The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL

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Bacterial dysbiosis accompanies carcinogenesis in malignancies such as colon and liver cancer, and has recently been implicated in the pathogenesis of pancreatic ductal adenocarcinoma (PDA)1. However, the mycobiome has not been clearly implicated in tumorigenesis. Here we show that fungi migrate from the gut lumen to the pancreas, and that this is implicated in the pathogenesis of PDA. PDA tumours in humans and mouse models of this cancer displayed an increase in fungi of about 3,000-fold compared to normal pancreatic tissue. The composition of the mycobiome of PDA tumours was distinct from that of the gut or normal pancreas on the basis of alpha- and beta-diversity indices. Specifically, the fungal community that infiltrated PDA tumours was markedly enriched for Malassezia spp. in both mice and humans. Ablation of the mycobiome was protective against tumour growth in slowly progressive and invasive models of PDA, and re-population with a Malassezia species—but not species in the genera Candida, Saccharomyces or Aspergillus—accelerated oncogenesis. We also discovered that ligation of mannose-binding lectin (MBL), which binds to glycans of the fungal wall to activate the complement cascade, was required for oncogenic progression, whereas deletion of MBL or C3 in the extratumoral compartment—or knockdown of C3AR in tumour cells—were both protective against tumour growth. In addition, reprogramming of the mycobiome did not alter the progression of PDA in Mbl−/− (also known as Mbl2) or C3-deficient mice. Collectively, our work shows that pathogenic fungi promote PDA by driving the complement cascade through the activation of MBL.

It has recently been reported that intrapancreatic bacteria expand by about 1,000-fold in PDA1. Here we show that there is a similar and marked increase in intratumoral fungi in PDA and in mouse models of this disease (Fig. 1a–d). Because there is direct communication between the gut and pancreatic duct via the sphincter of Oddi, we postulated that endoluminal fungi can access the pancreas. To test this, we administered Saccharomyces cerevisiae labelled with green fluorescent protein (GFP) to control and tumour-bearing mice via oral gavage. Fungi migrated into the pancreas within 30 min, which suggests that the gut mycobiome can directly influence the pancreatic microenvironment (Fig. 1e).

We next assessed whether there is evidence of fungal dysbiosis during tumorigenesis, using p48cre;Lsl−/−; KrasG12D (p48 is also known as Ptf1a) mice (hereafter referred to as KC mice), which express oncogenic Kras in their pancreatic progenitor cells and are a model for the development of slowly progressive PDA2. A comparison between the fungal communities of the gut and within the pancreas in 30-week-old KC mice, by principal coordinate analysis (PCoA), suggested that the mycobiomes of the gut and tumours clustered separately (Fig. 1f). We also observed reduced alpha-diversity in the transformed pancreas compared with the gut (Fig. 1g). Ascomycota and Basidiomycota were the only phyla that were detected in pancreatic tissue, whereas Mortierellomycota and Mucoromycota were also detected in the gut at a low abundance (Fig. 1h). The most-prevalent genus in the pancreata of KC mice was Malassezia, at about 20% abundance; this represents a marked increase in relative abundance compared to the presence of this genus in the gut (Fig. 1i). Of note, benign pancreatic inflammation did not increase fungal infiltration into the pancreas (Extended Data Fig. 1).

To determine whether the gut mycobiome is reprogrammed during the course of oncogenesis, we performed a longitudinal analysis of faecal samples from KC mice and littermate controls. PCoA suggested that, whereas wild-type and KC mice had similar fungal communities early in life, by 30 weeks of age there were differences in beta-diversity between the gut mycobiomes in the two backgrounds (Fig. 1j–l). Accordingly, fungal communities in the gut of KC and wild-type mice differed considerably at 30 weeks (Extended Data Fig. 2).

