Review of: "Transglutaminase 2 associated with PI3K and PTEN in a putative membrane-bound signalosome platform blunts cell death"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

This paper aims to investigate the role of tissue transglutaminase in cell death process induced by Arsenic trioxide (ATO) in all-trans retinoic acid (ATRA)-differentiated acute promyelocytic leukaemia (APL) NB4 cell line. In particular, by using NB4 WT (wildtype NB4 cells), NB4 TG2-C (virus control containing scrambled shRNA), TG2-deficient NB4 TG2-KD (shRNA-based knockdown), and NB4 TG2-KO cells (TALEN TG2 knockout), they for the first time in literature show plasma membrane-bound form of TG2 functions as a signalling hub that brings CD18, c-SRC, PI3K/p110, PI3K p85 into close proximity leading to activation of PI3K, which in turn results in AKT (phospho-AKT T308, S473)and mTORC2 (phospho-mTOR Ser2481, and Ser2448) activation and PTEN inactivation. The data, as far as they go, support the conclusion, but are less than compelling for several reasons. One of my major concerns are the lack of TG2 expression data (gene and protein level) in NB4wt, NB4 TG2-C, NB4 TG2-KD and NB4 TG2-KO cells with and without ATRA differentiation. Authors should indicate the downregulation fold for TG2 gene and protein expression in these cells. p-mTOR (2481, 2448) p-AKT (S473, T308), mTOR, AKT, TG2, and GAPDH protein expression levels in NB4 cell lines were only shown following 5 day-ATRA treatment, there is no information on the levels for these proteins in parental NB4 cells without ATRA treatment. Authors claim that GTP-bound TG2 acts an upstream signal activating a signalosome platform as treatment of TG2 inhibitor NC9 that lacks TG2 in open/transamidase active conformation abolishes all interactions between TG2 and its interacting proteins. Authors should perform Native-PAGE-Western Blot analysis to show whether TG2 is truly in open or closed form in ATRA and/or ATO treated NB4wt, NB4 TG2-C, NB4 TG2-KD and NB4 TG2-KO cells. In discussion authors should discuss their findings relevant to the literature (For example: There is no mention of Boroughs et al. (J Biol Chem 2014 Apr 4;289(14):10115-25) or Condello et al. (FASEB J. 2013 Aug;27(8):3100-12) who has done the pioneering work on TG2 on PI3-kinase signaling events.)

Minor comments are;

- For the uniformity of the paper, data values should either placed to all graphs or removed from graphs (i.e. graphs in Figure 2 b and d have data values while the ones in Figure a and c don not have any).
- It seems supplementary Figures are missing statistical evaluation and *P<0.05 indications.

- In methods under Plasma membrane preparation (authors should indicate whether they have used an Abcam Kit or not.