BRPC. Consideration of the regimen to be used and the duration of preoperative treatment are required. Furthermore, it is necessary to consider whether or not preoperative treatment should be administered for resectable pancreatic cancer.

3. Effect of preoperative therapy on pancreatic cancer tissues

From resected specimens following preoperative treatment, the effect of chemotherapy or CRT on pancreatic cancer tissue may be determined. Evans *et al* (59) published an appropriate grading system for the effect of CRT that has been widely used for pancreatic cancer. Pancreatic ductal adenocarcinoma tissues are characterized by universal desmoplastic reaction, featuring a fibrotic stroma and dysfunctional hypoperfused vascularity (60). The stroma is composed of extracellular matrix proteins, including collagen, hyaluronic acid, and secreted acidic and cysteine-rich proteins, and cellular elements, including cancer-associated fibroblasts (CAFs) (51). This altered stroma has been implicated in cancer development and maintenance, and also in the poor sensitivity of pancreatic cancer to chemotherapeutics. Furthermore, the interaction between carcinoma cells and stromal cells, including CAFs, influence stromal formation, invasion and metastasis (60,61).

Epithelial-mesenchymal transition (EMT) of tumor cells induced by stromal cells has been reported (62-64). EMT is a key event in tumor invasion and metastasis, whereby epithelial cell layers lose polarity and cell-cell contacts, and undergo marked cytoskeletal remodeling (65). Chemotherapy and radiotherapy are known to induce apoptotic cell death in malignant tumors (66). Additionally, it has been reported that anticancer treatments may also induce EMT in cancer cells, which may serve an important role in the aggressive behavior of tumors (67-69). Furthermore, EMT is induced in pancreatic cancer cells irrespective of whether the patient receives chemotherapy, and the interaction between cancer cells and stromal cells serves a crucial role in pancreatic cancer (70).

Low-dose paclitaxel (71-74), metformin (75-77), angiotensin receptor blocker (ARB) (61,78), statins (79,80) and histone deacetylase inhibitors (HDACis) (81,82) have all been indicated as agents that can inhibit the EMT of tumor cells or activation of stromal cells. There are a number of studies investigating the effects of these drugs and cancer treatments: For instance, metformin has been epidemiologically demonstrated to suppress tumor metastasis (83,84), whereas Nakai *et al* (85) demonstrated that inhibition of the renin-angiotensin system affects the prognosis of patients with advanced pancreatic cancer receiving GEM. A number of studies have focused on the inhibition of tumor EMT and stromal cell activation using paclitaxel (74,86,87). The results of these studies are consistent with the observation of tumor shrinkage and a decrease in stroma in tumors treated with nab-paclitaxel and GEM (88,89). Finally, HDACis have been tested in clinical trials of pancreatic cancer treatment combined with valproic acid and oral S-1 (90).

Chemotherapy for treating pancreatic cancer has diversified by adding further regimens including FOLFIRINOX and nab-paclitaxel plus GEM, but curing pancreatic cancer using chemotherapy or CRT remains difficult. Carcinoma cells within a tumor are heterogeneous, indicating that certain carcinoma cells may have slightly different properties from those of others (91). A concept has been proposed stating that a specific subpopulation of carcinoma cells with stem cell-like properties are responsible for tumor growth, whereas other carcinoma cells do not contribute to tumor expansion (92). In our previous study, it was demonstrated that residual pancreatic cancer tissues following preoperative chemotherapy were rich in chemoresistant cancer stem cells with the marker cluster of differentiation (CD)44 (70). A number of lines of evidence suggest an association between EMT and cancer stem cell characteristics in pancreatic cancer (93). Therefore, it may not be an exaggeration to say that suppression of EMT is the key for the treatment of pancreatic cancer.

Conversely, it has been reported that GEM affects antitumor immunity. Major histocompatibility complex class I-related chain A (MICA) expressed on the surface of cancer cells functions as a ligand for natural killer group 2 member D (NKG2D), an immune-receptor expressed on natural killer (NK) cells, and CD8 and $\gamma\delta$ T cells. The interaction between MICA and NKG2D stimulates NK cell-mediated cytotoxicity, and GEM stimulates the expression of MICA on the surface of cancer cells to enhance the cytotoxicity of NK cells (94). Furthermore, Miyashita *et al* (95) detected significantly increased immunohistochemical expression of MICA on the surface of pancreatic cancer cells and NKG2D-positive cells surrounding cancer cells in pancreatic cancer tissues following preoperative chemotherapy with GEM and oral S-1 compared with in untreated cancer tissues. However, cancer cells promote immune escape by ectodomain shedding of MICA and produce soluble MICA that competitively inhibits NKG2D expression on the surface of NK cells. The key molecule that activates the shedding protease, including a disintegrin and metalloproteinases 9, 10 and 17, is considered to be transforming growth factor (TGF)-β (96-98). Therefore, treatments that inhibit TGF-β are important in antitumor immunity (99). Metformin (100,101) and ARB (61,78,102) are well-known as drugs that inhibit TGF-β, in addition to inhibition of EMT. Although clinical effectiveness is unlikely to be achieved using one of these as an anticancer agent, it is reasonable to use these drugs for patients with comorbidities, including diabetes,

hypertension and hyperlipidemia, in the treatment of pancreatic cancer. When considering EMT and antitumor immunity, GEM and nab-paclitaxel therapy is currently the most suitable regimen for the treatment of pancreatic cancer.

4. Conclusions

Chemotherapy for pancreatic cancer has diversified following the addition of further regimens, but patient prognosis remains poor. Therefore, the possibility of prognostic improvement using NAC or NACRT is being actively investigated. According to the results of several clinical trials, NACRT improves resectability, R0 resection rate, OS rate and DFS rate, and suggests a survival advantage in patients with LAPC and BRPC compared with patients with NAC. For resectable pancreatic cancer, the necessity of preoperative treatment remains controversial.

It is beneficial to obtain various results from resected specimens following preoperative treatment. Residual pancreatic cancer tissues following preoperative chemotherapy are rich in chemoresistant cancer stem-like cells and EMT markers. Metformin, ARB, statins and low-dose paclitaxel are well known drugs that inhibit EMT and TGF-β. Although clinical effectiveness is unlikely to be achieved using one of these as an anticancer agent, it is reasonable to use these drugs for patients with comorbidities in the treatment of pancreatic cancer. Furthermore, GEM affects the antitumor immunity by stimulating the expression of MICA on the surface of cancer cells to enhance the cytotoxicity of NK cells. When considering EMT and antitumor immunity, there is the possibility that GEM and nab-paclitaxel therapy is the most suitable regimen for the treatment of pancreatic cancer. However, even as perioperative treatment progresses, R0 resection is the most important factor in the long-term survival time of patients with pancreatic cancer.

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