SMAD4 Loss is Associated with Response to Neoadjuvant Chemotherapy with Hydroxychloroquine in Patients with Pancreatic Adenocarcinoma

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Abstract

Background

SMAD4, a tumor suppressor gene, is inactivated or deleted in 60–90% of pancreatic adenocarcinomas (PDA). Loss of SMAD4 allows tumor progression by limiting cell cycle arrest and apoptosis and increasing metastases. SMAD4 deficient PDA cells are resistant to radiotherapy by upregulating autophagy, a cell survival mechanism that counteracts apoptotic mechanisms and allows intracellular recycling of macromolecules and organelles. Hydroxychloroquine (HCQ) is an orally available autophagy inhibitor with an established toxicity profile. We studied whether HCQ treatment in SMAD4 deficient PDA may prevent therapeutic resistance induced by autophagy upregulation.

Methods

We retrospectively analyzed the SMAD4 status of PDA patients enrolled in two prospective clinical trials evaluating administration of preoperative HCQ. The first dose escalation trial demonstrated the safety of preoperative gemcitabine with HCQ (NCT01128296). More recently, a randomized trial of gemcitabine/nab-paclitaxel +/- HCQ evaluated Evans Grade histopathologic response (NCT01978184). Immunohistochemistry of resected specimens for SMAD4 was previously performed. Patients not treated at the max HCQ dose (n = 5), not resected (n = 2) or with SMAD4 staining unavailable were excluded (n = 10). The effect of SMAD4 loss on response to HCQ and chemotherapy was studied for association with clinical outcome. Fisher’s exact test and log-rank test were used to assess response and survival.

Results

52 patients receiving HCQ with neoadjuvant chemotherapy were studied. 25 of these patients had SMAD4 loss (48%). 76% of HCQ treated patients with SMAD4 loss obtained a histopathologic response ≥ 2A, compared to only 37% with SMAD4 intact (p = 0.006). In contrast with prevailing views, loss of SMAD4 was not associated with a detriment in median overall survival in HCQ treated patients (34.43 months in SMAD4 loss vs. 27.27 months in SMAD4 intact, p = 0.18).

Conclusions

The addition of HCQ to neoadjuvant chemotherapy in patients with PDA may improve treatment response in those with SMAD4 loss. Further study of the relationship between SMAD4, autophagy and treatment outcomes in PDA is warranted.

Background
Pancreatic cancer has the third-highest cancer related mortality in the United States and is destined to be the second by 2025\textsuperscript{1}. Despite recent advances in available therapies, median overall survival of patients with pancreatic cancer is less than 6 months, and 5-year survival is less than 10\%\textsuperscript{2,3}. This dismal prognosis is driven by early metastatic spread and resistance to treatment, promoted by a unique tumor microenvironment. Pancreatic cancers rely on autophagy as a survival mechanism whereby damaged organelles are recycled and used for energy during metabolic stress\textsuperscript{4}. Pancreatic cancer cells utilize autophagy to support the abnormal nutrient demands of rapid growth in a hypoxic, acidotic tumor microenvironment\textsuperscript{5–7}. Autophagy also allows malignant cells to escape the cellular damage incurred by chemotherapy and radiation treatments\textsuperscript{9–10}. Beyond metabolic recycling as a tumor survival mechanism, autophagy may also promote tumor growth through other mechanisms. Autophagy also promotes formation of dense stroma by cancer associated fibroblasts, hindering the cytotoxic effects of chemotherapy on cancer cells\textsuperscript{11}. Higher levels of autophagy correlate with worse prognosis in pancreatic cancer\textsuperscript{12}.