We next analysed the faecal and tumour mycobiome in patients with PDA. As in mice, Ascomycota and Basidiomycota were the most common phyla in the gut and in tumour tissue of humans (Fig. 2a). At the genus level (and once again parallel to our mice data), Malassezia was more prevalent in tumour tissues than in the gut (Fig. 2b). Moreover, alpha-diversity analyses revealed considerable differences between the gut and PDA-tissue in humans (Fig. 2c). PCoA confirmed that there were distinct clusters of fungal communities in the tumour tissue and gut of patients with PDA (Fig. 2d). Furthermore, the mycobiome in pancreata from patients with PDA clustered separately from that in the pancreata of healthy individuals (Fig. 2e). Collectively, these data indicate that the mycobiome of PDA tumours is distinct from that of the gut or healthy pancreas.

To determine the influence of fungal dysbiosis on the progression of PDA, we ablated the mycobiome using oral administration of amphotericin B in the KC mouse model. Ablation of the mycobiome protected the mice against oncogenic progression (Fig. 3a). Similarly, amphotericin B was protective against tumour progression in an aggressive orthotopic model of PDA that uses tumour cells derived from Pdx1cre; KrasG12D; Tp53R172H (Tp53 is also known as Trp53) mice (hereafter, KPC mice)3 (Fig. 3b). Ablation of the mycobiome potentiated the effect of chemotherapy based on gemcitabine (Fig. 3c). Of note, treatment with flucanazole was also protective against tumour progression (Extended Data Fig. 3a). However, treatment with antifungal agents did not offer protection against tumour growth in germ-free mice (Extended Data Fig. 3b). Furthermore, consistent with absence of increased fungal infiltration in pancreatitis, treatment with antifungal agents did not ameliorate benign pancreatic inflammation (Extended Data Fig. 3c–e).

To confirm that fungal dysbiosis accelerates the progression of PDA, we re-populated mice treated with amphotericin B with Malassezia

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https://doi.org/10.1038/s41586-019-1608-2
**globsosa**, which is present at an increased abundance in PDA and in mouse models of this cancer (Figs. 1i, 2b). Of note, the *M. globosa* ATCC strain that we used in our repopulation experiments had 100% sequence identity to the *Malassezia* taxon that was the most abundant in PDA (Supplementary Table 1). Control mice were repopulated with *Candida* sp., *S. cerevisiae* or *Aspergillus* sp. or treated with vehicle. Of these, only *M. globosa* accelerated the growth of PDA tumours; the other taxa, and treatment with vehicle, had no effect (Fig. 3d). Repopulation with *Candida tropicalis* also did not accelerate the growth of PDA tumours (Extended Data Fig. 3f).

MBL is a mannose-binding lectin that recognizes fungal pathogens and activates the lectin pathway of the complement cascade. Expression of *MBL* (also known as *MBL2*) was associated with reduced survival in patients with PDA, on the basis of transcriptomic data from The Cancer Genome Atlas (TCGA) (Extended Data Fig. 4a). We postulated that *MBL* may promote tumorigenesis via activation of MBL. Accordingly, MBL-null KC mice exhibited delayed oncogenic progression (Fig. 4a). Deletion of *Mbl* was also protective against the growth of orthotopic tumours of KPC cells, and resulted in extended survival of the mice (Fig. 4b, c). Moreover, treatment with amphotericin B did not provide protection against tumour growth in MBL-null mice (Extended Data Fig. 4b). Similarly, *Malassezia*—which binds C-type lectin receptors—did not accelerate tumour progression in MBL-null mice (Extended Data Fig. 4c).

The C3 complement cascade has previously been investigated in PDA and other cancers, and is potent oncogenic by diverse mechanisms that include increasing the proliferation motility and invasiveness of tumour cells, and promoting adaptive immune responses. Because MBL initiates the lectin pathway of the complement cascade that triggers C3 convertase, we postulated that the fungus–MBL axis promotes the progression of PDA via complement activation. Similar to MBL, the expression of C3 was associated with a trend towards reduced survival in patients with PDA (Extended Data Fig. 4d). We found robust expression of C3a in the pancreas of KC mice, and this was nearly absent in wild-type or MBL-null KC mice (Extended Data Fig. 4e). Consistent with our hypothesis, recombinant C3a accelerated the proliferation of KPC cells in vitro (Extended Data Fig. 4f) and the growth of KPC tumours in vivo (Fig. 4d), whereas C3-deficient mice were protected against PDA progression (Fig. 4e). Similarly, knockdown of C3AR in PDA cells (Fig. 4f) mitigated tumour growth (Fig. 4g).

