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WORD COUNT 2120
TIME SUBMITTED 20-JUN-2022 05:05PM
PAPER ID 87466224

Name of Journal: *World Journal of Gastroenterology*
Manuscript NO: 76810
Manuscript Type: MINIREVIEWS

Effective combinations of anti-cancer and targeted drugs for pancreatic cancer treatment

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Abstract

Pancreatic cancer is highly aggressive and lethal. Due to the lack of effective methods for detecting the disease at an early stage, pancreatic cancer is frequently diagnosed late. Gemcitabine has been the standard chemotherapy drug for patients with pancreatic cancer for over 20 years, but its anti-tumor effect is limited. Therefore, FOLFIRINOX (leucovorin, fluorouracil, irinotecan, oxaliplatin) as well as combination therapies using gemcitabine and conventional agents, such as cisplatin and capecitabine, has also been administered; however, these have not resulted in complete remission. Therefore, there is a need to develop novel and effective therapies for pancreatic cancer. Recently, some studies have reported that combinations of gemcitabine and targeted drugs have had significant anti-tumor effects on pancreatic cancer cells. As gemcitabine induced DNA damage response, the proteins related to DNA damage response can be suitable additional targets for novel gemcitabine-based combination therapy. Furthermore, KRAS/RAF/MEK/ERK signaling triggered by oncogenic mutated *KRAS* and autophagy are frequently activated in pancreatic cancer. Therefore, these characteristics of pancreatic cancer are potential targets for developing effective novel therapies.

In this minireview, combinations of gemcitabine and targeted drugs to these characteristics, combinations of targeted drugs, combinations of natural products and

anti-cancer agents, including gemcitabine, and combinations among natural products are discussed.

Key Words: Pancreatic cancer; Gemcitabine; Targeted drug; Combination therapy

Nishimoto A. Effective combinations of anti-cancer and targeted drugs for pancreatic cancer treatment. *World J Gastroenterol* 2022; In press

Core Tip: Gemcitabine has been the standard chemotherapy drug for patients with pancreatic cancer; however, its effectiveness is limited. Therefore, various combination therapies involving gemcitabine and targeted drugs are being explored. A review of combination therapies based mainly on clinical studies has been published recently; therefore, this minireview focuses on the findings of basic studies and discusses combinations of gemcitabine and targeted drugs, combinations of targeted drugs, combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products.

INTRODUCTION

Pancreatic cancer is fatal and has a 5-year survival rate of approximate 10%[1]. It is estimated that pancreatic cancer will become the second most common cause of cancer-related deaths in the United States by 2030[2]. Pancreatic cancer is frequently diagnosed at a late stage owing to the lack of effective methods for detecting it at earlier stages and non-specific symptoms. Therefore, there is an urgent need to develop both novel effective therapies for pancreatic cancer that has progressed to a late stage and effective methods for detecting pancreatic cancer at an early stage.

Gemcitabine is the standard treatment for patients with pancreatic cancer. However, as the anti-tumor effect of gemcitabine is limited, FOLFIRINOX (leucovorin, fluorouracil, irinotecan, oxaliplatin) as well as combination therapies of gemcitabine and conventional agents, such as cisplatin and capecitabine, has also been administered[3–5]. Although these combination therapies improved overall survival compared to gemcitabine alone, they did not achieve complete remission. In addition, the incidence of toxicity associated with these combination therapies has increased[3–6].

Recent studies have attempted to identify effective combinations of gemcitabine and targeted drugs for pancreatic cancer. As gemcitabine, a well-known DNA-damaging agent, induced DNA damage response, the proteins related to DNA damage response can be suitable additional targets for novel gemcitabine-based combination therapy. Furthermore, KRAS/RAF/MEK/ERK signaling triggered by oncogenic mutated *KRAS* and autophagy, which are described as the characteristics of pancreatic ductal adenocarcinoma (PDAC), are frequently activated. Therefore, these characteristics are promising targets for effective novel therapeutic strategies[7–12]. An excellent review on effective combination therapies for pancreatic cancer was published recently[13]. This review was based mainly on the findings of preclinical and clinical studies[13]; therefore, this minireview focuses on the findings of basic studies and discusses combinations of gemcitabine and targeted drugs, combinations of targeted drugs, combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products.

COMBINATIONS OF GEMCITABINE AND TARGETED DRUGS

Gemcitabine, a well-known DNA-damaging agent, has been the standard first-line drug for patients with pancreatic cancer. However, the efficacy of gemcitabine in pancreatic cancer is limited and a novel gemcitabine-based combination therapy is required. In this section, combinations of gemcitabine and targeted drugs to enhance the anti-tumor effect are summarized.

