Proton pump inhibitors act synergistically with fluconazole against resistant *Candida albicans*

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Part 1 *In vitro* interaction of RAB combined with FLC against a susceptible *C. albicans* strain (CA4)

Method: Checkerboard microdilution assay was carried out to determine the interactions of RAB and FLC against CA4. Briefly, drugs were serially diluted 2-fold in RPMI-1640 medium, and drugs at final concentrations of 0.03-16 μg/mL for FLC and 2-128 μg/mL for RAB were added to the wells. Subsequently, the yeast at a final concentration of 2.5×10^3 CFU/mL was added to each well. The wells containing RPMI-1640 medium acted as negative controls, and a drug-free well was set as the growth control. After 24 h of incubation at 35 ℃, MICs were determined as mentioned above.

Results: Results were presented as the **Table S1** and **Fig. S1**. As shown in Table **S1**, FLC at the concentration of 0.5μg/ml caused about 80% growth inhibition, indicating a MIC_{80} of FLC 0.5μg/ml. In addition, more than 80% growth inhibition was induced by 4-32 μg/ml RAB plus 0.25μg/ml FLC. However, when 64-128 μg/ml RAB combined with 0.25μg/ml FLC, the growth rate was more than 60%, showing an antagonism effect. This result was also observed in CA 8, another susceptible strain.

Table S1 Growth rate of a susceptible *C. albicans* strain CA4 treated with the combination of RAB and FLC

Growth rate		FLC(μg/ml)											
		0	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	Blank
RAB(μg/ml)	128	99%	96%	94%	91%	92%	74%	27%	0%	0%	0%	0%	0%
	64	98%	97%	95%	92%	62%	32%	7%	0%	1%	0%	0%	0%
	32	98%	97%	95%	35%	12%	7%	7%	3%	0%	0%	1%	0%
	16	98%	95%	94%	34%	5%	6%	7%	5%	3%	3%	3%	0%
	8	100%	100%	99%	38%	8%	7%	4%	5%	5%	4%	3%	0%
	4	100%	100%	100%	26%	9%	7%	4%	3%	4%	3%	3%	0%
	2	100%	99%	100%	23%	15%	5%	3%	3%	4%	5%	4%	0%
	0	100%	100%	97%	27%	27%	21%	7%	5%	4%	4%	4%	0%

Part 2 Interaction of RAB combined with FLC and the effect of LAN on Yeast-to-Hyphae Morphogenesis of CA4.

Method: Effect of RAB combined with FLC on the yeast-to-hyphae morphogenesis of a susceptible *C. albicans* (CA4) was studied in a microplate-based assay as the manuscript. In this assay, the yeast cells (10^5 CFU/mL) were added to a 96-well microplate, and drugs at the final concentrations of 2 μg/mL for FLC, 64-512 μg/mL for RAB and 128-1024 μg/mL for LAN were then added. The microplate was incubated at 35 ℃ for 5 h, and then was placed directly under the inverted microscope.

Results: Interaction of RAB combined with FLC on the yeast-to-hyphae morphogenesis of CA4 was shown in the **Fig. S1**, and the effect of LAN was shown in the **Fig. S2**. From the **Fig. S1**, RAB was found to inhibit hyphae formation when it was used alone, and the higher the dose, the shorter the hyphae. The length of hyphae in the 512 μg/mL RAB group was the shortest. However, when RAB combined with FLC, the shortest hyphae was observed in the group of 256 μg/mL RAB + FLC, but not 512 μg/mL RAB + FLC. This result accorded with that of *in vitro* interaction, indicating that yeast-to-hyphae morphogenesis was involved in the interaction of PPIs combined with FLC against *C. albicans*.

For the effect of LAN on the yeast-to-hyphae morphogenesis of CA4, **Fig. S2** showed that the hyphae in the control group were longest among all groups, and a large area of hyphae gathered together. In the LAN-treated groups, as the dose of LAN increased, the hyphae were getting shorter and less hypha gathered. Although there was no difference between LAN_{1024} and LAN_{512} groups in the length of hyphae, the number of cells in the LAN_{1024} group was less than that of LAN_{512} group. These findings demonstrated LAN might possess a weak inhibitory effect on the yeast-to-hyphae morphogenesis of susceptive *C. albicans* strains.