Moreover, we found that targeting the mycobiome had no additional effect in C3-deficient animals (Fig. 4h). In aggregate, these data indicate that the pancreatic mycobiome requires the MBL–C3 axis to promote tumour growth.

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**Fig. 1** | **PDA is characterized by a distinctive intratumoral and gut mycobiome.** a. The abundance of intrapancreatic fungi was compared between healthy individuals and patients with PDA who were matched for age, gender and body mass index, using fluorescent in situ hybridization (FISH). n = 3 individuals per group. Representative images are shown. Scale bar, 20 μm. b. The abundance of intrapancreatic fungi was compared in three-month-old, littermate wild-type (WT) and KC mice by FISH. Representative images are shown. n = 3 mice per group. Scale bar, 20 μm. c. Fungal DNA content was compared in the pancreata of healthy individuals and patients with PDA who were matched for age, gender and body mass index, using quantitative PCR (qPCR). d. Fungal DNA content was compared in the pancreata of three-month-old wild-type and KC mice, using qPCR. e. GFP-labelled *S. cerevisiae* was administered to 30-week-old KC mice by FISH. The abundance of intrapancreatic fungi was compared in the pancreata of three-month-old wild-type and KC mice by FISH. f. The abundance of intrapancreatic fungi was compared in the pancreata of three-month-old wild-type and KC mice by FISH.

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**Notes:**

1. The Cancer Genome Atlas (TCGA).
2. Mannose-binding lectin.
3. Mannose-binding lectin.
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9. Mannose-binding lectin.
PDA in humans is associated with a distinct mycobiome. 

**Fig. 2** | **PDA in humans is associated with a distinct mycobiome.** 

**a-d.** Gut and tumour (n = 18 and 13 biologically independent specimens, respectively) specimens from patients with PDA were analysed by 18S ITS sequencing. Taxonomic composition of mycobiota assigned to the phylum level, on the basis of their average relative abundance. Data are mean ± s.e.m. **b.** Hierarchical tree cladogram, depicting differences between the gut and tumours in terms of the taxonomic composition of mycobiota assigned to the genus level (on the basis of their average relative abundance). **c.** The gut and tumour mycobiomes of patients with PDA (n = 13) fungal communities in patients with PDA, based on a Bray–Curtis dissimilarity matrix, as in Fig. 1f. **e.** PCoA plots of fungal communities in pancreata of patients with PDA (n = 13) and healthy individuals (n = 5), based on a Bray–Curtis dissimilarity matrix. P values determined by two-tailed Student’s t-test (a), two-sided Wilcoxon rank-sum test (c) or pairwise PERMANOVA (d, e).

Fungal dysbiosis promotes pancreatic oncogenesis. 

