Attenuating DNA damage response and immunosuppression radiosensitizes pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is a most devastating disease with the worst 5-year survival among all cancers in the US of around 10%.[1] Surgery is the only curative treatment for pancreatic cancer patients. However, less than 15% of patients are eligible for surgery upon diagnosis since the majority are already in the advanced stage. Neoadjuvant therapies, including chemotherapy and radiotherapy aim to shrink or restrain the tumours, have shown promise in borderline resectable or metastatic PDAC patients. Nevertheless, radiotherapy has been rarely reported to bring more advantage to PDAC patients due to the high incidence of radioresistance. Therefore, there is a vigorous need to understand the mechanism underlying pancreatic cancer radioresistance. Many signalling pathways are involved in the resistance to radiation therapy including DNA damage repair, cell cycle control, inflammation, and oxidative stress response pathways both within the tumour itself and in the surrounding tumourmicroenvironment.[2]

Genomic instability is the hallmark of cancer and the cause of tumour heterogeneity. Pancreatic cancer has a higher level of genomic instability than all other cancers. The genetic alterations in DNA damage repair pathways caused by genomic instability can influence the response of tumours to radiation.[2] A recent bioinformatics and cellular function analyses showed that PDK1-driven radiotherapy resistance positively correlated with activated PI3K signalling, upregulated stemness markers and suppressed DNA damage biomarkers including RAD50, with increased epithelial-mesenchymal transition(EMT) transition and tumour sphere formation in hepatocellular carcinoma.[3] Radiosensitizers may improve the therapeutic efficacy of radiotherapy and serve as a better choice for pancreatic cancer neoadjuvant therapies. However, there are no effective agents that function as radiosensitizers to treat PDAC in the clinic to date.

Recently reported in *EBioMedicine*, Seshacharyulu et al. showed that cholesterol biosynthesis (CBS) is involved in the radioresistance of PDAC.[4] In the CBS metabolic pathway, farnesyl diphosphate synthase (FDPS) is the key enzyme for isoprenoid biosynthesis, catalyzing isoprenylation of several small GTPases.[5] In this study, they found that inhibition of FDPS using zoledronic acid (Zol) sensitized pancreatic cancer cells to radiation and activated immune cells.[6] FDPS is overexpressed in PDAC tissue and cell lines and is associated with poor radiation therapy response and patient survival, making it a potentially suitable biomarker for radiation therapy response and an ideal target for developing PDAC therapy. Zol combined with radiation reduced PDAC cell colony formation, increased cell apoptosis, and withdrew the radiation-associated G2/M cell cycle arrest. Moreover, Zol treatment sensitized radiation therapy by significantly inhibiting pancreatic cancer growth in 3D mouse models, and patient-derived PDAC tumoroids *in vitro*. This result was validated in xenograft mouse models with a platinum-based fiducial marker embedded in the mouse pancreas to precisely locate the xenograft tumours to improve the radiation therapy efficacy and minimize damage to normal surrounding cells and organs.

They performed RNA-seq analysis of the PDAC xenografts and patient-peripheral blood mononuclear cells to decipher the underlying mechanisms. They found that Zol increased pancreatic cancer cell radiation therapy response by affecting Rac1 and CDC42 small GTPases prenylation, thereby modulating downstream Rad50 involved DNA damage and the radiation response signalling pathway. Furthermore, the signature fibroblast activation-associated genes in the tumour microenvironment such as *TGFβ3, FGF2, MIF, PDGFRβ*, and *IL6* were confirmed to be downregulated in Zol and RT-treated xenograft tumours.

In the ongoing phase I/II clinical trial of Zol combined with hypofractionated radiation therapy/5FU or capecitabine treatment for patients with borderline resectable and locally advanced PDAC patients, the safety, efficacy, overall survival, surgical resection rate and tumour response were evaluated. The results showed that the resection rate was increased to 40% with Zol compared to 33% without Zol after neoadjuvant therapy. Further CIBERSORT analysis of RNA-seq data from the PDAC xenografts and patients-peripheral blood mononuclear cells showed that Zol treatment activated multiple immune cell types and repressed the immunosuppressive cells, suggesting a new choice to increase PDAC patient's immunotherapy response using Zol.

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Cholesterol biosynthesis has been reported to be involved in the radioresistance of breast cancer and pancreatic cancer.[7,8] Statins, the HMG-CoA inhibitors, have been used to treat breast cancer with increased recurrence free survival in preclinical and clinical studies. Previous studies showed that simvastatin could sensitize radioresistant breast cancer cell lines to radiation and reduced EMT transition and cellular migratory abilities.[8] However, further detailed mechanistic studies are needed to examine the roles statins played in cholesterol and lipid-metabolism for the radioresistance regulation in cancers.

Using radiosensitizers to improve tumour systemic immune cell activation and reverse immune escape are considered to be promising strategies to improve current cancer treatment. Rech et al. found that CD40 pathway activation is required for radiotherapy response in PDAC. Radiation in *LSL-Kras*$^{G12D/+}$;*LSL-Trp53*$^{R172H/+}$;*Pdx-1-Cre* (KPC) mice stimulated an early pro-inflammatory response, and the combination of an agonist αCD40 antibody, radiotherapy, and dual immune checkpoint blockade abolished tumours and generated long-term immunity.[9] Therefore, a radiosensitizer combined with immune checkpoint inhibition converts the tumour microenvironment from suppressive to stimulatory and may provide a new rationale for PDAC treatment.

In summary, the study of Seshacharyulu et al. identified FDPS as a novel radioresistance target, which activated downstream DNA damage repair pathways by affecting small GTPase prenylation. Moreover, they provided preclinical evidence implicating the use of Zol as a potential radiosensitizer and immuno modulator to promote tumour immune response. Thus, projecting FDPS-targeting as a putative enhancer of radiosensitivity and a potential new therapeutic approach for PDAC patients with radiotherapy resistance. These new findings pave the way for developing PDAC neoadjuvant therapeutic strategies to benefit nonresectable patients. Nevertheless, these results need to be further solidified by validating in the spontaneous KPC mouse model and in further clinical trials enrolling more patients. Detailed mechanistic studies for the DNA damage pathways and immunomodulatory effects of FDPS also warrants further investigation.

Declaration of interests

Courtney W. Houchen has ownership interest in COARE Holdings Inc. Other authors declare no conflicts of interest.

Contributors

All authors were involved in the overall design, literature search and writing of this commentary.

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