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［摘要］ 目的 方法 - RT-PCR 结果 *BCR1* *NRG1* *TUP1* *HWP1* *EFG1* *CPH1* *ALS1* *ALS3* *CSH1* 结论

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Study on the antibiofilm activity of kaempferol in *Candida albicans*

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［**Abstract**］ **Objective** To study the action of kaempferol (KAE) against *Candida albicans* biofilms and explore the potential mechanisms. **Methods** Biofilm metabolic activity assay was used to investigate the action of KAE against *C. albicans* biofilm formation as well as mature biofilm. The inhibition of KAE in hyphal formation was examined by microscope. The water-hydrocarbon two-phase separation assay was used to test the effect of KAE on the cell surface hydrophobicity of *C. albicans*. The mRNA expression of the genes involved in biofilm formation was determined by real time RT-PCR. **Results** KAE showed inhibition effect on *C. albicans* biofilm formation in a dose-dependent manner. Moreover, KAE inhibited mature biofilm. The biomass of biofilm was reduced upon KAE treatment. KAE inhibited hyphal formation and reduced the cell surface hydrophobicity of *C. albicans*. In the presence of KAE, the mRNA expression of the genes involved in biofilm formation was changed, with the up-regulation of *BCR1,NRG1,TUP1* and down-regulation of *HWP1,EFG1,CPH1,ALS1,ALS3* and *CSH1*. **Conclusion** KAE showed antifungal activity against *C. albicans* biofilm. The mechanisms may relate to the inhibition of hyphal formation and reduction of cell surface hydrophobicity.

［**Key words**］ kaempferol *Candida albicans* biofilm adhesion hyphae

/ biofilm

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Candida albicans [1]

[2-3]

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kaempferol KAE

1

[4-6]

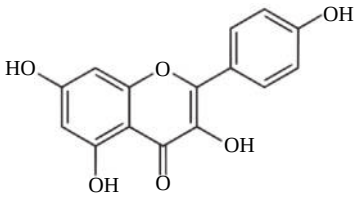


图 1 山奈酚的分子结构

1 材料与方法

1.1 材料

1.1.1

SC5314 *C. albicans* SC5314

Sigma (DMSO DMSO

RPMI1640 XTT 2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide Sigma PBS

RNA RNA PrimeScript RT Master Mix Perfect RealTime SYBR Premix ExTaq™ TaKaRa

(SDA) 10 g, *D*- 40 g 20 g 1 000 ml 121 15 min

YPD 10 g 10 g *D*- 20 g 1 000 ml 121 15 min

RPMI1640 RPMI1640 Gibco BRL 10 g MOPS 34.5 g $NaHCO_3$2.0 g 1 000 ml NaOH pH 7.0 1 000 ml 4

1.1.2

( ) (Eppendorf) ( ) 96 Corning Infinite M200 Austria TECAN ABI7500 RT-PCR (Applied Biosystems)

1.2 菌株活化

SC5314 SDA 30 3 d YPD 30 200 r/min 16 h

1.3 山奈酚抗生物被膜形成实验

[7]

PBS 3 RPMI1640 1×10^6 CFU/ml 96 100 μl 37 2 h 100 μl RPMIl640 37 24 h

1.4 山奈酚抗成熟生物被膜实验

PBS 3 RPMI1640 1×10^6 CFU/ml 96 100 μl 37 2 h RPMIl640 37 24 h PBS 2 100 μl RPMIl640 37 24 h

1.5 生物被膜代谢活性测定

PBS 2 200 μlXTT- 0.5 mg/ml XTT-1 μmol/L 37 2 h 492 nm *OD*

1.6 生物被膜基质含量测定

biomass [8] 1.5 cm×1.5 cm Bentec 12 2 ml 1×10^6 CFU/ml RPMI1640 2 h RPMI1640 37 24 h PBS 2 100 μl RPMIl640 37 24 h PBS 2

1.7 菌丝形成抑制实验

1% YPD 30
4 h PBS 2 16 μg/ml
YPD+FBS 10%
l×l0^6 CFU/ml 37
3.5 h

1.8 细胞表面疏水性测定
- [9]