**Fig. 3** | **Fungal dysbiosis promotes pancreatic oncogenesis.** 

**a.** KC mice treated with amphotericin B (ampho.) or vehicle were killed at three months old. Pancreatic weights (n = 9 mice) and tumour weights (n = 9 mice) were recorded. Representative sections stained with haematoxylin and eosin (H&E) or trichrome. The percentage of preserved acinar area, and the fraction of normal ducts, acinar ductal metaplasia (ADM) and graded (I and II) pancreatic intraepithelial neoplasia (PanIN) lesions were determined on the basis of H&E staining. The fraction of fibrotic area per pancreas was calculated on the basis of trichrome staining. **b.** Wild-type mice that bear orthotopic PDA tumours were treated with vehicle or amphotericin B (n = 16 mice per group, data pooled from 3 independent experiments) and killed three weeks later. Tumours were collected and weighed. Data are representative of at least five experiments. **c.** Wild-type mice that bear orthotopic PDA tumours were treated with vehicle (n = 9 mice), amphotericin B (n = 6 mice), gemcitabine (gem.) (n = 8 mice) or amphotericin B and gemcitabine (n = 6 mice). Tumour weight was recorded after three weeks of treatment. **d.** Wild-type mice treated with amphotericin B were repopulated with *M. globosa* (n = 8 mice), *S. cerevisiae* (n = 9 mice), *Candida* sp. (n = 8 mice), *Aspergillus* sp. (n = 10 mice) or vehicle (n = 8 mice), and killed three weeks later. Tumours were collected and weighed. Data are representative of two experiments. Scale bars, 200 μm (a), 1 cm (b–d). Data are mean ± s.e.m. P values determined by two-tailed Student’s t-test (a–d).
In summary, we found that fungi migrate from the gut to the pancreas, and PDA tumours contain a marked expansion in the pancreatic mycobiome. The composition of the PDA mycobiome was distinct from that of the gut or normal pancreas, and was enriched for *Malassezia* species in both mice and humans. Ablation of the mycobiome was protective against progression of PDA, and repopulation with species of *Malassezia*—but not with other commensal fungi—accelerated oncogenesis. Whether the reprogramming of the mycobiome is a cause or consequence of oncogenesis is difficult to answer fully. However, our fungal adoptive-transfer and fungal-ablation experiments suggest that particular species of fungi are sufficient to promote the progression of PDA. It is likely that—akin to observations regarding the microbiome—*inflammation induced by oncogenic Kras* leads to fungal dysbiosis, which in turn promotes tumour progression via the activation of the MBL–C3 cascade (Fig. 4i).

As the mycobiome influences the microbiome and vice versa, further study is required to assess this dynamic crosstalk in the pathogenesis of PDA. Our work suggests that the mycobiome may be a new target for therapeutic agents, and an area for the discovery of biomarkers.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at [https://doi.org/10.1038/s41586-019-1608-2](https://doi.org/10.1038/s41586-019-1608-2).

Received: 13 February 2019; Accepted: 5 September 2019; Published online 2 October 2019.

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 METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Mice and tumour models. KC mice, which develop spontaneous pancreatic neoplasia by targeted expression of mutant Kras in the pancreas,12 were a gift from D. Bar-Sagi. C57BL/6, MBL-null and C3 -/− mice were originally purchased from Jackson Laboratories and were bred in-house. All experiments were performed on animals that were sex- and age-matched within each experiment. All mice were negative for mycoplasma within the past two months. In select experiments, mice received a single intratumoral injection of recombinant mouse C3a (40 μg/kg; R&D) on day 14 after injections of orthotopic tumours. Mice with pancreatic tumours were monitored regularly for size, body weight, and survival. Mice were euthanized by CO2 inhalation. Tumours were excised and placed in 10% buffered formalin for histological analysis.

Human sample collection and data from TCGA. Human faecal samples and specimens of pancreatic tissue were collected under sterile conditions from healthy volunteers and patients undergoing surgery for PDA or for pancreatic endocrine tumours (benign disease) at NYU Langone Medical Center. Donors were de-identified. Samples were stored at −80°C until analysis. Patients who had received antibiotic or antifungal treatment within the past three months were excluded. Human specimen were collected under protocol and approval of the Institutional Review Board at NYU School of Medicine’s Institutional Review Board. Tumours (benign disease) at NYU Langone Medical Center. Donors were de-identified. Samples were stored at −80°C until analysis. Patients who had received antibiotic or antifungal treatment within the past three months were excluded. Human samples were collected in compliance with the policies and approval of the National Cancer Institute’s TCGA (https://portal.gdc.cancer.gov/). Survival was measured according to the Kaplan–Meier method, and analysed using the log-rank test.