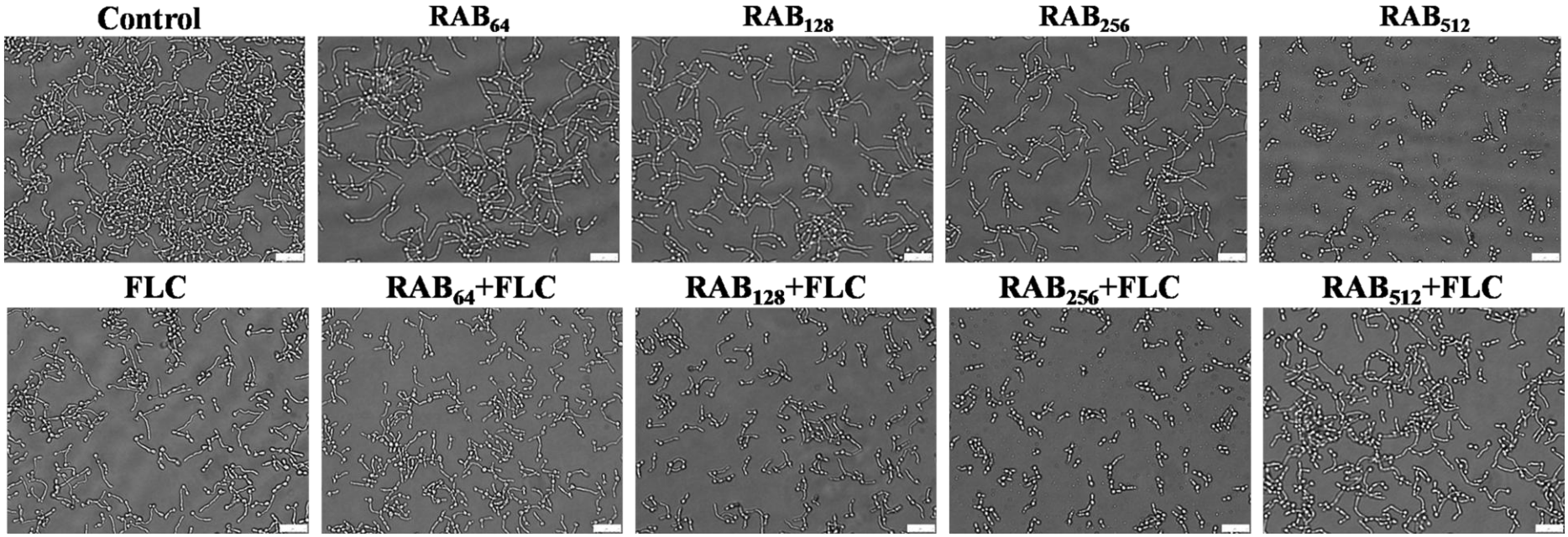

Fig. S1 Interaction of RAB combined with FLC on the yeast-to-hyphae morphogenesis of CA4. The yeast suspension (5×10^5 CFU/mL) were incubated in RPMI-1640 medium with PBS, FLC (2 μg/mL), RAB (64, 128, 256 and 512 μg/mL), RAB(64, 128, 256 and 512 μg/mL) plus FLC (2 μg/mL), respectively. After an incubation of 5 h at 35 ℃, cells were observed under the inverted microscope at a 40×10 multiplier, and the scale in the figure was 25 μm. RAB_{64}, RAB_{128}, RAB_{256} and RAB_{512} are RAB at the dose of 64, 128, 256 and 512 μg/mL, respectively.

