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World Journal of Gastroenterology
Editors-in-Chief
Damian Garcia-Olmo, MD, PhD, Doctor, Professor, Surgeon;
Stephen Strom, PhD, Professor; Andrzej Tarnawski, DSc, MD, PhD, Professor
Scientific editor: Jing Yu

Dear Professor Ma, Professor Garcia-Olmo, Professor Strom, Professor Tarnawski, and Professor Yu: 17 June, 2016

Thank you for considering our paper titled "Perspectives on the combination of radiotherapy and targeted therapy with DNA repair inhibitors in the treatment of pancreatic cancer" (Manuscript ID: 26185) for publication in the World Journal of Gastroenterology. We highly appreciate the reviewer comments and have revised our manuscript according to the provided suggestions. Furthermore, we have attached our point-by-point responses and have included the recommended revisions.

We appreciate your patience and generous help with editing our manuscript.

Respectfully yours,

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Reviewer(s)' Comments:

Reviewer 02460781

1. *This article reviews the perspectives on the combination of radiotherapy and targeted therapy with DNA repair inhibitors in the treatment of pancreatic cancer. There is an extensive review of literature and the content is rich. However, the length of abstract and whole text should be shorter.*

Authors' responses:

We are very appreciated with the comments. We had shortened the abstract to fit the limitation (206 words). In addition, we reduced the length and simplify the description in sections of "Introduction" and "Types of DNA damage in pancreatic cancer".

The revised abstract is as follows:

"Pancreatic cancer is highly lethal. Current research that combines radiation with targeted therapy may dramatically improve prognosis. Cancerous cells are characterized by unstable genomes and activation of DNA repair pathways, which are indicated by increased phosphorylation of numerous factors, including H2AX, ATM, ATR, Chk1, Chk2, DNA-PKcs, Rad51, and Ku 70/Ku80 heterodimers. Radiotherapy causes DNA damage. Cancer cells can be made more sensitive to the effects of radiation (radiosensitization) through inhibition of DNA repair pathways. The synergistic effects, of two or more combined non-lethal treatments, led to co-administration of chemotherapy and radiosensitization in *BRCA*-defective cells and patients, with promising results. ATM/Chk2 and ATR/Chk1 pathways are principal regulators of cell cycle arrest, following DNA double-strand or single-strand breaks. DNA double-stranded breaks activate DNA-dependent protein kinase, catalytic

subunit (DNA-PKcs). It forms a holoenzyme with Ku70/Ku80 heterodimers, called DNA-PK, which catalyzes the joining of nonhomologous ends. This is the primary repair pathway utilized in human cells after exposure to ionizing radiation. Radiosensitization, induced by inhibitors of ATM, ATR, Chk1, Chk2, Wee1, PP2A, or DNA-PK, has been demonstrated in preclinical pancreatic cancer studies. Clinical trials are underway. Development of agents that inhibit DNA repair pathways to be clinically used in combination with radiotherapy is warranted for the treatment of pancreatic cancer."

Reviewer 02543991

1. *The authors stated that it is still challenging to make an early diagnosis for pancreatic cancer due to lacking useful screening methods, the authors should focus on this point and give some perspectives on this or give a short discussion in this review.*

Authors' responses:

We highly appreciated with these comments, and have added some perspectives [reference 2-4] in the first section of "Introduction" as follows:

It remains challenging to make an early diagnosis for sporadic pancreatic cancer, because of the low lifetime risk of developing pancreatic cancer and the lack of adequate screening methods[2]. Endoscopic ultrasonography and MRI screening tools may play a limited detection role in patients with high risks, such as family history or known germline mutations[3, 4].

2. *In "types of DNA damage in pancreatic cancer" section, the authors cited the studies that the telomeres were much shorter in cancer cell, and stated that*

widespread DNA damage was found in this cancer, what the authors want to say?

Authors' responses:

Thanks for your comments. The telomeres were much shorter in pancreatic cancer cells than in PanIN-1 or PanIN-2 lesions. The activation of telomeres expression seen in the majority of pancreatic cancers may be resulted from the protective mechanism of telomeres against catastrophic DNA damage[22]. Previous studies also demonstrated that telomere shortening was closely associated with the DNA repair impairment[23.24]. Therefore, we want to provide evidence of widespread DNA damages and activation of DNA repair pathways from the earliest to late pancreatic neoplastic lesions. To induce more lethal damages to the partially defective cancer genomes, radiotherapy combining with inhibitors of DNA repair may be a good rationale to provide better therapeutic efficacy.

These information" The activation of telomeres expression seen in the majority of pancreatic cancers may be resulted from the protective mechanism of telomeres against catastrophic DNA damage[22]. Previous studies also demonstrated that telomere shortening was closely associated with the DNA repair impairment[23.24]" have been added to the "*types of DNA damage in pancreatic cancer" section* (Page 8 Lines 5-6).

Ref.