Extraction and sequencing of fungal DNA. Samples of pancreatic tissue were suspended in 500 μl sterile PBS, and pretreated by vortexing and sonication, followed by overnight treatment with proteinase K (2.5 μg/ml; Thermo Fisher) at 55°C. Total microbial genomic DNA was purified from tissue and faecal samples using the MoBio Power kit, as per the manufacturer’s instructions (MoBio Laboratories). DNA was quantified for concentration and purity using the NanoDrop 2000 spectrophotometer (Thermo Fisher) and stored at −20°C. DNA was extracted from human and mouse tissues by FISH. Fluorescence microscopic analysis was conducted with Nikon Eclipse 90i confocal microscope (Nikon) using a Cy3-labelled probe at 555 nm (extinction wavelength, 555 nm and emission wavelength, 570 nm; Molecular Probes) was used to detect the fungal colonization within human and mouse pancreatic tissues by FISH. Fluorescence microscopic analysis was conducted with Nikon Eclipse 90i confocal microscope (Nikon) using a Cy3-labelled probe at 555 nm (extinction wavelength, 555 nm and emission wavelength, 570 nm; Molecular Probes). The fraction and number of ducts that contained any grade of PanIN lesions were measured by examining 10 H&E-stained high-power fields (×200) and a final extension of 72°C (10 min). After each PCR cycle, purified libraries of AMPure XP beads were flushed for purity by Nanodrop, quantified by Picogreen assay and sizes were confirmed on agarose gels. Negative controls were included in all sequencing runs. Equimolar amounts of the generated libraries were combined and quantified fluorometrically. The pooled amplicon library was denatured, diluted and sequenced on an Illumina MiSeq platform using MiSeq Reagent Kit v3 (600 cycles) following the 2× 300-base pair-end sequencing protocol.

Bioinformatics and statistical analyses. The Illumina-generated fungal ITS data were processed using the QIIME (v1.9.1) and the reads were demultiplexed, quality-filtered and clustered into OTUs using default parameters. To maintain consistency, read 1 was used for the analyses, as previously described. Before demultiplexing, the 5′ primers of a total 16,647,630 R1 reads were trimmed forward, TAACCCAGTATGACCACTAAA and reverse, TGTTGAATTTGTG TGCATTTG.

Histology, immunohistochemistry and microscopy. For histological analysis, pancreatic specimens were fixed with 10% buffered formalin, dehydrated in ethanol, embedded with paraffin and stained with H&E or Gomori Trichrome. The characteristics of control KC mice have previously been detailed.15 Pancreatic oedema was calculated by measuring intralobular white space on H&E sections. Immunohistochemistry was performed using antibodies directed against CD45 (30-F11, BD Biosciences), C3a (F10-30, Novus), and DAPI (no. H-1200; Vector Laboratories). For paraffin-embedded slides, samples were dewaxed in ethanol, followed by antigen retrieval with 0.01 M sodium citrate with 0.05% Tween 20.

FISH. The D223 28S rRNA gene probe labelled with the 5′ Cy3 fluorophore (extinction wavelength, 555 nm and emission wavelength, 570 nm; Molecular Probes) was used to detect the fungal colonization within human and mouse pancreatic tissues by FISH. Fluorescence microscopic analysis was conducted with Nikon Eclipse 90i confocal microscope (Nikon) using a Cy3-labelled probe at 555 nm (extinction wavelength, 555 nm and emission wavelength, 570 nm; Molecular Probes). The fraction and number of ducts that contained any grade of PanIN lesions were measured by examining 10 H&E-stained high-power fields (×200) and a final extension of 72°C (10 min). After each PCR cycle, purified libraries of AMPure XP beads were flushed for purity by Nanodrop, quantified by Picogreen assay and sizes were confirmed on agarose gels. Negative controls were included in all sequencing runs. Equimolar amounts of the generated libraries were combined and quantified fluorometrically. The pooled amplicon library was denatured, diluted and sequenced on an Illumina MiSeq platform using MiSeq Reagent Kit v3 (600 cycles) following the 2× 300-base pair-end sequencing protocol.
using cutadapt (v.1.12), and sequences that were shorter than 100 bases or sequences including asparagine were discarded. The reads were filtered by quality at 20, using multiple_split_libraries_fastq.py (q = 19; defaults were used for the other parameters). The 1,989,618 quality reads (mean 8,575; n = 166) were then processed with QIIME. Chimeric sequences were removed using VSEARCH (v.2.4.3) with UNITE UCHIME reference dataset (v.7.2). OTUs were picked using the open-reference OUT picking method, with default parameters, against the UNITE reference database (v.7.2) to assign taxonomy using pick_open_reference出色的。 There were 126,862 OTUs, corresponding to 1,856,993 reads (about 93.57% of the total reads), that did not align to fungi; these OTUs were excluded from the downstream analyses. OTUs that were unidentifed in UNITE database were blasted to NCBI, and the taxonomy information of the best hit (similarity or coverage ≥ 97%) for each OTU was re-assigned. A total of 127,646 sequence reads were clustered into 1,899 OTUs (corresponding to 86,640 reads) for longitudinal faecal samples from mice; 390 OTUs (corresponding to 25,021 reads) for tissue samples from mice; 2,980 OTUs (corresponding to 15,349 reads) for faecal samples from humans; and 311 OTUs (corresponding to 636 reads) for tissue samples from humans. Sequence data were analysed at various levels of phylogenetic affiliations. Low-abundance OTUs in < 2 samples, and samples identified as outliers, were removed. Distinctions in the composition of the mycobiomes between cohorts and within samples over time were tested for significance using a Mann–Whitney U test. Alpha-diversity and beta-diversity were computed and plotted in Phyloroc. PCoA was performed on Bray–Curtis dissimilarity indices, and a one-way PERMANOVA was used to test for significant differences between cohorts (Adonis, R package Vegan v.2.4.5). P values < 0.05 were considered to be significant.