Gemcitabine and Chk1 inhibitor

Quinone-methide triterpenoid pristimerin induces lysosomal degradation of checkpoint kinase 1 (Chk1) and augments the expression of γ-H2AX, which is a biomarker of DNA damage following gemcitabine treatment[14]. Furthermore, the combination of gemcitabine and pristimerin was shown to increase apoptosis of pancreatic cancer cells[14]. The Chk1 inhibitor MK-8776 also enhances the sensitivity of multiple human cancer cell lines, including pancreatic cancer cells, to gemcitabine[15]. The DNA damage response mediated by Chk1 in pancreatic cancer stem cells was greater than that in non-pancreatic cancer stem cells, indicating that Chk1 inhibition selectively sensitizes pancreatic cancer stem cells to gemcitabine[16]. The combination of Chk1 inhibition and gemcitabine reduces the ability of tumor initiation in pancreatic cancer stem cells[16]. The anti-tumor effect of the combination of gemcitabine and doublecortin-like kinase 1 (Dclk1) inhibitor has also been reported[17]. The latter significantly decreased the expression of gemcitabine-induced phosphorylated Chk1 in pancreatic cancer cells. The combination of gemcitabine and Dclk1 inhibitor did not arrest the cell cycle at the S phase and allowed cell cycle progression[17]. In addition, the combination of gemcitabine and Dclk1 inhibitor increased the rate of γ-H2AX-positive cells compared to individual treatments. The combination of gemcitabine and Dclk1 inhibitor induced PARP1 cleavage as well as caspase-3 activation and significantly decreased the survival rate of pancreatic cancer cells compared to gemcitabine treatment alone[17].

Gemcitabine and KRAS antibody/MEK inhibitor

Oncogenic *KRAS* mutations are present in approximately 90% of pancreatic cancer cases. Consequently, KRAS and its downstream proteins, such as RAF, MEK, and ERK, are activated in pancreatic cancer cases and contribute to the progression of the disease. Therefore, inhibitors targeting these proteins may be effective in inhibiting this progression.

Antibodies that bind intracellularly to the activated GTP-bound form of oncogenic KRAS mutants have been developed and significantly sensitize pancreatic cancer cells to gemcitabine[18,19]. These antibodies synergistically increase the anti-tumor effect of gemcitabine by inhibiting the RAF/MEK/ERK signaling pathway downstream of KRAS[18,19]. The antibodies are internalized in the cytoplasm by endocytosis through the tumor-associated receptors of extracellular epithelial cell adhesion molecules[19]. These antibodies synergistically increase the anti-tumor effect of gemcitabine by inhibiting KRAS/RAF/MEK/ERK signaling in pancreatic cancer cells[18,19].

The MEK inhibitor, trametinib, both alone and in combination with gemcitabine, was shown to exhibit significantly enhanced anti-tumor effects compared to gemcitabine alone[20]. The combination of gemcitabine and trametinib also increased the inhibition of tumor growth in pancreatic cancer patient-derived orthotopic xenografts in nude mice compared to trametinib alone[20]. Moreover, the combination of the MEK inhibitors, trametinib and cobimetinib, prevented tumor growth in gemcitabine-resistant pancreatic cancer patient-derived orthotopic xenografts in nude mice[21]. These results suggest that such combinations have therapeutic potential against pancreatic cancer.

Gemcitabine and autophagy inhibitor

Gemcitabine has significantly been shown to increase autophagy induction in human pancreatic cancer cells, and combined treatment with gemcitabine and chloroquine, an autophagy inhibitor, triggered a marked boost in reactive oxygen species (ROS) levels and increased lysosomal membrane permeability[22]. Consequently, proteases, including

cathepsins, are released from lysosomes into the cytoplasm, leading to apoptosis. Thus, the combination of gemcitabine and chloroquine has an anti-tumor effect on pancreatic cancer cells through the apoptotic pathway by lysosomal dysfunction *via* a marked boost of ROS[22]. Cancer stem cells are considered to be responsible for the recurrence and chemoresistance of cancer. The expression of the markers of cancer stem cells, aldehyde dehydrogenase 1, CD44, and CD133, was found to be positively correlated with the expression of LC3 type II, an autophagy marker, in pancreatic cancer tissues[23]; this suggests an association between autophagy and cancer stem cells. Indeed, autophagy inhibition decreased the activity of sphere formation of pancreatic cancer stem cells, and gemcitabine and autophagy inhibition markedly reduced the populations of cancer stem cells[23].

COMBINATIONS OF TARGETED DRUGS

Recent studies have demonstrated that combinations of targeted drugs have potential for developing novel and effective therapy for pancreatic cancer. In this section, combinations of targeted drugs for PDAC therapy are summarized.

ERK and autophagy inhibitors

KRAS suppression or ERK inhibition was shown to decrease both glycolytic and mitochondrial functions and to increase autophagic flux in PDAC, suggesting that ERK inhibition enhances dependence on autophagy[24]. The combination of ERK and autophagy inhibitors synergistically enhanced anti-tumor activity in KRAS-driven PDAC *via* the dysfunction of the energy pathways consisting of glycolysis and autophagy[24]. It has been reported that inhibition of the KRAS/RAF/MEK/ERK signaling pathway elicits autophagy, resulting in protection of PDAC cells from cytotoxic effects[25]. The combination of MEK1/2 and autophagy inhibitors showed synergistic anti-tumor effects against PDAC cells *in vitro* and promoted the regression of patient-derived xenografts of PDAC in mice[25]. Furthermore, the effect of the combination of trametinib and chloroquine was not limited to PDAC and resulted in

similar responses in patient-derived xenografts of *BRAF*-mutated colorectal cancer and *NRAS*-mutated melanoma[25].

