Reviewer's report

**Title:** Identification of Molecular Pathways Affected by Pterostilbene, a Natural Dimethylether Analog of Resveratrol

**Version:** 1  **Date:** 7 January 2008

**Reviewer:** Dominique Sanglard

**Reviewer's report:**

This paper describes the use of *S. cerevisiae* (Sc) for establishing the mode of action of the compound pterostilbene, a plant analog of resveratrol. The authors exposed Sc to pterostilbene at the IC50 concentration for 3 h and used microarrays as experimental support to determine the effect of the drug at the transcriptional level. The authors found many genes with altered expression after drug exposure. The data mining analysis indicated several metabolic pathways grouped by GO terms that were affected by drug exposure, especially genes involved in methionine metabolism, mitochondrial functions, drug detoxification, and transcription factor activity. Additional analyses revealed also a large number of genes involved in lipid metabolism affected by pterostilbene treatment. The authors next tested a restricted number of Sc mutants in genes differentially expressed by pterostilbene treatment. Among them, only PDR3 was found to perturb drug response by enhancing susceptibility to pterostilbene. In conclusion, this study shows the usefulness of Sc for discovering drug mode of action. The paper is well written and experiments carefully performed. A few points need to be addressed.

1) It is difficult in this study to reduce the mode of action of the drug to a single target or a single pathway. This is probably due to the fact that the authors used a single time point for drug exposure and that this time point was quite late to guess the primary mode of action of the drug. The ideal experimental approach would have used several time points and even several drug concentrations for the purpose of guessing a principal mode of action. In the worse case, many of the differentially expressed genes listed by the authors may have been the result of drug side effects. Of course, one cannot exclude that pterostilbene may have multiple targets in Sc and that multiple experimental conditions would have revealed similar patterns than observed by the authors. However, the single time point and single drug concentration approach used by the authors is very restrictive and constitutes one a major weakness of the paper. I advice the authors to mention these remarks in their discussion.

2) The mutant testing revealed that a mutant for PDR3, which is upregulated after drug exposure, is more susceptible to pterostilbene than the wild type. This is the only mutant with this phenotype among those tested. The involvement of PDR3 in this phenotype certainly confirms that pterostilbene uses drug stress
response in Sc. Given that PDR3 (together with PDR1) regulates ABC transporters among which PDR5 is a major member, it would be of high interest to test whether the PDR5 mutant is also more susceptible to pterostilbene.

3) In relation with the above-mentioned remark, I suggest to mention that it is also possible to determine drugs mode of action by systematic screening of a Sc mutants library, which is available to the research community. Confounding microarray results with mutant library â##hitsâ## can establish genes relevant to the drug mode of action and principal drug targets. The result obtained with PDR3 in an illustrative example reported in this study but it may be applied to an entire mutant collection. This remark should also be added in the authorâ##s discussion.

4) Other details:
- Please clarify what is meant by â##large-scaleâ## culture (Methods section)
- Given the phenotype of the MET3 mutant on pterostilbene, it seems that methionine pathway has no major impact on pterostilbene susceptibility and therefore suggest a minor role of pterostilbene on this pathway. Did the authors perform tests with other MET mutants? Are results similar? Moreover, does the type of medium (YPD) has an impact on pterostilbene susceptibility of MET mutants (given that this medium contains several sulfate-containing metabolites overcoming the need of MET genes)?
- Microarray data should be provided in a BMC-compatible format

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests