nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Chromium Controller instrument v2 and v3 (10x Genomics); HiSeq X Version 2.5 (Illumina); GeoMx Digital Spatial Profiling instrument (NanoString)
Data analysis	snRNA-seq: bcl2fast2-v2.20, CellRanger v3.0.2, Scrublet v0.2.3, Scanpy v1.7.2, InferCNV v3.9, CellBender (snapshot 11), Harmony-Pytorch v0.1.7, scCODA v0.1.2.post1, PAGA v1.2, glmer v3.1-152 Multiplexed Ion Beam Imaging (MIBI): MIBItracker v1.9.2, histoCAT v1.76, Ilastik v1.3.3post2, CellProfiler v3.1.9, scvi-tools v0.8.1 Receptor-ligand analysis: CellPhoneDB v.2.0 Consensus non-negative matrix factorization: scikit-learn v0.22.2, cNMF v1.1 (with custom modifications found at https://github.com/karthikj89/humanpdac/blob/master/src/cnmf.py) Gene set enrichment analysis: MSigDB v7.1 Bulk RNA-seq and survival analysis: RSEM v1.3.3, STATA/SE 15.1 Digital spatial profiling analysis: GeoMx Digital Spatial Profiling software (Nanostring), custom R code, ssGSEA v2.0, Geomx Software version 2.3.0.268, SpatialDecon v0.99.1, Azorius and Hydra: https://github.com/whwanglab/PDAC/tree/main/src/R All code has been made available in Github at https://github.com/karthikj89/humanpdac (DOI 10.5281/zenodo.6496927) and https://github.com/whwanglab/PDAC (DOI 10.5281/zenodo.6496573).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All figures are associated with raw data. Raw images for MIBI and Nanostring GeoMx experiments are publicly available on Science Data Bank (DOI 10.57760/sciencedb.01706). Raw and processed sequencing data (single-nucleus RNA-seq) for patient-derived tumors and organoids, and Nanostring GeoMx reads and count matrices have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession numbers GSE202051 and GSE199102, respectively. Raw single-nucleus RNA-seq data are also available in the controlled access repository DUOS (https://duos.broadinstitute.org/), under dataset ID 000139. Processed single-nucleus RNA-seq data are also available on the Single Cell Portal at: https://singlecell.broadinstitute.org/single_cell/study/SCP1089 (untreated) https://singlecell.broadinstitute.org/single_cell/study/SCP1096 (treated)

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	This study involved excess human specimens and data exclusively. Sample sizes were determined based on availability of patient specimens with a goal of at least 5 patients in each group (untreated, CRT, CRTL, other treatment) based on prior studies.
Data exclusions	For analysis of publicly-available bulk RNA-seq data from TCGA/ICGC/PanCuRx, patients with metastatic disease, neuroendocrine histology, or neoadjuvant treatment were excluded. For snRNA-seq/MIBI/DSP, patients with metastatic disease were not included in the study. We did not exclude any data from study patients.
Replication	Broad replication could not be undertaken due to the nature and scarcity of the unique human specimens used in this study. However, an assessment of the effect of sequencing depth was undertaken for one representative specimen where re-sequencing to higher depth yielded similar results without adding significant new biological information above and beyond the initial sequencing run. Bulk RNA-seq was performed on a subset of snRNA-seq specimens and comparison of the matched bulk RNA-seq and aggregate snRNA-seq data yielded comparable results.
Randomization	There was no randomization in this study. As described above, excess patient-derived specimens were obtained based on availability. Patients were included in the treated cohort if they received some form of neoadjuvant chemotherapy and/or radiotherapy. Future studies will include integration of the techniques and biological insights derived from this study into randomized controlled trials.
Blinding	Blinding was not possible due to acquisition of patient samples based on clinical availability. However, blinding was less relevant because the primary outcomes of interest were related to molecular differences between the treated and untreated cohorts and how these differences were associated with treatment efficacy and other clinical outcomes.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

MIBI (IONpath): subset of Epithelial i-Onc panel with the following custom conjugated antibodies
- dsDNA, 3519 DNA, channel 89
- CD31, EP3095, channel 152
- vimentin, D21H3, channel 163
- pan-cytokeratin, AE1/AE3, channel 165
- CD45, 2B11+PD7/26, channel 175