Inhibition of autophagy promotes apoptosis and represents a novel treatment target in pancreatic cancer\textsuperscript{13–15}. Hydroxychloroquine (HCQ) is an inexpensive, orally available, well-tolerated medication that inhibits the final step of autophagy and therefore may potentiate anti-neoplastic therapies\textsuperscript{16,17}. A recent phase 1/2 clinical trial added high dose hydroxychloroquine to neoadjuvant gemcitabine in patients with localized pancreatic adenocarcinoma. The combination was safe and well tolerated with no dose-limiting toxicity. 77\% of patients achieved R0 resection, which was superior when compared with historical controls. Patients who had a CA 19–9 response to treatment also had improved overall and disease free survival\textsuperscript{18}. A follow up, randomized phase 2 clinical trial of hydroxychloroquine added to preoperative gemcitabine and nab-paclitaxel in patients with potentially resectable tumors noted that Evans grade histopathologic and CA 19–9 biomarker responses were significantly improved in patients receiving HCQ\textsuperscript{19}. The success of these early phase trials suggests a potential benefit to HCQ autophagy inhibition in pancreatic cancer.

SMAD4, a tumor suppressor gene, is mutated or deleted in 55\% of pancreatic cancers\textsuperscript{20}. Loss of SMAD4 is associated with pancreatic tumor progression, metastases\textsuperscript{12,21}, and is an important negative prognostic factor for overall survival\textsuperscript{22,23}. Increased levels of autophagy have been observed in pancreatic cancer cells with loss of SMAD4 and SMAD4 mediated autophagy has been implicated in treatment resistance in pancreatic cancer\textsuperscript{3}.

Because SMAD4 mutated or deleted pancreatic cancers have an increased reliance on autophagy for treatment resistance, we hypothesized that patients with SMAD4 tumor loss/mutation would derive the greatest benefit from autophagy inhibition with HCQ. In this retrospective analysis of two sequential prospective clinical trials, patients who previously received HCQ with neoadjuvant chemotherapy were evaluated according to SMAD4 status for associations with survival, Evans grade histopathologic response, R0 resection rates, and CA 19–9 biomarker response.
**Methods**

**Study Design:**

This was a retrospective analysis of two prospective clinical trials evaluating hydroxychloroquine in the preoperative setting for pancreatic cancer patients\(^4,24\). Institutional review board approval was obtained from the University of Pittsburgh for the clinical trials analyzed in the current work (PRO10010028, PRO13080444). The trials were registered with the National Cancer Institute (NCT01128296, NCT01978184). All patients signed informed consent prior to participation. Both trial protocols and consent forms included approval for analysis of tissue specimens and correlation with oncologic outcomes as performed in the current study. The first trial was a safety phase dose escalation (UPCI 09-122, NCT01128296) demonstrating safety and tolerability of 1 month of preoperative gemcitabine with up to 1200 mg/day of HCQ. This was followed with a randomized trial of two months of gemcitabine/nab-paclitaxel with or without 600 mg twice daily of HCQ in the preoperative setting (UPCI 13-074, NCT01978184) that demonstrated a significant increase in histopathologic and biochemical responses in patients receiving HCQ.

**Immunohistochemical Analysis of SMAD4 Expression:**

Assessment of SMAD4 was performed blinded to any other patient data including outcome. Standard automated immunohistochemical labeling on formalin-fixed, paraffin-embedded, 4 μm thick tissue sections was performed for SMAD4 (clone B-8, 1:500, Santa Cruz Biotechnology, Dallas, TX). Following deparaffinization with serial xylene treatments and rehydration in ethanol, the slides were stained using the Ventana BenchMark XT; the enzymatic reactivity was visualized with the iVIEW DAB Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ). Immunohistochemical scoring of SMAD4 expression was performed similar to those published previously\(^21,25\). Normal SMAD4 staining of stromal cells surrounding the malignant glands were used as an internal positive control. The SMAD4 staining was scored as follows: intact (strong nuclear and cytoplasmic staining in >10% of cells, *Figure 1B*) or lost (lack of staining in both the nuclear and cytoplasmic compartments, *Figure 1D*). Representative H&E staining for pancreatic tumors are show in *Figures 1A* and *1C*.