**Quality control.** For quality control, we used best practices for microbiome- and mycobiome-based studies, as previously described. All the samples were collected using sterile techniques. All PCR reagents were regularly checked for environmental contaminants using ITS universal primers. All qPCR reactions had appropriate controls (without template) to exclude DNA contaminants. To control for the quality of our sequencing, we used both predetermined mock communities (such as C. tropicalis) and ‘negative’ (reagent-only) controls, to check background contamination and the rate of sequencing errors. We included both of these controls in each of the sequencing runs. We further confirmed the quality of our sequencing by including community controls composed of predetermined ratios of DNA from a mixture of three fungal species.

**Figure preparation.** Figures were prepared using BioRender software and Indesign (Adobe).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**

The sequence datasets analysed in this article are publicly available in the NCBI BioProject database, under the accession number PRJNA57226. Raw data for all experiments are available as Source Data to the relevant figures. Any other relevant data are available from the corresponding authors upon reasonable request.

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**Acknowledgements** We acknowledge the use of the Experimental Pathology and Microscopy core facilities at NYU School of Medicine. These shared resources are partially supported by the Cancer Center Support Grant, P30CA016087, at the Laura and Isaac Perlmutter Cancer Center. This work was supported by NIH grants CA168611 (G.M.), CA206105 (G.M. and D.S.), CA215471 (G.M.), CA193311 (G.M.), DK106029 (G.M.), DE025992 (D.S. and X.L.) and U11TR001445 (J.I.K.), Department of Defense grant CA170450 (G.M.) and Deutsche Forschungsgemeinschaft Grant AY 126/1-1 (B.A.).

**Author contributions** B.A. carried out in vivo and in vitro experiments, study design, PCR, analysis and interpretation, manuscript preparation and statistical analysis; S.P. carried out fungal DNA sequencing, analysis and interpretation, manuscript preparation, microbiology study design and statistical analysis; R.C. carried out in vivo experiments, histological analysis and manuscript preparation; Q.L. performed computational analyses and provided critical review; R.A. carried out in vivo experiments and provided technical assistance; J.I.K. carried out in vivo experiments and provided critical review; S.A.S. carried out mouse breeding and histology; D.W. performed tissue culture and cell-line generation; PP. provided technical assistance and carried out in vivo experiments; N.V. carried out knockdown experiments; Y.G. carried out PCR; A.S. performed FISH and provided critical review; M.V. carried out DNA extraction and contributed to computational analysis; B.D. carried out in vivo experiments and critical review; W.W. provided technical assistance; J.L. provided critical review and contributed to study design; E.K. carried out in vivo experiments and contributed to study design; J.A.K.R. provided technical assistance and contributed to study design; M.H. carried out in vivo experiments; C.Z. carried out human-sample collection; X.L. provided technical assistance; D.S. and G.M. conceived, designed, supervised, analysed and interpreted the study, prepared the manuscript and provided critical review.

