SUPPLEMENTARY METHODS

Patient cohorts

The present study included the analysis of 180 frozen surgical tissue specimens from patients with stage I-III PDAC enrolled between 1998 and 2017 in 3 institutions: Saitama Cancer Center, Japan, Nagoya University Hospital, Japan, and Kumamoto University Hospital, Japan. At all institutions, all tissue specimens were aseptically placed in a sterile box immediately after surgery and transported into the lab on ice. Each specimen was subsequently divided into small pieces using surgical blades and immediately frozen at -80°C in sterile tubes. The clinical data included patient demographics, tumor characteristics, recurrence events, and survival data. Written informed consent was obtained from all patients, and the study complies with the Declaration of Helsinki and received the approval of the Institutional Review Board of all participating institutions.

DNA extraction and nested PCR

The genomic DNA was extracted from the frozen cancer tissues using an AllPrep DNA/RNA/miRNA Universal kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All experiments were aseptically performed in a dedicated cabinet to reduce the possibility of contamination. The extracted DNA was quantified using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and stored at -80°C. DNA samples were analyzed by nested PCR, and primers for amplifying the ITS regions of fungal ribosomal DNA were designed using the Genbank database and previously published articles[1-3]. All PCR experiments included the multiple PCR wells of clean water as negative controls. The first PCR was performed using 100-200 ng of DNA incubated at 95°C for 15min as an initial denaturation step, followed by 40 cycles of 30sec at 94°C, 45sec at 52°C, and 1min at 72°C, with a final extension at 72°C for 10min. Primers for the first PCR were ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The second PCR was performed by the real-time qPCR assays using the SensiFAST SYBR Lo-ROX Kit (Bioline, London, UK) and QuantStudio 6 Flex RT-PCR System (Applied Biosystems, Foster City, CA, USA). Two microliters of the first PCR product were incubated at 95°C for 5min, followed by 50 cycles of 1min at 95°C and 45sec at 64°C using primers for *M. globosa* forward (5'-GGCCAAGCGCGCTCT -3'), and reverse (5'- CCACAACCAAATGCTCTCCTACAG -3'). The *M. globosa* burden was normalized by the expression levels of the *HBB* gene using the ΔCt method, and primers for the *HBB* gene were as follows: Forward (5'- TATTGGTCTCCTTAAACCTGTCTTG -3') and Reverse (5'-CTGACACAACTGTGTTCACTAGC -3').

PCR products sequencing

PCR amplified products were purified using the BigDye XTerminator Purification kit (Applied Biosystems). Subsequently, sequencing of the purified PCR products was performed using the corresponding primer pairs and BigDye Direct Cycle Sequencing kit (Applied Biosystems). The products of the sequencing PCR were analyzed in the SeqStudio Genetic Analyzer (Applied Biosystems), and the obtained sequences were analyzed using Chromas 2.6.6 software.

Statistical analyses

Fisher's exact test and two-sided Student's t-test were used for analyzing the differences in categorical and continuous variables, respectively. A *P*-value of <0.05 was considered statistically significant. Survival curves were constructed using the Kaplan–Meir method and were compared with the log-rank test. The cutoff points for continuous values were divided by the mean values. The factors acquired from univariate analyses (*P* <0.05) were included in the multivariate binary Cox regression analyses. All statistical analyses were performed with the EZR version 1.55 - a graphical user interface for R version 4.0.3[4].

SUPPLEMENTARY REFERENCES

1. Sugita T, Tajima M, Tsubuku H, et al. Quantitative analysis of cutaneous malassezia in atopic dermatitis patients using real-time PCR. Microbiol Immunol 2006;50:549-52.
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3. Turlier V, Viode C, Durbise E, et al. Clinical and biochemical assessment of maintenance treatment in chronic recurrent seborrheic dermatitis: randomized controlled study. Dermatol Ther (Heidelb) 2014;4:43-59.
4. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452-8.

Supplementary Table S1. Clinicopathological correlation of *M. globosa* level in the cohort of 180 PDAC patients.

	***M. globosa* negative** (n =102)		***M. globosa* positive** (n =78)		*P* value
	n	(%)	n	(%)	
Age, mean (±SD) (years)	65	(±9)	65	(±9)	0.66
Sex					0.09
Male	57	(56)	54	(69)	
Female	45	(44)	24	(31)	
Location					0.11
Head	73	(72)	56	(72)	
Body & Tail	29	(28)	22	(28)	
Tumor size, mean (±SD) (cm)	3.5	(±1.7)	3.6	(±1.9)	0.76
Differentiation					0.82
Well/Moderate	88	(86)	69	(89)	
Poor	14	(14)	9	(12)	
T stage					1.00
1-2	10	(10)	7	(9)	
3-4	92	(90)	71	(91)	
Lymph node metastases					0.53
Negative	33	(32)	29	(37)	
Positive	69	(68)	49	(63)	
Lymphovascular invasion					1.00
Negative	23	(23)	17	(22)	
Positive	79	(78)	61	(78)	
CEA					0.72
≤5.0 ng/mL	78	(77)	62	(80)	
>5.0 ng/mL	24	(24)	16	(21)	
CA19-9					0.25
≤37.0 U/mL	34	(33)	19	(24)	
>37.0 U/mL	68	(67)	59	(76)	

M. globosa, Malassezia globosa; PDAC, pancreatic ductal adenocarcinoma; SD, standard deviation; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9