**Statistical Analysis:**

Data analysis was performed using SAS 9.1 (SAS Institute, Cary, NC) and R (version 3.6.3, R Foundation, Vienna, Austria). Descriptive statistical analyses were performed to summarize patient’s characteristics including summary tables, proportions, median, means, and standard deviations. Fisher exact test was used in the data analysis of SMAD4 status with other categorical variables, while Wilcoxon rank sum test was used in the data analysis of SMAD4 status with continuous variables. Kaplan-Meier method and log-rank test were used to examine overall survival (OS) and disease-free survival (DFS) by SMAD4 status. All statistical tests were two-sided and \(p < 0.05\) was considered statistically significant.

**Results**
Patient Selection

Of 93 patients enrolled in the prospective clinical trials, 17 patients were excluded from this analysis (Fig. 2). Five patients were not treated with the maximum dose of HCQ during the dose escalation phase, 10 patients did not have SMAD4 staining performed, and two patients were not resected and therefore had no tumor available for SMAD4 staining. Of patients treated with HCQ as part of these trials, 25 of the 52 had SMAD4 loss (48%), compared with 15 of the 24 patients treated with chemotherapy alone (63%) (p = 0.32). Patient demographics and clinical are reported in Table 1. Male patients made up a significantly lower percentage of the cohort with SMAD4 loss (36% versus 70%, p = 0.01). No other demographic differences between SMAD4 groups were identified.

Table 1
Patient Demographics stratified by SMAD4 status for Chemotherapy and HCQ treatment

|                      | SMAD4 Preserved (n = 27) | SMAD4 Loss (n = 25) | p-value |
|----------------------|--------------------------|---------------------|---------|
| Male (%)             | 19 (70)                  | 9 (36)              | 0.01    |
| Age (SD)             | 66 ± 10                  | 64 ± 8              | 0.19    |
| Pre-Treatment Ca 19 - 9 (SD) | 1821.6 ± 2927         | 1697.3 ± 3660       | 0.45    |
| CT Vascular Involvement (%) | 10 (37)               | 11 (44)             | 0.61    |
| EUS Size in cm (SD)  | 2.768 ± 0.711            | 2.85 ± 0.86         | 0.64    |
| EUS Stage > 2B (%)   | 15 (56)                  | 17 (68)             | 0.64    |
| Tumor Size in cm (SD)| 3.0815 ± 1.37           | 2.648 ± 1.36        | 0.13    |
| Adjuvant Chemo (%)   | 22 (81)                  | 23 (92)             | 0.27    |
| Tumor Stage (%)      |                          |                     |         |
|                      | 1                        | 2                   | 0.69    |
|                      | 2                        | 3 (12)              |         |
|                      | 3                        | 17 (63)             |         |
|                      | 4                        | 1 (3.1)             |         |
|                      | n/a                      |                     |         |
| Nodal Involvement (%)| 17 (63)                  | 17 (68)             | 0.7     |
| Evans Grade (%)      |                          |                     |         |
|                      | 3                        | 8 (30)              | 0.85    |
|                      | 2                        | 19 (70)             |         |
| Angiolympathic Invasion (%) | 19 (70)         | 20 (80)             | 0.42    |
| Perineural Invasion (%) | 24 (89)             | 20 (80)             | 0.49    |

*CT = Computed Tomography, EUS = Endoscopic Ultrasound
Impact of SMAD4 Status on Outcomes for Patients Treated with HCQ

Among the patients treated with HCQ, a higher rate of Evans grade histopathologic response was noted in those with SMAD4 loss as compared to SMAD4 intact (76% vs 37%, \( p = 0.006 \)) (Fig. 3). 92% of patients with SMAD4 loss obtained an R0 resection compared to only 67% with intact SMAD4 (\( p = 0.04 \)) (Table 2). There were no significant differences in CA 19 – 9 response between patients based on SMAD4 status.

