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Tytuł w jęz. angielskim:

The effect of ergosterol on the activity of Cdr1 transporter and selected plasma membrane parameters in *Candida albicans*

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STRESZCZENIE W JĘZYKU ANGIELSKIM (SUMMARY)

Candida albicans is an opportunistic fungal pathogen in humans. In the presence of certain comorbidities, fungal infection has an almost 50% mortality rate. The available antifungal drugs target either ergosterol present in plasma membranes (polyenes) or its biosynthetic pathway (azoles). Although highly effective against fungal cells, polyene drugs are also toxic to the host. Azoles generally display less cytotoxicity, but almost 20% of clinically isolated *Candida* spp. strains are resistant to these compounds. Among the resistance mechanisms, activation of the Cdr1 protein (Cdr1p) is the most common. Cdr1p is the major multidrug resistance transporter in *C. albicans*. One of the critical elements of developing new mycoses treatment strategies is investigating the cellular processes related to Cdr1p activity. This study aimed to investigate the effect of ergosterol on plasma membrane parameters and Cdr1p activity.

The first aims of the thesis were to obtain *C. albicans* cells deprived of ergosterol and determining their growth in standard growth media. This research model was obtained by removing the *ERG11* gene, which encodes lanosterol 14α-demethylase, an enzyme in the ergosterol biosynthesis pathway. The encoded protein is also a direct target of the azoles. The two-step recombination method of the *SAT1*-Flipper gene cassette was employed to delete the *ERG11* gene, followed by selection on complete YPD medium. This approach demonstrated that *C. albicans* strains obtained in this way exhibited resistance towards azoles and polyenes. They also showed reduced growth under aerobic conditions on complete YPD medium compared to the parental strain.

Additionally, *C. albicans* failed to grow in a minimal YNB medium when deprived of ergosterol. This effect was revealed to be an auxotrophy towards adenine and uracil. These findings demonstrate that previously obtaining the *C. albicans erg11Δ/Δ* had been impossible due to the usage of minimal media as selection. Simultaneous deletion of *ERG3* and *ERG11* genes in *C. albicans* cells resulted in an increased growth defect on a complete YPD medium compared to cells lacking the *ERG11* gene only.

The next goal of the thesis was to examine the plasma membrane structure of *C. albicans* cells deprived of ergosterol. The following parameters were selected for evaluation: fluidity, sterol profile and their transport process, phospholipid profile, asymmetry, and plasma membrane potential.

Deletion of the *ERG11* gene in *C. albicans* resulted in increased plasma membrane rigidity. One of the reasons was the accumulation of 14-α methylergosta8,24,28-dienol, lanosterol, and other C-14 methylated derivatives of lanosterol. Unlike in the parental strain, the amount of sterols in the *ERG11*-deficient mutant increased proportionally with growth time. This was linked to the increased expression of the ergosterol biosynthesis genes associated with non-canonical metabolism of lanosterol, namely, *ERG25*, *ERG251*, *ERG27,* and *ERG6*.

Next, the expression of genes encoding proteins associated with intra- and extracellular sterol transport was examined. The obtained results indicated that in ergosterol-deprived *C. albicans* cells, cytoplasm-locating binding proteins (Osh proteins) play a more significant role in sterol transport than Lam and Arv1 proteins, which are found at sites where the plasma membrane contacts the endoplasmic reticulum. In addition, the data highlighted an increase in the expression of genes involved in the esterification of sterols and their storage as reserve material. A weaker role was observed for genes involved in the process of transporting acetylated sterols out of the cell.

The plasma membrane phospholipid profile was also affected in ergosterol-deprived cells. An increased amount of phosphatidylcholines, phosphatidylinositols, and unsaturation in fatty acids were observed. A reduction in the length of fatty acids and quantity of phosphatidylethanolamines were also detected. Additionally, phosphatidylethanolamines accumulated mainly in the outer monolayer of the plasma membrane. These findings prompted further investigation of plasma membrane potential and related processes in ergosterol-deprived cells. A method of estimating membrane potential with the usage of di-4-ANEPPS fluorescence probe was developed in order to fulfill this task. The results indicated that the lack of ergosterol caused the depolarization of cell membranes. One of the identified reasons was the delocalization of H^+-ATPase to the vacuole, its reduced amount, and activity.

The primary aim of this work was to determine the effect of ergosterol deprivation on the behavior of the Cdr1 transporter. This was achieved by investigating the expression of the *CDR1* gene, comparing the amount of Cdr1p in the cell lysates, and studying the localization and efflux activity of Cdr1p. Cells deprived of ergosterol showed an increased expression of the *CDR1* gene, as well as an increased abundance of the protein. Simultaneously, increased expression of genes encoding the positive regulators of *CDR1* (*UPC2* and *ZNC1*) and the reduced expression of the gene encoding the negative regulator *CDR1* (*FLO8*) were detected using RNA-Seq. However, in ergosterol-deprived cells, Cdr1p mislocalized from the plasma membrane to the vacuole in the early phase of exponential growth. This mislocalization was associated with the reduced efflux activity of the Cdr1p compared to the parent strain. Cells lacking both *ERG11* and *CDR1* genes were used to prove that strain lacking only *ERG11* gene displayed reduced Cdr1p activity, but the protein had partial efflux activity.

The final aim of the thesis was to present a practical application of the obtained results. Experimenting with the *C. albicans erg11Δ/Δ* strain allowed the selection of two combinations of drugs. One of which had beneficial effects (gentamicin stimulated fluconazole activity) whereas the other possessed adverse effects (capric acid induced resistance towards amphotericin B). The mechanism of the fluconazole-gentamicin combination was determined to be associated with the cell surface hydrophobicity of the *C. albicans* cells. When treated with fluconazole, cells with increased surface hydrophobicity overproduced membrane phosphatidylinositols, which in turn are directly recognized by gentamycin. The mechanism of the antagonistic action of the capric acid-amphotericin B combination has been identified as the following: treatment of *C. albicans* cells with capric acid leads to an increased fluidity of cell membranes, which are compensated by ergosterol overproduction. This leads to higher resistance of capric acid-treated cells against amphotericin B.

The obtained results provided new insights into the role of ergosterol in maintaining the structure of the cell membrane in *C. albicans*. The findings presented here demonstrate that plasma membrane disruption due to lack of ergosterol is associated with the loss of the correct location and activity of the Cdr1p. These conclusions may contribute to the development of new forms of treatment for mycoses, which in this thesis was presented as an example of newly described drug combinations.