Author's response to reviews

Title: Tamoxifen Elicited Uterotrophy: Cross-Species and Cross-Ligand Analysis of the Gene Expression Program

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Author’s Response to Reviewer’s Comments

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Review 1: George Daston
- As noted, on Page 7 Line 12 explanations have been added for defining the terms CAS, CAD, DAS, DAD.
- Information about the kinetics of EE and TAM as it relates to the timing of gene expression responses has been added to the text on Page 13, Line 1 per reviewers suggestion.
- Reviewer inquired about data in Table 1 and Figure 1 representing the same data. They do not; the first is dose response data and the second is time course data from independent animal studies.
- Information regarding maximum fold change has been incorporated into Table 3 per reviewer’s suggestion.
- end of review comments

Review 2: Todd Skaar
- Table 1 and Table 2 were entered incorrectly in the initial version and have been re-submitted with correct data.
- The reviewer expressed concern that these studies do not adequately mimic the human treatment regimen; this study does not have that as its aim as an acute/sub-acute design. Certainly, the extrapolations from sub-acute exposures to sub-chronic/chronic dosing regimens need to be done, but the purpose of this study is to utilize a sensitive model of estrogen signaling in a mammalian uterus, likely the most widely used model for this type of study, in order to query the temporal expression changes for tamoxifen in comparison to EE. Ovariectomy is not necessarily intended to mimic post-menopausal populations, although interpreting the data in that context is none the less plausible and interesting. Ovariectomy has been shown to increase sensitivity to estrogen exposure, thus the activities of tamoxifen in this context are useful in determining its potentially unique transcriptional behavior or monitoring just how much of an agonist it is in this model.
- With regard to dosage, again it is not the goal of this study to mimic chronic exposures, this is clearly an acute/sub-acute experimental study, designed to capture the early temporal expression cascades due to the known mechanism of tamoxifen action through the ER as a transcription factor. As the reviewer noted, we do not have perfect understanding of the metabolic profile of tamoxifen for both rodent species which is why we calibrated the time-course dosage using a dose response according to the maximal physiological effect observed in uterotrophy. The authors deemed a higher dose to be unnecessary as the proliferative phenotype was already at or approaching maximum.
Regarding the metabolite profile in rodents, various other studies have been performed in rats, mice and humans [1-5] suggesting rats and mice have divergent metabolic profiles with rats more closely modeling the human case, thus the use of both rats and mice in this study reflect a cross-model approach of identifying the expression changes associated in both species given this knowledge of their somewhat divergent metabolic profiles. In light of these concerns additional explanations have been added to the text (pg 4 ln 24, pg 5 ln 8) to clarify any confusion regarding the aims of the manuscript and provide discussion of the metabolic profiles in the mouse and rat relative to humans.

- source of tamoxifen (Sigma) indicates Z isomer, percentage is not provided. (http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/T5648)
- the assumptions for 2-way ANOVA are 1) normally distributed populations, 2) homogeneity of variances, 3) independent errors, 4) no interaction between blocks and treatments. Our samples and analysis meet all of these assumptions. Data at neighboring time points are from independent biological units and are not correlated as a part of the statistical analysis. Temporal correlations are calculated on gene by gene basis after statistical analysis is complete. Correlations are only used as an assessment of the similarity of gene expression profiles across treatment groups, not between time points. Statistical methods are valid as described.

-end of review comments

REFERENCES


