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Genome-wide screenings and subcellular localization analyses uncover major events in the mechanism of action antitumour lipids in *Saccharomyces cerevisiae*

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Antitumor lipids (ATLs) are a family of proapoptotic drugs with antineoplastic activity. Despite the promising therapeutic potential of ATLs, their mode of action and the differences between individual family members are not fully understood. *Saccharomyces cerevisiae* was used as a model organism to identify genes involved in cytotoxicity induced by clinically-relevant ATLs. A single-gene knockout collection was screened for strains resistant to the ATL prototype edelfosine.

Relevant gene clusters showed the involvement of drug uptake, endocytosis, vesicle transport and mitochondria in ATL-mediated cytotoxicity. Comparison between wild-type and respiratory-deficient ρ-cells showed mitochondria to be essential for drug toxicity and involved in the production of reactive oxygen species and the fragmentation of organelles.

Drug tracking by use of fluorescent analogues revealed endocytosis-independent uptake and accumulation in the endoplasmic reticulum. Novel genes essential for drug uptake were identified. Edelfosine caused the proton pump Pma1p to become ubiquitinated, internalized and degraded in the vacuole, leading to cytosolic acidification. Our working model proposes the disappearance of such a vital protein as a key event for drug toxicity. Edelfosine reorganized lipid rafts and displaced Pma1p from rafts, leading to its endocytosis and the endosomal sorting complex required for transport-mediated degradation. Retrograde transport was also involved in the process. In addition to this, parallel screenings and cell biology experiments identified differences in the way different ATLs promoted cell death in yeasts.

Our data support redistribution of raft proteins as a mechanism of action for edelfosine, which eventually leads to cell death. These data highlight the usefulness of yeasts as a model for providing further insight into the action of antitumour drugs.

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