**Competing interests** The authors declare no competing interests.

**Additional information**

**Supplementary information** is available for this paper at https://doi.org/10.1038/s41586-019-1608-2.

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**Peer review information** Nature thanks Marina Pasca di Magliano and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Extended Data Fig. 1 | Fungal infiltration of the pancreas in benign disease. Fungal DNA content was tested using qPCR in pancreata from control (ctl) mice (n = 5) and mice induced to develop caerulein-induced pancreatitis (n = 5). ns, not significant. Data are mean ± s.e.m. Two-tailed Student's t-test.
Extended Data Fig. 2 | Dysbiosis of the gut mycobiome in a mouse model of PDA. Hierarchical tree cladogram depicting changes in the taxonomic composition of the mycobiome (assigned to the genus level) in the guts of 30-week-old KC ($n = 14$) compared to wild-type ($n = 12$) mice, based on the average percentage relative abundance of genera as determined by 18S ITS sequencing.
Extended Data Fig. 3  | Efficacy of antifungal treatments in pancreatic disease. a, Wild-type mice that bear orthotopic PDA tumours were treated with vehicle (n = 7 mice) or fluconazole (n = 8 mice), and killed three weeks later. Tumours were collected and weighed. Data are representative of experiments that were performed twice. Scale bar, 1 cm. b, Germ-free wild-type mice were treated with amphotericin B (n = 6 mice) or vehicle (n = 10 mice), and orthotopic tumours from KPC mice were administered to them. Mice were killed three weeks later, and tumours were collected and weighed. Scale bar, 1 cm. c–e, Wild-type mice induced to develop caerulein-induced pancreatitis were serially treated with amphotericin B (n = 5 mice) or vehicle (n = 3 mice). c, Representative H&E-stained sections of pancreata are shown, and pancreatic oedema was quantified by measuring the percentage of the area that was white space. Scale bar, 100 μm. d, CD45+ inflammatory cell infiltration was determined by immunohistochemistry. Scale bar, 20 μm. e, Serum levels of amylase were measured. n = 5 mice treated with amphotericin B, n = 3 mice treated with vehicle and n = 3 mock-treated (control) mice. f, Wild-type mice treated with amphotericin B were repopulated with C. tropicalis (n = 4 mice) or vehicle (n = 4 mice), and killed three weeks later. Tumours were collected and weighed. Scale bar, 1 cm. Data are mean ± s.e.m. P values determined by two-tailed Student’s t-test (a–f).
Extended Data Fig. 4 | Fungal dysbiosis drives the progression of PDA via the lectin pathway. a, Kaplan–Meier survival curve of patients with PDA, stratified by high (n = 16 patients), medium-high (n = 24 patients), medium-low (n = 26 patients) and low (n = 17 patients) expression of MBL on the basis of data from TCGA. b, Orthotopic tumours from KPC mice were administered to MBL-null mice treated with vehicle (n = 3 mice) or amphotericin B (n = 4 mice), and killed three weeks later. Tumours were collected and weighed. Data are representative of three separate experiments. c, MBL-null mice treated with amphotericin B were repopulated with M. globosa (n = 5 mice) or sham-repopulated (n = 4 mice), and killed three weeks later. Tumours were collected and weighed. Data are representative of experiments that were repeated twice. d, Kaplan–Meier survival curve of patients with PDA, stratified by high (n = 18) versus low (n = 15) expression of C3, on the basis of data from TCGA. e, Pancreata from three-month-old wild-type, KC and KC, MBL-null mice were stained using a monoclonal antibody against C3a. Representative images from two experiments are shown. Scale bar, 20 μm. f, KPC tumour cells were seeded in 96-well plates with vehicle or recombinant mouse C3a. n = 5 cells per group for each time point. Cellular proliferation was measured at serial time points using the XTT assay. Data are representative of experiments that were repeated three times. Data are mean ± s.e.m. P values determined by two-tailed log-rank test (a, d) or two-tailed Student's t-test (b, c, f).
Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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  - 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

- BioRad CFX Manager 3.1, SoftMaxPro 5.4.6, Microsoft Excel 2016, SoftMaxPro 5.4.6, BioRad CFX Manager 3.1, QIIME 1.9.1, Nikon i Series Support Tools Ver2.4.4, Illumina

