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Intratumoral *Malassezia globosa* levels predict survival and therapeutic response to adjuvant chemotherapy in patients with pancreatic ductal adenocarcinoma

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Fungi are microeukaryotes, estimated to encompass between 2.2 to 3.9 million species that inhabit various anatomic sites within the human body with relatively stable colonization – collectively forming the mycobiome[1, 2]. Despite their low abundance in the human microbiome[1, 2], emerging evidence highlights the significant influence of mycobiome on the host's health, including nutrition, metabolism, immunity, and the physiological functions of various body organs[3, 4]. More specifically, the associations between mycobiome and human cancer have garnered increasing attention in recent years due to the significant enrichment of specific fungi in patients with malignant tumors. However, the biological underpinnings of human mycobiome in cancer pathogenesis and disease progression continue to evolve and remain unclear.

A recent pre-clinical and clinical study revealed that pancreatic ductal adenocarcinomas (PDACs) harbor significant enrichment of one such specific fungi in mice models and human specimens relative to normal pancreatic tissues[5]. Furthermore, this study revealed that the commensal gut fungi – *Malassezia globosa* (*M. globosa*) – migrates from the gut to

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the pancreas and promotes pancreatic carcinogenesis via the activation of the complement cascade[5, 6]. More interestingly, this study elegantly demonstrated that antifungal drugs inhibited the oncogenic progression of PDAC, and the combination of an antifungal drug and chemotherapy exhibited a synergistic anti-cancer effect against PDAC in animal models[5]. Likewise, another commensal fungus, *Candida tropicalis*, was implicated in the pathogenesis of colorectal cancer (CRC) through the accumulation of myeloid-derived suppressor cells[7]. In light of such groundbreaking studies, alterations in the mycobiome have gained increasing attention as functional determinants of disease and potential diagnostic and prognostic biomarkers in various cancers. For instance, as fecal biomarkers, a higher abundance of multiple fungi, including *M. globosa*, has been shown to distinguish CRC patients from healthy individuals accurately[8]. Furthermore, more recent analytical studies demonstrated that tumor-specific mycobiome could predict prognosis in multiple gastrointestinal malignancies[9]. Given this evidence, we hypothesized that high *M. globosa* levels in tissue specimens from patients with PDAC might serve as critical prognostic biomarkers.

Accordingly, this study explored the feasibility of tissue-based fungal biomarkers for predicting prognosis in patients with PDAC. Using quantitative nested polymerase chain reaction (PCR) assays in PDAC patient cohorts, we first determined the frequency of *M. globosa*-positivity in PDACs, followed by the quantitative associations between the levels of this fungus and its potential in predicting cancer-related death and tumor recurrence in patients with this malignancy.

To examine the *M. globosa* levels, surgically resected tissue specimens from a cohort of 180 PDAC patients with stages I-III disease were analyzed using quantitative nested PCR assays. Following extraction of DNA, the specific target amplification for the internal transcribed spacer (ITS) regions of *M. globosa* ribosomal DNA was confirmed using the BigDye Terminator Cycle Sequencing (Fig. 1A). Subsequently, in the quantitative nested PCR assays normalized by the *HBB* gene expression, 78 of 180 (43.3%) cases exhibited a Ct value of less than 33, indicating high levels of this fungus, and were defined as *M. globosa*-positive. In contrast, no signal was observed for the remaining 102 cases, even after 50 PCR cycles (Fig. 1B), and they were defined as *M. globosa*-negative. Interestingly, this frequency of *M. globosa*-positivity in PDAC tissues was similar to that in stool samples of healthy individuals (36–53%), which were reported in a previous sequencing-based human gut mycobiome project[10].

First, the correlation between *M. globosa* levels and clinicopathological factors was assessed to determine their prognostic potential. In these analyses, *M. globosa* levels did not significantly correlate with other key clinicopathological factors, including patient demographics, tumor characteristics, and serum tumor markers (Supplementary Table. S1). However, male patients tended to have a higher burden of *M. globosa*-positive cases vs. female patients (P =0.09). This higher abundance of *Malassezia* species in male patients was also found in previous studies that quantified *Malassezia* colonization of the skin[11, 12]. It was unclear whether this higher abundance of *M. globosa* in male patients may affect differential gender burden and prognostic outcomes in PDAC. Therefore, further studies

are warranted on this topic, including investigating larger global populations to confirm the findings further.

Next, to determine whether the intratumoral *M. globosa* levels might serve as predictors of prognosis in patients with PDAC, Kaplan-Meier analyses were performed for overall survival (OS) and recurrence-free survival (RFS). Interestingly, *M. globosa*-positive PDACs exhibited a significantly poor OS (hazard ratio [HR]: 1.69; 95% confidence interval [CI]: 1.21–2.38; log-rank P <0.01; Fig. 1C), as well as markedly poor RFS (HR: 1.51; 95% CI: 1.07–2.11; P =0.02; Fig. 1D). Of note, multivariate Cox regression analyses revealed that the *M. globosa* levels were significant and independent predictors for both OS (HR: 1.66; 95% CI: 1.17–2.35; P <0.01; Fig. 1E) and RFS (HR: 1.51; 95% CI: 1.07–2.13; P =0.02; Fig. 1F), highlighting that intratumoral *M. globosa* levels have the potential to serve as prognostic biomarkers in patients with PDAC.

