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【博士学位論文の要約】

論文題目：Study on the effect of curcumin and its analogues against *Candida albicans*

Lee Yean Sheng

Candida albicans is a fungal pathogen that can cause both superficial and systemic infections. The rise of antifungal-resistant strains, as well as the scarcity of effective and selective antifungals, have posed a significant problem in the treatment of fungal infection in clinics recently. This alarming condition has prompted the development of innovative antifungal medicines that are both safe and effective in the treatment of fungal infections. Curcumin is one of the novel compounds for the treatment of fungal diseases.

Curcumin has been reported that possesses several biological functions which benefit human health. One of it is anti-fungal activity against fungal pathogen. In this study, microdilution assay showed that the curcumin was able to inhibit growth of *C. albicans* planktonic cells at 250 µg/ mL. It was also capable of inhibiting the 50% growth of *C. albicans* biofilm cells at 100 µg/ mL. However, its mechanism is still unclear. In human cancer cells, curcumin was reported to influence the human *HSP90* gene expression and Hsp90 protein. Hence, it was expected that curcumin also might affect the *C. albicans* Hsp90. In this study, the mRNA level test showed that the *C. albicans HSP90* was reduced greatly by curcumin. This is the first report that the curcumin downregulated the transcription level of *HSP90* of *C. albicans*. The importance of *HSP90* in resistance to curcumin has been confirmed by using *tetO-HSP90/hsp90Δ* and *HSP90*-overexpressed mutants. Using checkerboard assay and microdilution assay, *tetO-HSP90/hsp90Δ* was shown susceptible to curcumin while *HSP90*-overexpressed mutant was shown resistant to curcumin. In this study, the depletion of *HSP90* resulted in the reduction of *HOG1* has been confirmed by the genetic depletion of *HSP90* in *tetO-HSP90/hsp90Δ* strain. This defect resulted in the reduced survivability of the cells at high temperature and NaCl concentration. Similarly, the pharmacological depletion of *HSP90* by curcumin led to transcriptional reduction of *HOG1* and phenocopied *tetO-HSP90/hsp90Δ* in term of thermal and osmotic tolerances. Nonetheless, the effect of the curcumin to *C. albicans* was counteracted by overexpression of HSP90. These findings suggested that curcumin reduced *HSP90* resulted in the decrease in *HOG1* expression and impaired the stress responses of *C. albicans.* This study also showed that the depletion of *HSP90* by curcumin was not due to the reduction of transcriptional factors of *HSF1* and *AHR1*. However, the curcumin inhibit the *HSP90* expression at post-transcriptional level.

Antifungal resistance has been a major issue in antifungal chemotherapy. ATP-binding cassette (ABC) transporters such as Cdr1 and Cdr2 play important role in the antifungal resistance. Previous studies showed that the curcumin was able to inhibit the ABC transporters. Besides, previous studies showed that the reduction of *HSP90* reduced the protein of Cdr1 in *C. albicans*. Hence, it was expected that curcumin might influence the efflux pump activity. In this study, mRNA expression of *CDR1* was reduced by the curcumin. Using Nile red accumulation assay, curcumin was shown to disrupt the efflux pump activity of wild-type strain after the cells incubated with curcumin for 2 hours. The disruption was due to the reduction of *HSP90* and *CDR1*. This has been confirmed by the *tetO-HSP90/Δhsp90* strain and *HSP90*-overexpressed strain. The genetic depletion of *HSP90* in the *tetO-HSP90/Δhsp90* strain phenocopied the pharmacological inhibition of *HSP90* by curcumin. However, the overexpression of *HSP90* helped to improve the efflux pump activity of *HSP90*-overexpressed strain in the presence of curcumin. These data revealed that the curcumin impaired the efflux pump activity of *C. albicans* via affecting the gene expression of *HSP90.* Besides, in this study, the curcumin was found to accumulate the Nile red in the cells without affecting the *HSP90* expression. This showed that curcumin was also found to inhibit the efflux pump activity directly. This direct pharmacological blocking of efflux pump is one of the ways to address the antifungal resistance.

Although curcumin was found to possess several good biological functions, it has several limitations such as low bioavailability, poor cellular uptake and low stability in aqueous solution. Design and synthesis of novel structural analogue could be a good way to address these shortcomings. One of the

analogues is GO-Y030. It has been proved to possess more effective functions than curcumin in human cancer cells. However, GO-Y030 has low water solubility. As a result, another 2 compounds, compound A and B, have been synthesized with higher water solubility. The antifungal activity of these 3 analogues are still unclear. In this study, microdilution assay showed that three curcumin analogues were able to exhibit the antifungal inhibitory activity against *C. albicans* planktonic cells as curcumin did. Besides, the data also showed that the curcumin analogues were able to exhibit the anti-biofilm activity against the biofilm cells. A and B exhibited greater antifungal activity than GO-Y030. This might be due to the difference in their structures. More antifungal effectiveness of A and B might be due to the presence of extra nitrogen atoms which increased the polarity of the compounds. Previous research showed that polar compound was more bioactive compared to the non-polar compound. Polar compounds showed more fungicidal activity as compared to non-polar compounds as polar compounds were more electronegative and interfered in biological processes involving in electron transfer and thus affected the organism. Besides, this study showed that the exposure of *C. albicans* to the curcumin analogues decreased the gene expression of HSP90. Similar to curcumin, the downregulation of *HSP90* was not due to the inhibition of the transcription factors *HSF1* and *AHR1*. Besides, curcumin analogues downregulated *HOG1* and *CDR1*. As a result, this finding is the first report of the reduction of HSP90 gene expression by curcumin analogues for C. albicans. Nile red accumulation assay showed that the curcumin analogues reduced the efflux pump activity via the depletion of the *HSP90* gene. However, the only curcumin and GO-Y030 inhibited the efflux pump activity competitively. It was possible that the high polarity of A and B results in decreased in the inhibition of transporter activity.

Overall, curcumin and curcumin analogues were able to exhibit antifungal activity via disruption of *HSP90* expression. This led to the disruption of thermal and osmotic stress responses. Besides, they were also able to disrupt the efflux pump activity. Taken together, this study sheds new light on the functions of curcumin. However, the complex mechanism by which curcumin affects *C. albicans* needs to be further explored.