|                          | SMAD4 Intact (n = 27) | SMAD4 Loss (n = 25) | \( p \)-value |
|--------------------------|-----------------------|---------------------|--------------|
| **Evans Grade**          |                       |                     |              |
| 1                        | 17 (63)               | 6 (24)              | 0.006        |
| \( \geq 2A \)             | 10 (37)               | 19 (76)             |              |
| **R0 Resection (%)**     |                       |                     |              |
| Yes                      | 9 (33)                | 2 (8)               | 0.039        |
| No                       | 18 (67)               | 23 (92)             |              |
| **Decrease in Ca 19 – 9 (%)** |                 |                     |              |
| < 50%                    | 7 (26)                | 4 (16)              | 0.4          |
| \( \geq 50–74\% \)       | 3 (11)                | 6 (24)              |              |
| \( \geq 75–89\% \)       | 11 (40)               | 6 (24)              |              |
| \( \geq 90\% \)          | 3 (11)                | 6 (24)              |              |
| N/A                      | 3 (11)                | 3 (12)              |              |
| **Percent Decrease of Ca 19 – 9 (Mean)** | 12.4 | 7.4 | 0.62 |
| **Median Ca 19 – 9 Post Treatment (IQR)** | 200.4 (39–547) | 42.7 (28–385) | 0.23 |
| **Median OS (mon)**      | 27.27                 | 34.43               | 0.18         |
| **Median DFS (mon)**     | 13.23                 | 15.43               | 0.49         |

Assessment of Survival in HCQ Treated Patients

Disease-free and overall survival curves for HCQ treated patients are reported in Supplemental Figs. 1 and 2, respectively. There was a nonsignificant trend toward improved median overall survival in patients treated with HCQ with SMAD4 loss (34.43 months vs. 27.27 months, \( p = 0.18 \)). There were no significant differences in disease free survival.

Discussion

Autophagy is emerging as an increasingly important therapeutic target in pancreatic cancer. The tumor suppressor gene SMAD4, mutated or deleted in 55% of pancreatic cancer, has been implicated in
treatment resistance via upregulation of autophagy\textsuperscript{3,20}. During radiation therapy, high volumes of intracellular free radicals are generated, producing cytotoxic oxidative damage in cancer cells\textsuperscript{26}. Cancer cells demonstrate increased expression of autophagy related genes and accumulation of autophagosomes after radiation exposure\textsuperscript{27}. The recycling of organelles during autophagy serves as a rescue from radiation damage, perhaps contributing to radio-resistance\textsuperscript{28}. Blockade of autophagy related genes results in radio-sensitization of carcinoma cells\textsuperscript{27}. Pancreatic cancer cells with SMAD4 knockdown demonstrate increased levels of autophagy and enhanced tolerance to irradiation. Both the restoration of SMAD4 expression and inhibition of autophagy using chloroquine results in increased radiation sensitivity\textsuperscript{3}.

Similar trends have been demonstrated during chemotherapy treatment. After treatment with gemcitabine, cellular markers of autophagy are upregulated\textsuperscript{9}. Studies \textit{in vitro} and \textit{in vivo} have proven that autophagy prevents pancreatic carcinoma cells from entering the apoptotic pathway after stimulus with gemcitabine, contributing to treatment resistance\textsuperscript{14}. Chloroquine and hydroxychloroquine serve as late inhibitors of autophagy by preventing fusion of the autophagosome and lysosome to block recycling of organelles\textsuperscript{29}. As an inhibitor of autophagy, hydroxychloroquine may improve tumor response to chemotherapy\textsuperscript{16,17}. In glioblastoma and chronic myeloid leukemia, the addition of chloroquine has improved response to chemotherapeutics and tyrosine kinase inhibitors respectively\textsuperscript{30,31}. Given the autophagy mediated treatment resistance in SMAD4 mutated or deleted pancreatic cancer cells, this study retrospectively examined the effect of HCQ with neoadjuvant chemotherapy according to SMAD4 status.

