Reviewer's report

**Title:** Ccp1, a FGF2 downstream gene, regulates cell proliferation and apoptosis in neuroblastoma cells

**Version:** 1 **Date:** 29 June 2010

**Reviewer:** John K Heath

**Reviewer's report:**

The paper reports a study of CCP1, a gene reported to be regulated downstream of FGF signaling. The paper examines the consequences of both ectopic expression and depletion of ccp1 and comes to the conclusion that ccp1 accelerates progress through G1 partially via activation of the MAPK pathway and protects from apoptosis. This is accentuated by FGF treatment.

In general terms the paper reports interesting results on a previously little studied gene. However, there are a number of technical deficiencies and unresolved issues which need to be addressed before publication.

P5 Natural immortalisation of MEFs requires further description as the concomitant genetic changes may have permitted non standard responses - was this a pool or clones? were the ells grown under hyperbaric conditions etc.?

P8 Fig1 1c lakes scale bars & DAPI co staining. Some operational definition of "morphological transformation" is required I dont find the images particularly informative or compelling - cytoskeletal staining? growth in soft agar?

P9 Fig2 I dont follow the design of experiments of figs 2 B and D - How can we compare the effect of BUDR incorporation in serum versus cell multiplication in the absence of serum - should not BUDR in the absence of serum be more informative? The fact there is high level BUDR incorporation and a modest effect of ccp1 expression may be due to the serum. In any case we cant compare the two sets of data. This is done in figure 3. Essentially the proliferative data should be cleaned up so that meaningful comparison can be made. At present the effects of ccp1 seem minor.

P11. Given there is an antibody SiRNA depletion must be demonstrated at the protein level. The usual caveats about off target effects should be included.

P11 It would be good to have some evidence that inhibition of caspases reversed the "apoptotic effect" - and other markers of apoptosis distinguishing extrinsic from intrinsic pathways.

p12 The evidence that Mek is involved in ccp1 induced proliferation essential relies on one inhibitor and the baseline labelling seems to be different from previous figures - unless this was done in the presence of serum which would be a strange experiment to try. The value of this work would be extended by
inhibitors of AKT and an FGF kinase inhibitor control.

P13 The ERK time courses are poorly justified and not quantified. Given that in no case does the signal peak and decay it is hard to draw any conclusions. It would be good to have densitometric data on the blots.

Crucial issue is that since ccp1 action is augmented by FGF they are clearly using different pathways - or FGF induction of ccp1 yields higher levels of ccp1 expression than the forced system employed here. It would be interesting to look at ccp1 depletion in some long term FGF system such as neurite outgrowth.

The paper contains some grammatical errors and incomplete references

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare i have no competing interests