Finally, we examined the impact of intratumoral *M. globosa* levels on treatment response in PDAC patients treated with adjuvant chemotherapy following curative resection of their tumors, using Kaplan-Meier analyses. In line with our findings for all cases, *M. globosa*-positive PDACs exhibited a significantly poor OS (HR: 2.00; 95% CI: 1.31–3.06; P <0.01; Fig. 1G) and RFS (HR: 1.55; 95% CI: 1.02–2.37; P =0.04; Fig. 1H) in the subset of patients treated with adjuvant chemotherapy. These results illustrate that patients with *M. globosa*-positive PDAC might have greater resistance to adjuvant chemotherapy, rendering an overall poor prognosis in this malignancy.

In summary, we, for the first time, demonstrate that intratumoral *M. globosa* levels are associated with cancer-related survival and tumor recurrence in patients with PDAC. In addition, our study provides novel evidence that *M. globosa* levels are a significant predictor of therapeutic response to adjuvant chemotherapy in PDAC patients. This study highlights that *M. globosa* levels are a significant predictor of prognosis in PDAC patients and might be a potential target of antifungal intervention in patients with PDAC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability:

Data is contained within the article.

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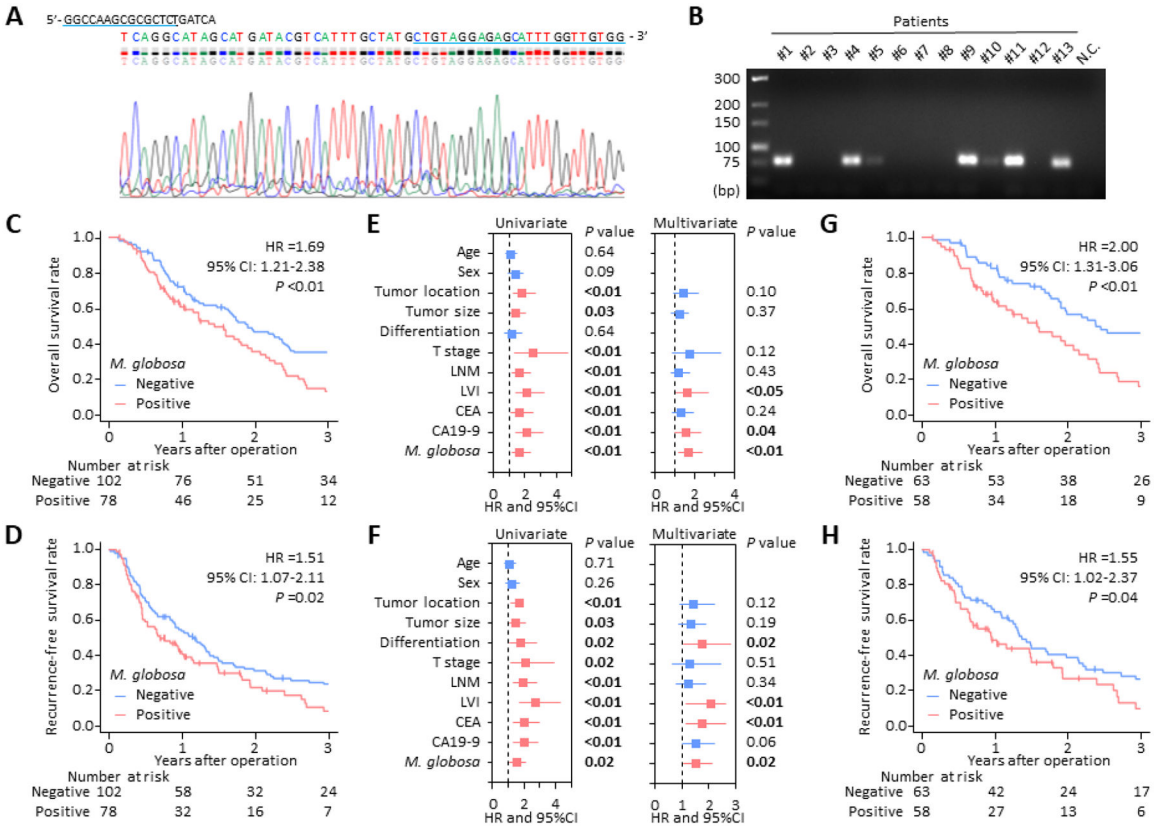

Figure 1.

Intratumoral *Malassezia globosa* levels and prognostic significance in PDAC. **(A)** Representative sequences of PCR amplified products in nested PCR assays for detecting *M. globosa* in PDAC specimens. Forward and reverse primer location is indicated by a blue underline. **(B)** Representative agarose gel electrophoresis (4% agarose) of PCR amplified products in nested PCR assays. Lane 1 is a low-range DNA ladder, lanes 2–14 are PCR amplified products from PDAC tissue specimens, and lane 15 is negative control. **(C, D)** Kaplan-Meier curves depicting the overall survival (C) and recurrence-free survival (D) for patients with *M. globosa* negative (n =102) and positive (n =78) tumors. **(E, F)** Forest plots with HR for each key clinicopathological factor and *M. globosa* level in univariate and multivariate Cox regression analysis for overall survival (E) and recurrence-free survival (F) in the clinical cohort (n =180) **(G, H)** Kaplan-Meier curves depicting the overall survival (G) and recurrence-free survival (H) for PDAC patients who were treated with adjuvant chemotherapy (n = 121). PDAC, pancreatic ductal adenocarcinoma; PCR, polymerase chain reaction; *M. globosa*, *Malassezia globosa*; N.C., negative control; HR, hazard ratio; CI, confidence interval; LNM, lymph node metastases; LVI, lymphovascular invasion; CEA, carcinoembryonic antigen; CA19–9, carbohydrate antigen 19–9.