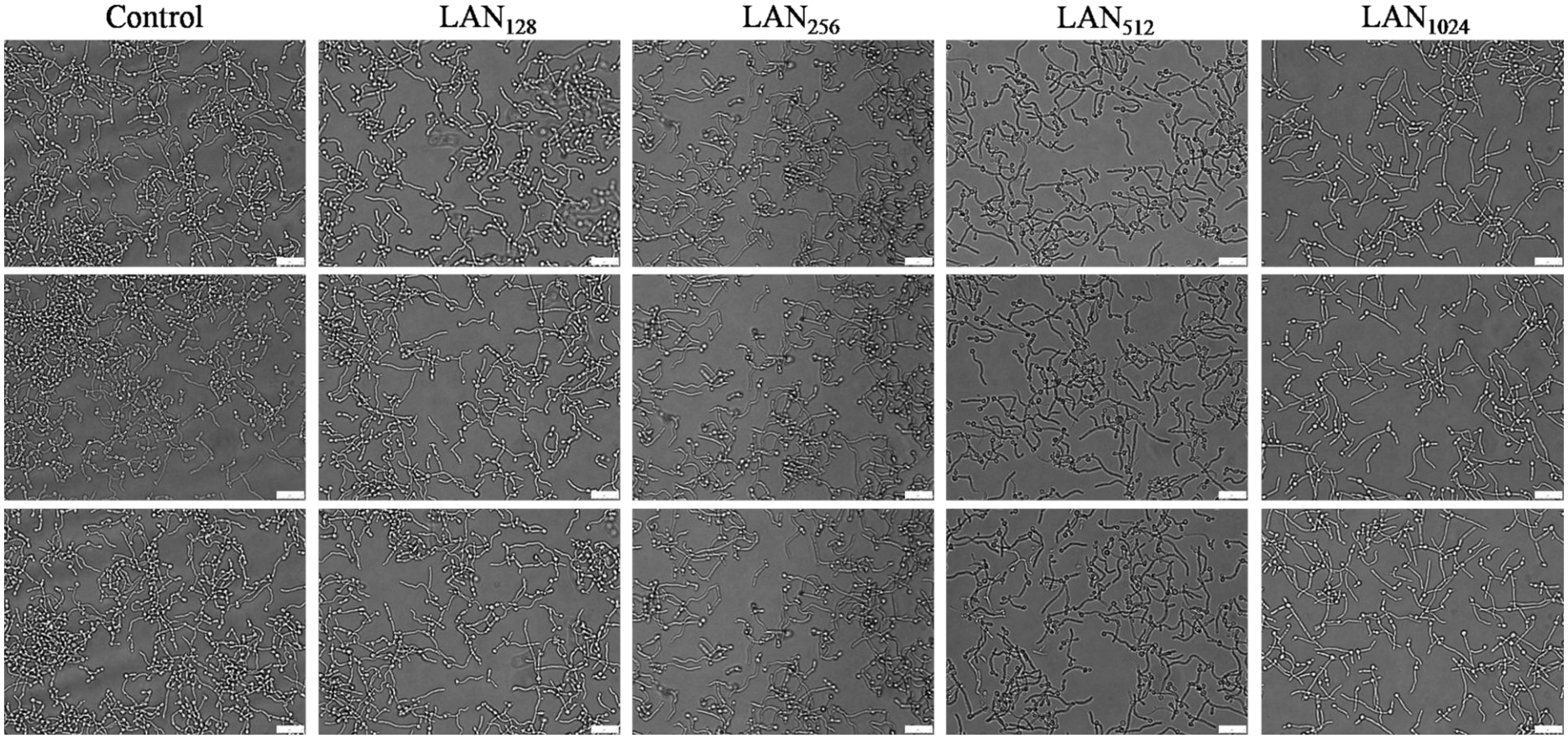

Fig. S2 Effect of LAN on the yeast-to-hyphae morphogenesis of CA4. The yeast suspension (5×10^5 CFU/mL) were incubated in RPMI-1640 medium with PBS, RAB (128, 256, 512 AND 1024 μg/mL), respectively. After an incubation of 5 h at 35 ℃, cells were observed under the inverted microscope at a 40×10 multiplier, and the scale in the figure was 25 μm. LAN_{128}, LAN_{256}, LAN_{512} and LAN_{1024} are LAN at the dose of 128, 256, 512 and 1024 μg/mL, respectively.

Part 3 Interaction of OME and RAB combined with FLC on phospholipase activity of CA4.

Methods: Effects of OME or RAB combined with FLC on the extracellular phospholipase activity of CA4 were detected by egg yolk agar plates. The yeast cells (10^6 CFU/mL) were incubated with no drug, FLC (1 μg/mL), OME (32 μg/mL), RAB (16 μg/mL), OME (32 μg/mL) plus FLC (1 μg/mL), RAB (16 μg/mL) plus FLC (1 μg/mL) for 24 h at 35 ℃. After the incubation, 10 μL of the cell suspensions were inoculated onto egg yolk agar plates and the plates were then incubated for 72 h at 35 ℃. The colony diameter and precipitation zone diameter were measured.