NanoString (DSP): conjugated antibodies
- pan-cytokeratin, AE1/AE3, AF532, cat. no. NBP2-33200AF532 (Novus Biologicals)
- CD45, D9M8I, AF594, cat. no. 13917S (Cell Signaling Technology)
- alpha-SMA, 1A4, AF647, cat. no. IC1420R (Novus Biologicals)

Multiplexed IF: antibodies from Invitrogen
- NRXN3, rabbit polyclonal IgG, cat. no. PAS-101708
- Goat anti-rabbit IgG AF 647, cat. no. A-21245

Validation

MIBI (IONpath): Each lot of conjugated antibody was quality control tested by MIBIscopeTM analysis of stained tissue microarray using the appropriate positive and negative tissue fields of views and were pathologist verified. This included IHC and MIBI staining on FFPE human placenta, human tonsil, mouse liver, and/or human thymus. In this study, these antibodies were used to stain human pancreatic tumor.
Nanostring (DSP) and multiplexed IF: All conjugated antibodies were validated for human FFPE samples as described previously in Merritt CR et al., Nat Biotech 2020

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Cultrex HA-R-Spondin1-Fc 293T cell line; PDAC_U_12 primary human pancreatic ductal adenocarcinoma organoid line

Authentication

The Cultrex HA-R-Spondin1-Fc 293T cell line was authenticated by titration of conditioned media concentration that demonstrates the expected dose-dependent growth of organoids in culture.
The PDAC_U_12 organoid line was grown in complete organoid media as defined in Freed-Pastor et al., Cancer Cell 2021 and authenticated as malignant epithelial in origin by spheroid formation in Matrigel and single-nucleus RNA-seq analysis.

Mycoplasma contamination

The above cell/organoid lines tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Human subjects who had resected, non-metastatic primary pancreatic ductal adenocarcinoma (PDAC) and provided informed consent for protocol 2003P001289 (Massachusetts General Hospital IRB) were included in this study and divided into an untreated group (no pancreatic cancer-directed therapy prior to surgical resection) and treated group (chemotherapy, radiotherapy, and/or additional therapies prior to surgical resection). Age, sex, and genotypic information were not part of the inclusion criteria.

Recruitment

Protocol 2003P001289 has been banking frozen primary PDAC specimens for almost two decades and broad consent for molecular studies (including transcriptomic and genomic analyses) was obtained prior to resection. The protocol includes consent for the generation of immortalized tumor cell lines (patient-derived organoids). Patients were not explicitly recruited for this study but chosen based on their status of having non-metastatic PDAC and having a specimen with an RNA integrity number (RIN; Agilent RNA 6000 Pico Kit) greater than an empirically determined threshold of 6 as described in the Methods. No other sources of significant selection bias were identified.

Ethics oversight

Massachusetts General Hospital Institutional Review Board

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

1. NCT01821729; 2. NCT03563248

Study protocol

1. Available on ClinicalTrials.gov (updated August 7, 2018); 2. Not yet published (anticipated will be available later in 2022)

Data collection	1. This Phase II study began in July 2013 and had an actual primary completion date of July 2018. The study took place at Massachusetts General Hospital, in collaboration with the National Cancer Institute. Participants were 18+ years of age with cytologic or histologic proof of PDAC, locally advanced unresectable disease, and life expectancy of at least 3 months. 2. This Phase II study began on August 10, 2018 and had an estimated primary completion date of December 31, 2021. The study is sponsored by Massachusetts General Hospital and is taking place in multiple locations throughout the United States. Participants were 18+ years of age with confirmed localized PDAC that is borderline/potentially resectable or locally advanced. Details regarding additional inclusion criteria can be found on ClinicalTrials.gov. Specimens from this trial that were included in this study were from patients treated at Massachusetts General Hospital.
Outcomes	1. The primary outcome measured the number of participants that received treatment with proton radiation along with FOLFIRINOX-Losartan and then subsequently underwent attempted surgery and achieved R0 resection. The results indicated 34 out of 49 (69.4%) participants achieved R0 resection. The secondary outcome was to determine the progression-free survival of patients with locally advanced disease who receive FOLFIRINOX-Losartan and proton beam radiation therapy. Disease progression was assessed using Response Evaluation Criteria in Solid Tumors (RECIST 1.1). The time frame was measured from the start of treatment until death or progression, and the median duration was 17.5 months. 2. The primary outcome measures the proportion of participants with R0 resection. The secondary outcomes included progression-free survival, overall survival, pathologic complete response, and the number of participants with treatment related serious adverse events. Results have not yet been published.