1% YPD 30
4 h 30
4 h PBS
2 YPD OD_{600}=
1.0 1.2 ml
0.3 ml 3 min
OD_{600}
YPD

=[(OD_{600} −OD_{600})/OD_{600}]×
100%

1.9 引物设计
Primer Premier5
RT-PCR 1

1.10 实时定量 RT-PCR 实验

PBS 3 RPMI1640
l×l0^6 CFU/ml
37 2 h
16 μg/ml RPMIl640 37
24 h PBS
3 RNA
RNA
100 μl RNA DEPC
RNA RNA
A_{260}/A_{280} 1.8~2.0
TaKaRa
RNA cDNA
PCR 18S rRNA
95 30 s 40
95 5 s 60 20 s 72 30 s

表 1 引物序列

5′-3′	
HWP1-F	TGGTGCTATTACTATTCCGG
HWP1-R	CAATAATAGCAGCACCGAAG
EFG1-F	TATGCCCCAGCAAACAACTG
EFG1-R	TTGTTGTCCTGCTGTCTGTC
CPH1-F	ATGCAACACTATTTATACCTC
CPH1-R	ATGCAACACTATTTATACCTC
ALS1-F	TTGGGTTGGTCCTTAGATGG
ALS1-R	ATGATTCAAAGCGTCGTTC
ALS3-F	CTAATGCTGCTACGTATAATT
ALS3-R	CCTGAAATTGACATGTAGCA
CSH1-F	CTGTCGGTACTATGAGATTG
CSH1-R	GATGAATAAACCCAACAACT
TUP1-F	GATTGACGAG TCCTCCAACG
TUP1-R	AAACCAACCTATCGCCATCA
NRG1-F	TATCAGTATG CTGCTCCTCC
NRG1-R	GGAGTTGGCCAGTAAATCAC
BCR1-F	AGTATAATGCTCCTGGTAAGAA
BCR1-R	ACGTAAAGGAGGCACGGCATA
18S rRNA-F	AATTACCCAATCCCGACAC
18S rRNA-R	TGCAACAACTTTAATATACGC