Data analysis

- GraphPad Prism 7, Microsoft Excel 2016, BioRad CFX Manager 3.1, SoftMaxPro 5.4.6, R 3.5.2, LeSe, cutadapt v.1.12, VSEARCH v.2.4.3, Phylseq 1.27.0, Adonis, Vegan v.2.4.5, igraph 1.2.4, R 3.5.2, UNITE database v. 7.2, QIIME 1.9.1

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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 list of figures that have associated raw data
- A description of any restrictions on data availability

Sequence data will be available in the Sequence Read Archive (SRA) database at NCBI at the time of publication. All other datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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 study design

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

| Sample size | Power analyses were done based on estimates |
|-------------|---------------------------------------------|
| Data exclusions | Data points were not excluded. |
| Replication | All experiments were repeated multiple times as indicated in each figure legend. Attempts at replication were successful. |
| Randomization | No formal randomization was carried out in experiments involving multiple genotypes. For all other experiments, animals were randomly divided into experimental groups. |
| Blinding | Administration of compounds was carried out as a blinded experiment (all information about the expected outputs and the nature of used compounds were kept from the animal-technicians). |

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 | Methods |
|---------------------------------|---------|
| n/a | n/a |
| ☑ Antibodies | ☑ ChIP-seq |
| ☑ Eukaryotic cell lines | ☑ Flow cytometry |
| ☑ Palaeontology | ☑ MRI-based neuroimaging |
| ☑ Animals and other organisms | |
| ☑ Human research participants | |
| ☑ Clinical data | |

**Antibodies**

Antibodies used

rat anti CD45 (clone 30-F11, BD Biosciences) catalog number 553076 (1:100); rabbit anti C3a (clone JF10-30, Novus) catalog number NBP2-66994 (1:100). Secondary antibodies: Rat IgG HRP-conjugated Antibody (Vector Labs) catalog number MP-7404 (1:1000); Rabbit IgG HRP-conjugated Antibody (Vector Labs) catalog number MP-7401 (1:1000).

Validation

Antibody specificity was evaluated using the proper negative controls (rat IgG2b, x for rat anti CD45 and rabbit IgG for anti C3a).

**Eukaryotic cell lines**

Policy information about [cell lines](#)

Cell line source(s)

Cells used for orthotopic tumor injections were generated by our group from endogenous tumors. HEK293FT were purchased from Thermo Fisher Scientific (catalog number: R70007).

Authentication

None of the cell lines were authenticated.

Mycoplasma contamination

Mycoplasma testing was performed within the past 2 months and was negative.

Commonly misidentified lines

(See [ICLAC register](#))

No commonly misidentified cell lines were used.

**Animals and other organisms**

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research

**Laboratory animals**

CS7BL/6, MBL-null (B6.1295-Mbl1tm1Kata Mbl2tm1Kata/), and C57/6-/- (B6.129S4-C3tm1Crrr/). mice were originally purchased from Jackson Labs [Bar Harbor, ME]. 8-10 week old males and females were used. KC mice, which develop spontaneous pancreatic neoplasia by targeted expression of mutant Kras in the pancreas, were a gift from Dafna Bar-Sagi [New York University].

**Wild animals**

This study did not involve wild animals.
Field-collected samples | This study did not involve samples collected from the field.
---|---
Ethics oversight | All animal experiments were approved by the New York University School of Medicine Institutional Animal Care and Use Committee (IACUC).

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

Human research participants

| Policy information about | studies involving human research participants |
| Population characteristics | Human fecal samples and pancreatic tissue specimens were sterilely collected from healthy volunteers and patients undergoing surgery for either PDA or benign disease (pancreatic endocrine tumors) at NYU Langone Medical Center. |
| Recruitment | Human fecal samples and pancreatic tissue specimens were sterilely collected from healthy volunteers and patients undergoing surgery for either PDA or benign disease (pancreatic endocrine tumors) at NYU Langone Medical Center. |
| Ethics oversight | Human specimens were obtained using an Institutional Review Board approved protocol, conducted in accordance with the Declaration of Helsinki, the Belmont Report, and U.S. Common Rule and donors of de-identified specimens gave informed consent. |

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