Results: As shown in the **Table S2**, P_Z values of control group and drug-monotherapy groups were 0.65-0.66, showing a very high phospholipase activity. For the combination groups, the very high phospholipase activity was also observed with the P_Z values both 0.68±0.01. There was no difference between control and drug-monotherapy groups or drug combination groups ($P > 0.05$), demonstrating that drug monotherapy or drug combination has no inhibitory effect on the phospholipase activity of CA4.

Table S2 Phospholipase activity of CA4 treated with drugs

Drugs	P_z value ± SD[b]	Phospholipase activity
No drug	0.65±0.02	Very high
FLC	0.66±0.02$^{n.s}$	Very high
OME	0.65±0.01$^{n.s}$	Very high
RAB	0.66±0.02$^{n.s}$	Very high
OME+FLC	0.68±0.01$^{n.s}$	Very high
RAB+FLC	0.68±0.01$^{n.s}$	Very high

[a] FLC, fluconazole (1 μg/mL); OME, omeprazole (32 μg/mL); RAB, rabeprazole (16 μg/mL).

[b] P_z values were the median of three independent experiments; Pz ≤ 0.69, very high phospholipase activity, Pz = 0.70-0.79, high activity; Pz = 0.80-0.89, low activity; Pz = 0.90-0.99, very low activity; Pz = 1, negative activity; SD, standard deviation; Compared with the control group, $^{n.s}$ $P > 0.05$.

Part 4 Relative expression efflux gene in CA10

Methods: The transcriptional expression of *CDR1*, *CDR2* and *MDR1* in the tested *C. albicans* SC5314 or CA10 was measured using qPCR as previously reported (PMID: 27503639). The primers used are shown in the Table S6. 18S rRNA served as the internal control in *C. albicans*. The transcript levels of the detected genes were calculated using the formula $2^{-\Delta\Delta CT}$.

Table S3 Primers used in this assay

Genes	Primer sequences (5'→3')
18S rRNA	F: AATTACCCAATCCCGACAC
	R: TGCAACAACTTTAATATACGC
CDR1	F: TAACACTTATGGTTTCCACAT
	R: AGCATAAGTTTCTCTGTCGA
CDR2	F: GAGTGTTGGTGATACTTTGG
	R: CACTCAAAGAAGCTTCAGCA
MDR1	F: AGATAATCAAGGTGAACCCAA
	R: GCTGATCCCATATAAACTGAA

Results: In this study, we measured the transcriptional expression of *CDR1*, *CDR2* and *MDR1* in both *C. albicans* SC5314 and CA10. *C. albicans* SC5314 was a wild isolate with no gene mutation. Compared with this wild isolate, gene expression of efflux pumps (*CDR1*, *CDR2* and *MDR1*) in CA10 was much higher ($P < 0.001$) (**Fig. S3**). In this case, the efflux pump was generally considered to be over-expressed.

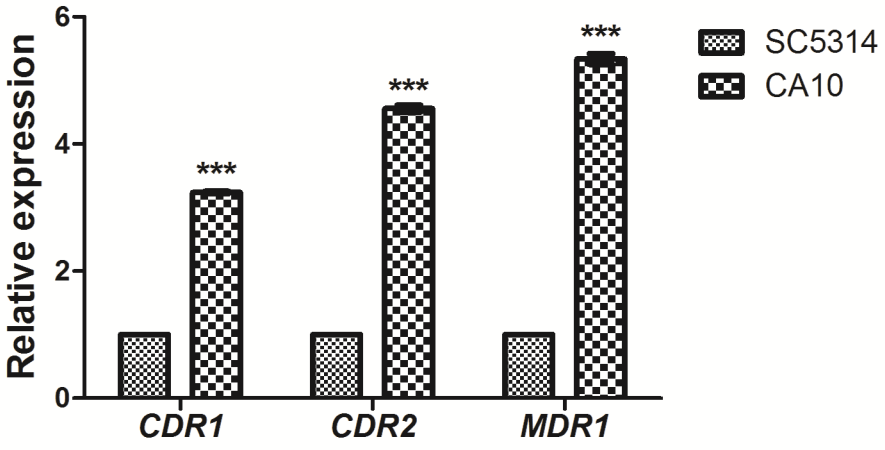

Fig. S4 Relative expression efflux gene in CA10. *C. albicans* SC5314 was a wild isolate with no gene mutation. Error bars indicated standard errors of the means. Statistical significances were determined by Student's t-test. $^{***}P < 0.001$ when compared with the respective controls.