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Anomalies and Possible Data Fabrication/Falsification in the Paper Entitled "Greatwall-phosphorylated Endosulfine is both an inhibitor and a substrate of PP2A-B55 heterotrimers." Authored by Williams, B.C., Filter, J.J., Blake-Hodek, K.A., Fuda, N.J., Shalloway, D. and Goldberg, M.L. and Published in the Journal eLife [eLife (2014) e01695, doi: 10.7554/elife.01695].

Author: H.Y. Lim Tung, PH.D., Peptide and Protein Chemistry Research Laboratory, Nacbraht Biomedical Research Institute, 3164 21St Street, Suite 117, Astoria (NYC), NY 11106, USA. E mail: *hyltung2010@nacbrahtbiomedresins.net*

Abstract. In the paper entitled "Greatwall-phosphorylated α-Endosulfine is both an inhibitor and a substrate of PP2A-B55 heterotrimers." Authored by Williams, B.C., Filter, J.J., Blake-Hodek, K.A., Fuda, N.J., Shalloway, D. and Goldberg, M.L. and Published in the Journal eLife [eLife (2014) e01695, doi: 10.7554/elife.01695], the authors claimed that when α-Endosulfine was phosphorylated on serine 67 by Greatwall kinase, it became a potent inhibitor of PP2A-B55α and that phospho serine 67 of α-Endosulfine was also a substrate of PP2A-B55 α. It is submitted that the authors failed to show that serine 67 of α-Endosulfine was dephosphorylated by the enzyme that it inhibited. The phosphorylation dependent inhibition of PP2A-B55 by α-Endosulfine has more to do with science fiction than science as the authors did not seem to know how to perform a protein phosphatase inhibition assay. Anomalies and possible Data Fabrication/Falsification are discussed

The paper entitled "entitled “Greatwall-phosphorylated α-Endosulfine is both an inhibitor and a substrate of PP2A-B55 heterotrimers", authored by Williams, B.C., Filter, J.J., Blake-Hodek, K.A., Fuda, N.J., Shalloway, D. and Goldberg, M.L. and published in the Journal eLife [eLife (2014) e01695, doi: 10.7554/elife.01695] was meant to extend the work of Mochida et al. [1,2] which did not show that α-Endosulfine was a specific inhibitor of PP-2A-B55 when α-Endosulfine was phosphorylated on serine residue by Greatwall kinase (critiqued in [3]). Indeed, several studies have questioned the role of Greatwall kinase in the control of entry of mitosis and the resumption of meiosis [4- 8]. Rangone et al. [4] reported that that they could not demonstrate "a substantial inhibitory effect of phospho-Endos upon the ability of PP-2A to dephosphorylate histone H1 phosphorylated by Cdk1". One of the authors's paper entitled "Greatwall phosphorylates an Inhibitor of protein phosphatase-2A that is essential for mitosis", and published in Science [Science (2010) Vol 330, pp1670-1673] was quoted as stating that "the use of other substrates to assess for the inhibition of PP-2A by phospho-Endos was apparently less effective". Kim et al. [5] was very forceful in stating that "Despite the importance and evolutionary conservation of this pathway (i.e Gwl-Endos-PP2A pathway), it can be bypassed in two situations. First, heterozygosity for loss-of-function mutation by twins which encode the Drosophila B55 protein suppressed the effects of Endos or gwl mutants. Several types of cell division occur normally in twins heterozygotes in the complete absence of Endos or the near absence of Gwl. Second, this module was non essential in the nematode Caenorhadlis elegans. The worm genome does not contain an obvious ortholog of gwl1, although it encodes a single Endos protein with a surprisingly well conserved Gwl target site. Deletion of this site had no obvious effects on cell divisions involved in viability or reproduction under normal laboratory condition". Although, the title of the paper by Hara et al. [6] tried to hide the content of the paper, it showed clearly that "Greatwall kinase is not essential for G2/M transition and full activation of cyclin B-Cdk1 in Starfish oocytes treated with 1-MeAde". The paper by Okumura et al [7] was quite direct at stating that Greawall kinase was not essential for resumption of meiosis in Star fish oocytes. While the title of the paper by Adhikari did not announce it, the paper by Adhikari et al. [8] did show quite remarkably that (i) Mastl kinase (equivalent of Greatwall kinase) was not required for the resumption of meiosis

and progression to metaphase I in mouse oocytes, (ii) Mastl-deficient ooctes did not fail to progress through meiosis I but underwent GVBD with kinetics and efficiencies that were indistinguishable from those of $Mastl^{+/+}$ oocytes, and (iii) Mastl has no essential role during meiotic resumption or prometaphase I progression in mouse oocytes,

In view of the fact that Greatwall kinase was not essential in mitosis or resumption of meiosis and that in some organisms, the phosphorylation of α-Endosulfine by Greatwall kinase appears to not be essential neither, it is not clear why the authors of the paper did not take these papers into account instead of persisting in trying to extend and validate the papers by Mochida et al. [1,2] which are at best misleading and at worse dishonest (See a critical assessment of the papers by Mochida et al in [3]). Irrespective, as shown below, the paper by the authors being critiqued here were riddled with anomalies and Data Fabrication/Falsification and fantastical conclusions.

