Author's response to reviews

Title: Identification of Oxidized Protein Hydrolase as a Potential Prodrug Target in Prostate Cancer

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Author's response to reviews: see over
Dear Editor,

We appreciate the feedback we have received from you and the reviewers. We have taken the comments provided to us under consideration and have made the following corrections and explanations to address the reviewers concerns:

**Reviewer 1**

Comment 1: We have edited the results and conclusion to more clearly indicate that LNCaP cells have the highest expression levels of OPH and that PC3 and DU145 cells have slightly less OPH expression compared to RWPE-1 cells. While examining normal and prostate cancer tissues are an interest to our group, we believe that the citation and data from the Human Protein Atlas adequately support that differential OPH expression occurs between normal and cancerous prostate tissues.

Comment 2: We have more clearly indicated in the second paragraph of the Results section that the general esterase activity visualized with α-naphthyl acetate is higher in LNCaP, PC3, and DU145 cell lines compared to RWPE-1 and RWPE-2. The third paragraph describes the results of staining with the chiral ANAA substrates. The OPH activity revealed by the ANAA substrates is higher in LNCaP cells compared to RWPE-1 cells. PC3 and DU145 cells have slightly lower OPH activity than RWPE-1 cells.

Comment 3: Due to the high protein concentrations used in our native gel experiments (120 µg) and the nature of n-PAGE, we are unable to provide normalized protein levels using antibodies or a generalized stain. The amount of protein loaded in native gels is typically normalized by protein concentration, as we have carried out. Our attempts to visualize proteins with Coomassie blue in the native gel simply result in large unquantifiable streaks due to the large amount of protein added to the gel and the decreased band resolution of native gels. Likewise, the use of immunoblotting as a normalization standard in native electroblots is very difficult. In a native gel, a given protein often forms multiple bands as seen with OPH. Since SDS is not present in a native gel, proteins will migrate based on several factors including size, 3D structure, charge, and protein-protein interactions, often resulting in multiple bands. We have provided anti-GAPDH as a normalization control in our western blots comparing OPH levels in the various cell lines.

**Reviewer 2**

Comment 1: Regarding Figure 3, the figure has been replaced with the correct figure (Fig 3C). This should eliminate the confusion the reviewer had when comparing Figure 3C with Figure 3A.

Thank you again for your comments. We appreciate your time and effort and are happy to address any concerns that the reviewers and editorial staff have with the manuscript.