Reviewer’s report

**Title:** Terminalia catappa attenuates urokinase-type plasminogen activator expression through Erk pathways in Hepatocellular carcinoma

**Version:** 1  **Date:** 8 November 2013

**Reviewer:** Giuseppina De Petro

**Reviewer’s report:**

Minor Essential Revisions. I appreciated the manuscript by Ming-Ju Hsieh et al - Terminalia catappa attenuates urokinase-type plasminogen activator expression through Erk pathways in Hepatocellular carcinoma - The authors have raised interesting issues on the molecular mechanism underlying the antimetastatic effects of Terminalia catappa leaf extract (TCE). Since the overexpression of urokinase (at mRNA and protein level) in human hepatocellular carcinoma (HCC) is an unfavourable prognostic factor for HCC patients, the u-PA targeting by molecular technologies (AS-RNA, shRNA: Tavian D et al Cancer Gene Therapy, 2003; Salvi et al, Molecular Cancer Ther. 2004), also in human HCC xenografts (Salvi A et al Tumor Biology 2007) decreases the aggressive behaviour of HCC cells inhibiting their migration and invasion capabilities, the authors have decided to investigate the effects of TCE on uPA expression. Actually the authors in the text refer to antimetastatic effects of TCE (as I mentioned above); I think this term (antimetastatic) is inappropriate because the experimental design and the results shown have been obtained in cultured HCC cells and not in tumor animal models. I suggest to change this term. I appreciated the results obtained by the authors concerning the decrease of uPA expression, also those on the impairment of transcriptional uPA activity (uPA promoter assay, Chip IP data).

--I would suggest a minor essential revision.

--Level of interest: an article of importance in its field, in HCC field, but probably also in other cancer types that might be examined in the future

--Quality of written English: Acceptable

--Background:

--line 6: I suggest to eliminate the information on the 2 alpha elices and 2 antiparalle beta strands; this information is not necessary. The list of uPA domain should refer to the position in the uPA molecule: from the N-terminus to the C-terminus (that is, GF domain, kringle domain and serine protease domain). Further in the background the authors should summarize the information available on uPA as putative target for HCC therapy in animal model by siRNA.

--Results:

--title of the first paragraph: ..... and their endogenous inhibitors PAI-1. PAI1 is one inhibitor and the authors show results on this inhibitor; Therefore the sentence would be ..... and its endogenous inhibitor PAI-1.
Effects of TCE on the protein levels of uPA and their endogenous inhibitor PAI1: the results shown in Fig 1 show uPA expression as enzymatic activity and at protein level as they were detected in the conditioned media. It is necessary to state if the conditioned medium did not contain serum or it contained a small % of serum; the author refer to ref 24 (materials and Methods p 8), but it is not easy to find this article. Further I would like to know the amount of conditioned media loaded on the SDS-PAGE (I think they were comparable amounts in the two experiments, enzymatic activity and Western blotting, but it seems that a major amount has been loaded on SDS gel for Western blotting. It is necessary to give these sharp informations in the text. Further since uPA and PAI1 are secreted proteins, beta actin is not an appropriate control. It is possible to add, as supplementary figure, the protein staining of the gels to ascertain the comparable amount loaded. It is not possible to adjust the secreted uPA and PAI1 protein levels with the beta actin protein level. Since it is very difficult to have a control secreted protein, it is possible to consider the control sample as 100% and refer the tested samples to this.

Discussion:
P 16, line 2: …TCE has an inhibitory effect (it lacks this term).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests' below.