In Figure 2 of the paper, the authors showed that thiophospho ser 67-α-Endosulfine (Ensa) inhibited PP-2A-B55α with IC_{50} of ~.2 nM whereas phospho ser 67- α-Endolsulfine (Ensa) inhibited PP-2A-B55α with IC_{50} of ~ 1 nM under their assay condition. The authors also stated that dephospho-α-Endosulfine (Ensa) could inhibit PP-2A-B55α with an IC_{50} of ~800 nM casting doubt on the phosphorylation dependent inhibition of PP-2A-B55α by α-Endosulfine (Ensa) (Whether these values are to be believed is another matter which needs to be resolved. See below).

The idea that that PP-2A-B55α dephosphorylated serine 67 of α-Endolfine (Ensa) with a Km value of 0.0009–0.0017 µM was more in the realm of science fiction than science. Based on the fact that 0.5 nM of PP-2A-B55α was used per assay and the results of Figure 7B and 7C showed that a Vmax value ~0.000007 nmol phosphate/min/0.5 nM PP-2A-B55α, it can be estimated that the specific activity of PP-2A-B55α was ~0.00007 nmol phosphate released/min/mg of enzyme when phospho ser 67-α-Endolfine (Ensa) was used as substrate, indicating that the latter is an extremely poor substrate of PP-2A-B55α.. The value of ~0.00007 nmol phosphate released/min/mg of enzyme is 1.4 million times lower than what one would observe with the dephosphorylation of an Inhibitor of

PP-1 such as DARPP-32. Indeed phospho-thr35-DARPP-32 is a very poor substrate of the protein phosphatase (PP-1) that it inhibits. An important and pertinent questions that the authors of the paper must ask is how can one measure PP-2A-B55α that had a specific activity of ~0.00007 nmol phosphate released/min/mg. It is beyond normal comprehension and in the realm of science fiction to be able to measure ~0.00007 nmol phosphate release/min/0.1 mg of PP-2A-Bα. It is simply against the laws of nature to be able to measure ~0.00007 nmol phosphate released/min. Any data and calculation that was based on the measurement of ~0.00007 nmol phosphate released/min must be either fabricated and falsified or due to gross incompetence.

As the concentration of α-Endolfine (Ensa) has been estimated to be ~150 to 300 nM in Xenopus oocyte extracts and of PP-2A-B55α to be ~50 to 70 nM it can be surmised that PP-2A-B55α would be inhibited by α-Endosulfine (Ensa) in the absence of its phosphoryation by Greatwall kinase. It is indeed quite odd to use thiophospho ser 67-α-Endosulfine (Ensa) when phospho ser 67- α-Endosulfine (Ensa) inhibited PP-2A-B55ɗ with IC_{50} of ~ 1 nM!. Did the authors determine the stoichiometry of phosphorylation of α-Endossilfine by Greatwall kinase using ATP-ɣ-S as the phosphate donor.

In order to show that serine 67 phosphorylated by Greatwall kinase (if it can be shown to be of any physiological relevance) is a substrate of the enzyme that it inhibits, the authors must compare its dephosphorylation by PP-2A-B55α with that of its potential dephosphorylation by PP-2A-B55ß, PP-2A-B55ɣ, PP-2A-B55ɗ, PP-2A-B56'α, PP-2A-B56'ß, PP-2A-B56'ɣ, PP-2A-B56'ɗ, PP-2A-B56'Ɛ, PP-2A-B"70 and PP-2A-B"130, PP-1, PP-1I, PP-2B, PP-2C, PP-4, PP-5 and PP-6, and show that the Km and Vmax for the dephosphorylation of phospho serine 67 of α-Endosulfine (Ensa) by PP-2A-B55α is indeed much lower and higher respectively. The author should also use a standard amount of protein phosphatase that can be measured in the laboratory setting, preferably in the range of 0.066 to 0.100 unit and not 0.00007 unit or less (one unit of protein phosphatase activity is that amount that causes the release of 1 nmol phosphate released per min).

In Figure 4 of the paper, the authors stated that the "Drosophila larvae lacking B55 are deficient in anti-Endos". That is a misleading and possibly dishonest statement because the authors did not measure the activity of PP-1_I which requires phosphorylation by PP-1_I-ACK to exhibit its activity [9-17]. The authors did not also determine the activity of PP-$2A_0$ which requires activating molecules to express its full activity [18-21]. As shown in Figure 4 of the paper, a large amount of PP-1 catalytic subunit coincided with ant-Endos activity. Why the authors ignored the role of PP-1 in the dephosphorylation of "phoshpo-Endos" is beyond comprehension. Instead of coming up with a conclusory statement to rule out PP-1, the authors should have determined the activity of PP-1_I. Even without determining the activity of PP-1_I, the authors showed that when PP-1C (PP196A) was knocked out, ~50% of anti-Endos activity was also knocked out. Since the anti-Endos activity of PP-1 was determined against spontaneously active PP-1 and not against PP-1I, the ~50% anti-Endos activity reduction was a gross underestimation.

In summary, the paper by Williams et al. being critiqued here contained too many fantastical results that have more to do with science fiction than science. It therefore cannot be taken seriously.

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