22. **Seki K**, Suda T, Aoyagi Y, Sugawara S, Natsui M, Motoyama H, Shirai Y, Sekine T, Kawai H, Mita Y, Waguri N, Kuroiwa T, Igarashi M, Asakura H. Diagnosis of pancreatic adenocarcinoma by detection of human telomerase reverse transcriptase messenger RNA in pancreatic juice with sample qualification. *Clin Cancer Res* 2001; **7**: 1976-1981 [PMID: 11448913]

23. **Uziel O**, Beery E, Dronichev V, Samocha K, Gryaznov S, Weiss L, Slavin S, Kushnir M, Nordenberg Y, Rabinowitz C, Rinkevich B, Zehavi T, Lahav M. Telomere shortening sensitizes cancer cells to selected cytotoxic agents: in vitro and in vivo studies and putative mechanisms. *PLoS One* 2010; **5**: e9132 [PMID:20161752 DOI: 10.1371/journal.pone.0009132]
24. **Frías C**, García-Aranda C, De Juan C, Morán A, Ortega P, Gómez A, Hernando F, López-Asenjo JA, Torres AJ, Benito M, Iniesta P. Telomere shortening is associated with poor prognosis and telomerase activity correlates with DNA repair impairment in non-small cell lung cancer. *Lung Cancer* 2008; **60**: 416-425 [PMID:18077053 DOI:10.1016/j.lungcan.2007.11.001]

3. *It is known that some proteins maybe silenced during DNA damage, such as cdc25, which was referred in in checkpoint kinase inhibitors section, is there any strategies that activation of these modulators for cancer therapy? The authors may review or discuss this.*

Authors' responses:

We highly appreciated your comments. According to your suggestions, we have added the new section of "Wee1 and PP2A inhibitors" in the main text, Table, Figure 1, and reference 120-127 as follows:

WEE1 AND PP2A INHIBITORS

Chk1 activates Wee1 kinase1 at the G2-M checkpoint, upon DNA damage. Activated Wee1 phosphorylates CDC2 $(Cdk1)^{Tyr15}$, which enables CDC2/Cdk1 inactivation; this process contributes to G2-M arrest[120]. At the same time, activated Chk1 phosphorylates and inactivates Cdc25 to prevent

the dephosphorylation and inactivation of CDC2/Cdk1. In contrast, protein phosphatase 2A (PP2A) is able to dephosphorylate and inhibit Cdc25, through 14-3-3 protein[121]. Therefore, inhibitors of Wee1 or PP2A can be used to maintain the activity of Cdc25; thus, they theoretically allow cell cycle progression, without adequate time for DNA repair.

In vitro studies, in p53-defective MCF-7 cancer cells derived from breast cancer cell lines, show that compared to p53-intact cells, these cells were much more sensitive to Wee1 inhibition by MK-1775 (AZD1775) [122]. In addition, radiosensitization was observed in the p53-defective MCF-7 cells in a clonogenic assay. Cells pretreated with MK-1775 (AZD1775) had a reduction in $Cdk1^{Tyr15}$ phosphorylation and 53BP1 foci; however, they had an increase in γ-H2AX after IR[122]. The defective DNA repair was through inhibition of HR, but not NHEJ[122]. MK-1775 (AZD1775) monotherapy, in patient-derived pancreatic cancer xenografts, was ineffective[123]. However, a combination of gemcitabine and MK-1775 (AZD1775) abrogated G2-M checkpoint arrest, which was accompanied by pancreatic tumor regression, increased mitotic entry and apoptosis in pancreatic cancer cells[123]. Mk-1775 (AZD1775) increased gemcitabine-induced radiosensitization, in MiaPaCa-2 pancreatic cancer cell lines, through inhibition of $Cdk1^{Tyr15}$ phosphorylation and upregulation of γ-H2AX expression[124]. However, the radiosensitization phenomenon was not observed in HR and *BRCA2*-defective Capan-1 cells. A combination of AZD1775, gemcitabine, and radiotherapy enhanced a delay in tumor growth and impaired RAD51 focus formation, in xenografts derived from patients with pancreatic cancer[124]. In fact, MK-1775 (AZD1775) is the first-in-class Wee1 inhibitor, with high specificity and potency, to enter

clinical trial development. A phase I study, which used single agent MK-1775 (AZD1775) to treat refractory solid tumors, had activities in patients with a BRCA mutation; however, myelosuppression and supraventricular tachycardia were dose-limiting toxicities [125]. Clinical trials combining MK-1775 (AZD1775) and radiotherapy in various cancer types are underway.

Knockdown of the PP2A and PPP2R1A subunit, in MiaPaCa-2 and Panc-1 cell lines, resulted in significant radiosensitization and persistent γ-H2AX expression. The main mechanisms, of radiosensitization by PP2A inhibition, are through the activation of CDC25C/Cdk1^{Tyr15} and inhibition of HR[126]. The aforementioned phenomenon was reproduced using the PP2A inhibitor, LB-100, which increased CDC25C (T130,) but decreased Cdk1^{Tyr15} phosphorylation[127]. The synergistic effects of LB-100 and radiotherapy on delayed tumor growth were also observed in the MiaPaCa-2 xenograft model[126]. At present, a phase I clinical trial, in which LB-100 is administered alone or in combination with docetaxel, is ongoing. Initial outcomes show that one patient, with stage IV disease, had a long, stable disease[127].

4. *The short names in this manuscript are difficult to understand, the full name at the first time would be appreciated.*

Authors' responses:

We highly appreciate your comments. We have provided the full name of DNA repair associated proteins, for example, Ataxia telangiectasia mutated (ATM), cell cycle checkpoint kinase 2 (Chk2), and etc. at the first time.