Identification of positive and negative mediators of fluconazole tolerance in *Candida albicans*

E. Delarze¹, C. Pralong¹, F. Maranzano¹, S. Znaidi², M. Legrand², M. Arendrup³, C. Munro⁴, C. d'Enfert², D. Sanglard¹;

¹University of Lausanne and University Hospital Center, Lausanne, SWITZERLAND, ²Pasteur Institute, Paris, FRANCE,

³Statens Serum Institut, Copenhagen, DENMARK, ⁴University of Aberdeen, Aberdeen, UNITED KINGDOM

Candida albicans and tolerance

- Problems of **resistance** to antifungal treatments
 - Increased **Minimum Inhibitory Concentration** (MIC, first concentration inhibiting ≤ 50% of growth)
- Tolerance** → ability to **survive high drug concentrations without** development of resistance mechanisms [1]
 - Increased residual growth [1]
- Tolerance mainly observed for **fluconazole** (FLC), the **most widely used antifungal**
- Tolerance may play a role in **treatment failure** and **disease persistence**
- Mechanisms of tolerance are still poorly understood**

Aims of the project

- Identification and characterization of mediators of tolerance to FLC
- Mediators of tolerance → **new drug targets** and **treatment strategies improvement**

Identification of putative tolerance mediators

- Collection of **582 barcoded (BC) Doxycycline (Dox)-dependent overexpression (OE) C. albicans** strains
- Collection constructed in *C. albicans* BWP17 genetic background

Single strain FLC susceptibility assays

- Enrichment assay and individual strain susceptibility assay gave **12 putative activators** and **15 putative repressors**
- Determination of the Dox+/Dox- growth ratios** in presence of 8µg/ml FLC at pH6
- Since BWP17 basal tolerance was too low to detect reduction of tolerance, **tolerant clinical isolates were used putative tolerance repressors**
 - DSY2110** (TI = 27.4% of growth control)
 - DSY4754** (TI = 68.5% of growth control)

Gene name	Function
GZF3	GATA-type transcription factor
CRZ1	Calcineurin-regulated C2H2 transcription factor
YCK2	Plasma membrane protein similar to <i>S. cerevisiae</i> casein kinase I

Gene name	Function
CSK1	Putative mitogen-activated protein (MAP) kinase with an unknown role
CPH1	Transcription factor; for mating, filamentation on solid, pheromone-stimulated biofilms
SFL2	Transcription factor involved in regulation of morphogenesis

- CRZ1, GZF3, CSK1, CPH1** and **SFL2** showed **consistent increase/decrease of tolerance** when OE independently of the pH tested

- Inactivation of CRZ1** in clinical strains **decreases tolerance**. Inactivation of **GZF3** has **no significant impact** on tolerance in these strains.

Conclusions

- The **Dox-dependent OE system** and our **enrichment strategy** allowed us to **identify 2 potential activators** and **3 potential repressors** of FLC tolerance
- CRZ1** and **GZF3** were observed to **increase the residual growth by 2-fold** in BWP17 when OE at pH6.
- Overexpression of **YCK2** did not reach our tolerance cutoff (TI≥0.2)
- Overexpression of **CSK1** **reduced tolerance by 5-fold** (in DSY4754), while **CPH1** and **SFL2** yielded 6- and 2-fold tolerance reduction (in DSY2110).
- The **deletion of CRZ1** in clinical strains with high tolerance profiles **reduces tolerance**.