The addition of neoadjuvant HCQ was associated with improved R0 resection rates and higher degree of histopathologic response in patients with SMAD4 loss compared with SMAD4 intact. Previous studies have noted improved pathologic response rate\textsuperscript{19} and overall response rate\textsuperscript{32} in patients receiving concurrent neoadjuvant HCQ and chemotherapy. This analysis is the first to suggest specific benefit in patients with SMAD4 loss. This may indicate a role for delivery of HCQ especially to patients with SMAD4 loss in order to improve tumor resectability and inform patient selection for future studies on HCQ or other emergent and experimental autophagy inhibitors\textsuperscript{32–36}.

Both R0 resection and histopathologic response have been associated with improved survival in pancreatic cancer\textsuperscript{37}. While the clinical studies examined were not sufficiently powered to identify survival benefit, the observed trends are of interest. Loss of SMAD4 is generally associated with decreased overall survival\textsuperscript{22,23}, while in patients treated with HCQ as part of these studies, survival trends were similar regardless of SMAD4 status. SMAD4 loss also did not appear to be associated with a detriment in disease-free survival in patients receiving HCQ. A study in patients with advanced pancreatic cancer did not detected survival benefit with the addition of HCQ to gemcitabine and nab-paclitaxel\textsuperscript{19,32}, however a dedicated sub-group analysis to SMAD4 has not been performed. Additional studies according to SMAD4 mutational status could be considered to explore possible survival benefits in patients with SMAD4 loss.
This study is limited by its retrospective nature. The use of combined data from two individual chemotherapy regimens and durations from the included prospective clinical trials may also confound findings. In addition, the relatively small sample size precludes a multivariate analysis to determine independent predictors of histopathologic response. As a result, these data must be interpreted with caution and conclusions are limited. Prospective studies on the role of autophagy inhibition and SMAD4 loss in pancreatic cancer are warranted.

**Conclusion**

Prognosis in PDA is worsened by loss of the tumor suppressor gene SMAD4. SMAD4 deficient PDA escape radiotherapy and chemotherapy by upregulation of autophagy. In patients with SMAD4 loss, the addition of HCQ to neoadjuvant chemotherapy improved R0 resection rates and resulted in higher degree of histopathologic response. Patients with SMAD4 who received HCQ with neoadjuvant chemotherapy also displayed improved disease free survival and overall survival trends, though significance was not met. Further study of autophagy inhibition with HCQ in PDA with SMAD4 loss is warranted.

**Abbreviations**

PDA
Pancreatic Adenoarcinoma
HCQ
Hydroxychloroquine

**Declarations**

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE:**

Institutional review board approval was obtained from the University of Pittsburgh for the clinical trials analyzed in the current work (PRO10010028, PRO13080444). The trials were registered with the National Cancer Institute (NCT01128296, NCT01978184). All patients signed informed consent prior to participation. Both trial protocols and consent forms included approval for analysis of tissue specimens and correlation with oncologic outcomes as performed in the current study.

**CONSENT FOR PUBLICATION:**

The authors consent to publication of this material by the *Journal of Cancer Research and Clinical Oncology*. The authors guarantee that the contribution to the work has not been previously published elsewhere.

**AVAILABILITY OF DATA AND MATERIAL:**
The data that support the findings of this study are available from the corresponding author, BB, upon reasonable request.

COMPETING INTERESTS:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHORS CONTRIBUTIONS:

RR, MH, AZ, ML, NB, AS, HZ, and BB conceived and planned the experiments. NF and SW analyzed the data. NF wrote the manuscript with input from all authors. BB, HZ, ML, NB and AZ conceived the study and were in charge of overall direction and planning. BB supervised the project.

