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P2206 Analysis of copper toxicity towards *Cryptococcus neoformans* based on quantitative proteomics

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Background:

Cryptococcus neoformans, one of the three deadliest human fungal pathogens, is a cause of pneumonia and meningoencephalitis, which contributes about 600,000 deaths each year. It has been reported that copper toxicity is a critical part of host innate immunity defending fungal infection, and its toxicity may be associated with redox processes. However, the molecular mechanism remains unclear.

Materials/methods:

The process of copper suppressing *Cryptococcus neoformans* was analyzed based on quantitative proteomics in *mt1/2ΔΔ*, which is a mutant generated by knocking out metallothionein mt1 and mt2 from H99. Fungal cells were routinely grown in YPD medium (1% yeast extract, 2% peptone, 2% dextrose). 0.5mM CuSO4 was used as copper stress, and 30 mM NAC was used for blocking ROS. By combining iTRAQ with LC-MS/MS, the proteomic profile of copper suppressing process was constructed. LC-MS/MS data was collected using the Orbitrap Q Exactive plus MS platform coupled with EASY-nLC 1000 liquid chromatography. The generated MS/MS data was searched against UniProt *Cryptococcus neoformans* H99 database using Mascot (2.5.1) and Scaffold (4.6.2) software.

Results:

mt1/2ΔΔ was sensitive to copper toxic *in vitro*, as a deficiency of metallothioneins. While, this suppression of *mt1/2ΔΔ* was significantly recovered after NAC treatment. For quantitative proteomics analysis, a total of 3529 proteins were quantified, among which 175 proteins were differentially expressed under copper stress compared with normal culture. NAC treatment had the opposite regulation on most of these differential proteins. Intensive bioinformatic analysis was then carried out to annotate those quantifiable targets. Bioinformatic analysis showed that differential pathways were involved in the process. 'Ribosome', 'proteasome' and 'protein processing in endoplasmic reticulum' were significantly enriched. Especially, in 'proteasome' and 'ribosome' pathways, 66% and 37% of the annotated proteins were significantly changed, respectively. In 'protein processing in endoplasmic reticulum' pathway, the differential proteins were mainly concentrated on 'ER associated degradation' process.

Conclusions:

In this study, proteomic data of a copper sensitive strain under the influence of copper toxicity was analysed. We found that the copper inhibition of *Cryptococcus neoformans* resulted from changes in three ROS mediated pathways. These results shed new light on the copper antifungal mechanisms.