60~95 0.1
ABI 7500 SDS
$2^{-(\ \ Ct)}$

1.11 统计学分析
GraphPad Prism 6.0
($\bar{x} \pm s$)
3 P<0.05 P<0.01

2 实验结果

2.1 山奈酚抑制白假丝酵母生物被膜的形成

2 h 37 24 h
XTT
8 μg/ml
32 μg/ml
35% 128 μg/ml

2A

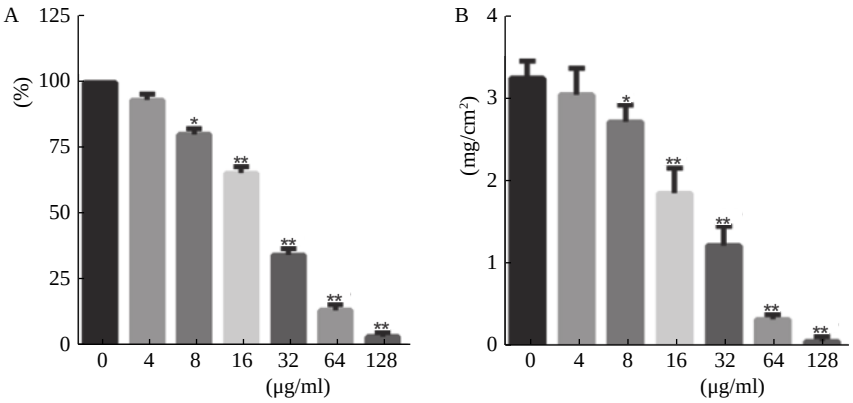


图 2 山奈酚对白假丝酵母生物被膜形成的影响

A. B.

*P<0.05 **P<0.01

32 μg/ml

50% 2B

2.2 山奈酚抗成熟白假丝酵母生物被膜活性

24 h 37 24 h

16 μg/ml

32 μg/ml

60% 128 μg/ml

28% 3

2.3 山奈酚对白假丝酵母菌丝形成的影响

10% YPD

16 μg/ml

4

2.4 山奈酚对白假丝酵母细胞表面疏水性的影响

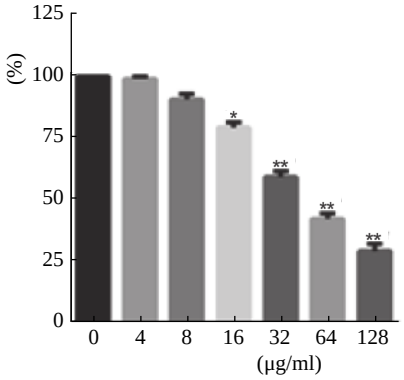


图 3 山奈酚抗成熟白假丝酵母生物被膜活性

*P<0.05 **P<0.01

4 μg/ml

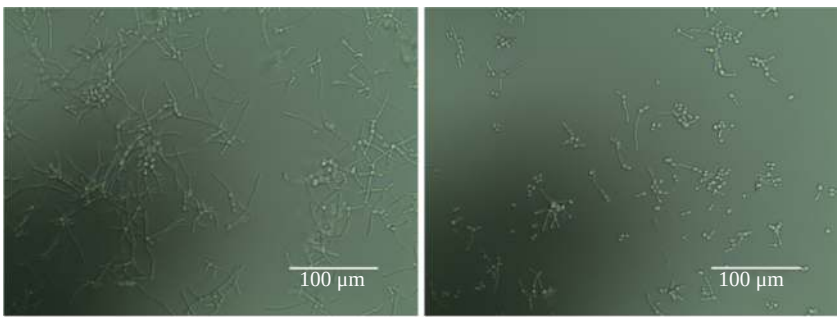
8 μg/ml

5

2.5 山奈酚对白假丝酵母生物被膜形成相关基因表达的影响

RT-

PCR



16 (μg/ml)

图 4 山奈酚抑制白假丝酵母菌丝形成的影响

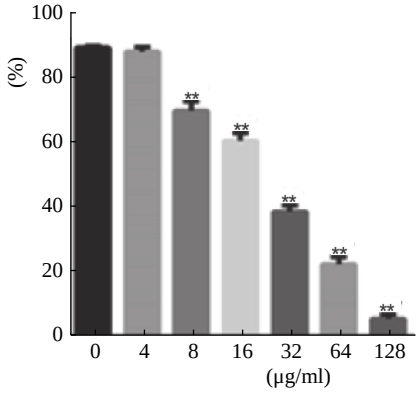


图 5　山奈酚对白假丝酵母细胞表面疏水性的影响

$^{**}P<0.01$

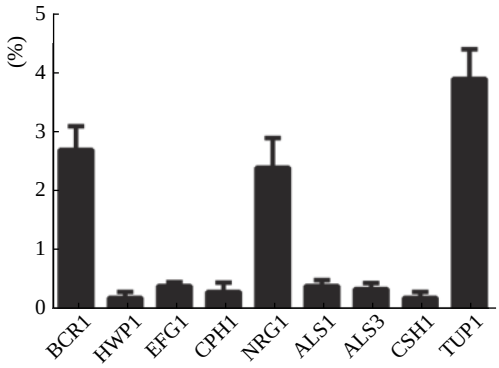


图 6　山奈酚对白假丝酵母生物被膜形成相关基因 mRNA 表达的影响

6

16 μg/ml

BCR1 *NRG1* *TUP1* 2.7 2.4 3.9 *HWP1* *EFG1* *CPH1* *ALS1* *ALS3* *CSH1*

3　讨论

DNA

B

[11-13]

3

RT-PCR

HWP1

[14]

HWP1

2

EFG1 *CPH1* *TUP1*

TUP1

[15]

farnesol *TUP1*

[7] *NRG1*

mRNA

cAMP/PKA *NRG1* *TUP1*

[16-18] *TUP1* *NRG1*

3

[19]

Shao

[10] *CSH1*

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[ ]

(p 417)

ALS [20]

ALS1 *ALS3* *BCR1*

ALS1 *ALS3*

ALS1 *ALS3*

BCR1 *BCR1*

ALS1 *ALS3* [21]

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