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References

1. Rawla P, Sunkara T, Gaduputi V: Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. World journal of oncology 10:10-27, 2019
2. McGuigan A, Kelly P, Turkington RC, et al: Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol 24:4846-4861, 2018
3. Wang F, Xia X, Yang C, et al: Gene Mutation Renders Pancreatic Cancer Resistance to Radiotherapy through Promotion of Autophagy. Clinical Cancer Research 24:3176-3185, 2018
4. Boone BA, Zeh HJ, 3rd, Bahary N: Autophagy Inhibition in Pancreatic Adenocarcinoma. Clin Colorectal Cancer 17:25-31, 2018
5. Kim SE, Park HJ, Jeong HK, et al: Autophagy sustains the survival of human pancreatic cancer PANC-1 cells under extreme nutrient deprivation conditions. Biochem Biophys Res Commun 463:205-
10. Yang S, Wang X, Contino G, et al: Pancreatic cancers require autophagy for tumor growth. Genes Dev 25:717-29, 2011

7. Hashimoto D, Blauer M, Hirota M, et al: Autophagy is needed for the growth of pancreatic adenocarcinoma and has a cytoprotective effect against anticancer drugs. Eur J Cancer 50:1382-90, 2014

8. Donadelli M, Dando I, Zaniboni T, et al: Gemcitabine/cannabinoid combination triggers autophagy in pancreatic cancer cells through a ROS-mediated mechanism. Cell Death Dis 2:e152, 2011

9. Mukubou H, Tsujimura T, Sasaki R, et al: The role of autophagy in the treatment of pancreatic cancer with gemcitabine and ionizing radiation. Int J Oncol 37:821-8, 2010

10. Saglar E, Unlu S, Babalioglu I, et al: Assessment of ER Stress and autophagy induced by ionizing radiation in both radiotherapy patients and ex vivo irradiated samples. J Biochem Mol Toxicol 28:413-7, 2014

11. Chen X, Yu Q, Liu Y, et al: Synergistic cytotoxicity and co-autophagy inhibition in pancreatic tumor cells and cancer-associated fibroblasts by dual functional peptide-modified liposomes. Acta Biomater 99:339-349, 2019

12. Bardeesy N, Cheng KH, Berger JH, et al: Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. Genes Dev 20:3130-46, 2006

13. Mirzoeva OK, Hann B, Hom YK, et al: Autophagy suppression promotes apoptotic cell death in response to inhibition of the PI3K-mTOR pathway in pancreatic adenocarcinoma. J Mol Med (Berl) 89:877-89, 2011

14. Papademetrio DL, Cavaliere V, Simunovich T, et al: Interplay between autophagy and apoptosis in pancreatic tumors in response to gemcitabine. Target Oncol 9:123-34, 2014

15. Kenzelmann Broz D, Spano Mello S, Bieging KT, et al: Global genomic profiling reveals an extensive p53-regulated autophagy program contributing to key p53 responses. Genes Dev 27:1016-31, 2013

16. Livesey KM, Tang D, Zeh HJ, et al: Autophagy inhibition in combination cancer treatment. Curr Opin Investig Drugs 10:1269-79, 2009

17. Amaravadi RK, Lippincott-Schwartz J, Yin X-M, et al: Principles and Current Strategies for Targeting Autophagy for Cancer Treatment. Clinical Cancer Research 17:654-666, 2011

18. Boone BA, Bahary N, Zureikat AH, et al: Safety and Biologic Response of Pre-operative Autophagy Inhibition in Combination with Gemcitabine in Patients with Pancreatic Adenocarcinoma. Ann Surg Oncol 22:4402-10, 2015

19. Zeh HJ, Bahary N, Boone BA, et al: A Randomized Phase II Preoperative Study of Autophagy Inhibition with High-Dose Hydroxychloroquine and Gemcitabine/Nab-Paclitaxel in Pancreatic Cancer Patients. Clin Cancer Res, 2020

20. Hahn SA, Schutte M, Shamsul Hoque ATM, et al: <em>DPC4</em>, A Candidate Tumor Suppressor Gene at Human Chromosome 18q21.1. Science 271:350-353, 1996
21. Boone BA, Sabbaghian S, Zenati M, et al: Loss of SMAD4 staining in pre-operative cell blocks is associated with distant metastases following pancreaticoduodenectomy with venous resection for pancreatic cancer. J Surg Oncol 110:171-5, 2014