ERK, Chk1, and autophagy inhibitors

Screening of druggable genes by genetic loss-of-function using CRISPR-Cas9 and small interfering RNA revealed that components of the ATR-Chk1 DNA damage response pathway were modulators of sensitivity to ERK inhibitor treatment in *KRAS-mutant* PDAC[26]. Chk1 inhibition suppressed the growth of both PDAC cell lines and organoids and activated ERK signaling and autophagy, suggesting that Chk1 inhibition enhances dependence on ERK signaling and autophagy[26]. These findings provide a mechanistic basis for the effectiveness of the inhibition of Chk1, ERK, and autophagy. Indeed, this triple combination of inhibitors synergistically enhanced the anti-tumor effect in *KRAS-mutant* PDAC[26].

2-deoxyglucose and MEK inhibitor

Pooled shRNA library screening was used in an orthotopic xenograft model to identify multiple glycolysis genes as potential targets that may sensitize PDAC cells to MEK inhibition[27]. Apoptosis in KrasG12D-driven PDAC cells was synergistically induced, in vitro, *via* the combination of 2-deoxyglucose, a glycolysis inhibitor, and a MEK inhibitor; the same also inhibited tumor growth of PDAC xenografts, leading to prolonged overall survival in a genetically engineered PDAC mouse model[27]. Molecular and metabolic analyses revealed that the combined inhibition of glycolysis and ERK signaling synergistically caused apoptosis by inducing lethal stress in the endoplasmic reticulum. These results indicate that the combination of 2-deoxyglucose and a MEK inhibitor may be an effective approach for targeting KRAS-driven PDAC[27].

Replication stress response and autophagy inhibitors

PDAC exhibits high basal lysosomal activity and relies on lysosome-dependent recycling pathways, such as autophagy, to generate substrates for metabolism[28].

Kinase inhibitor screening revealed that the replication stress response inhibitor and chloroquine, an autophagy inhibitor that works *via* the functional inhibition of lysosomes, were synthetically lethal in PDAC cells[28]. Chloroquine induces replication stress due to aspartate depletion, and a replication stress response inhibitor and chloroquine synergistically inhibit tumor growth in PDAC[28].

Immune checkpoint and autophagy inhibitors

Major histocompatibility complex class I (MHC-I) molecules are selectively degraded *via* autophagy in PDAC[29]. Consequently, the expression of MHC-I at the cell surface is reduced, and MHC-I is localized predominantly within autophagosomes and lysosomes. Autophagy inhibition was shown to restore the expression of MHC-I at the cell surface and improve antigen presentation[29]. Furthermore, autophagy inhibition synergizes with dual immune checkpoint blockade therapy (anti-PD1 and anti-CTLA4 antibodies) to enhance the anti-tumor immune response and reduce tumor growth in syngeneic host mice[29].

COMBINATIONS OF NATURAL PRODUCTS AND ANTI-CANCER AGENTS, INCLUDING GEMCITABINE

The use of natural products as adjunctive therapies for pancreatic cancer has a great potential due to the anti-cancer efficacy and low toxicity. Yue *et al* summarized combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products[30]. They focused on the following: combinations of natural products and gemcitabine (for example, cucurbitacin B and gemcitabine[31], glaucarubinone and gemcitabine[32], escin and gemcitabine[33], and gum mastic and gemcitabine[34]); combinations of natural products and other agents, such as all-trans retinoic acid and sulindac (for example, 12-O-tetradecanoylphorbol-13-acetate and all-trans retinoic acid[35], parthenolide and sulindac[36], and triptolide and hydroxycamptothecin[37]); and combinations among natural products (for example,

sulforaphane and quercetin[38], wogonin, apigenin, and chrysin[39], and metformin and aspirin[40]).

While agents from purified chemical compounds generally target single molecules, natural products mostly consist of multiple components that concurrently act on various molecular targets. Therefore, natural products are expected to have various functions, including improvement of anti-cancer efficacy, enhancement of immune system, and reduction of side effects[41].

CONCLUSION

In general, it is widely accepted that combination therapy is more effective than monotherapy. In this minireview, combinations of gemcitabine and targeted drugs, combinations of targeted drugs, combinations of natural products and anti-cancer agents, including gemcitabine, and combinations among natural products are described. Hereafter, preclinical and clinical studies are needed to examine the possibility for clinical applications. Concurrently, additional basic studies that attempt to identify combinations that synergize anti-cancer effects are needed to find the better treatment options.

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