22. Blackford A, Serrano OK, Wolfgang CL, et al: <em>SMAD4</em> Gene Mutations Are Associated with Poor Prognosis in Pancreatic Cancer. Clinical Cancer Research 15:4674-4679, 2009

23. Tascilar M, Skinner HG, Rosty C, et al: The SMAD4 Protein and Prognosis of Pancreatic Ductal Adenocarcinoma. Clinical Cancer Research 7:4115-4121, 2001

24. Lotze MT, Boone BA, Zureikat AH, et al: Phase I/II trial of autophagy inhibition in combination with neoadjuvant gemcitabine in patients with high-risk pancreatic adenocarcinoma: Safety, clinical response, and correlative studies. Journal of Clinical Oncology 32:218-218, 2014

25. Singhi AD, Foxwell TJ, Nason K, et al: Smad4 loss in esophageal adenocarcinoma is associated with an increased propensity for disease recurrence and poor survival. Am J Surg Pathol 39:487-95, 2015

26. Chen Y, McMillan-Ward E, Kong J, et al: Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells. Cell Death Differ 15:171-82, 2008

27. Apel A, Herr I, Schwarz H, et al: Blocked autophagy sensitizes resistant carcinoma cells to radiation therapy. Cancer Res 68:1485-94, 2008

28. Amaravadi RK, Yu D, Lum JJ, et al: Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 117:326-36, 2007

29. Mackenzie AH: Dose refinements in long-term therapy of rheumatoid arthritis with antimalarials. Am J Med 75:40-5, 1983

30. Sotelo J, Briceño E, López-González MA: Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. Ann Intern Med 144:337-43, 2006

31. Bellodi C, Lidonnici MR, Hamilton A, et al: Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. J Clin Invest 119:1109-23, 2009

32. Karasic TB, O'Hara MH, Loaiza-Bonilla A, et al: Effect of Gemcitabine and nab-Paclitaxel With or Without Hydroxychloroquine on Patients With Advanced Pancreatic Cancer: A Phase 2 Randomized Clinical Trial. JAMA Oncol 5:993-998, 2019

33. Sharma G, Ojha R, Noguera-Ortega E, et al: PPT1 inhibition enhances the antitumor activity of anti-PD-1 antibody in melanoma. JCI Insight 5, 2020

34. Cechakova L, Ondrej M, Pavlik V, et al: A Potent Autophagy Inhibitor (Lys05) Enhances the Impact of Ionizing Radiation on Human Lung Cancer Cells H1299. Int J Mol Sci 20, 2019

35. Amaravadi RK, Kimmelman AC, Debnath J: Targeting Autophagy in Cancer: Recent Advances and Future Directions. Cancer Discovery 9:1167-1181, 2019

36. Rebecca VW, Nicastri MC, Fennelly C, et al: PPT1 Promotes Tumor Growth and Is the Molecular Target of Chloroquine Derivatives in Cancer. Cancer Discovery 9:220-229, 2019
37. Chun YS, Cooper HS, Cohen SJ, et al: Significance of pathologic response to preoperative therapy in pancreatic cancer. Ann Surg Oncol 18:3601-7, 2011

Figures

Figure 1

Representative images of SMAD4 staining. Representative H&E staining in (A) and (C) of pancreatic cancer specimens. SMAD4 was scored intact with strong nuclear and cytoplasmic staining in >10% of cells (B) or lost with lack of staining in both the nuclear and cytoplasmic compartments (D).

Figure 2

Patients enrolled in two prospective clinical trials, retrospectively stratified by SMAD4 status.
Figure 3

Evans Grade histopathologic response in HCQ treated pancreatic cancer patients stratified by SMAD4 status. Patients with loss of SMAD4 had significant higher histopathologic response to treatment than patients with SMAD4 intact.

Supplementary Files

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