Reviewer's report

**Title:** Pim1 promotes human prostate cancer cell tumorigenicity and c-MYC transcriptional activity

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**Reviewer:** Jaideep Chaudhary

**Reviewer's report:**

The authors demonstrate that Pim1 a serine/threonine kinase acts as a putative oncogene in prostate cancer possibly through a c-Myc dependent pathway based on in vivo studies. The experimental approach consisted of over-expressing Pim1 in three human prostate cell lines representing different disease stages: benign (RWPE1), androgen-dependent cancer (LNCaP) and androgen-independent cancer (DU145). The authors then analyzed tumorigenicity in vitro and in vivo as well as the effect of Pim1 over-expression on c-Myc transcriptional activity by reporter assays and gene expression profiling using an inducible MYC-ER system. Their results suggested that Pim1 alone was not sufficient to convert the benign RWPE1 cell to malignancy although it enhanced their proliferation rates when grown as xenografts in vivo. However, Pim1 expression enhanced the in vitro and in vivo tumorigenic potential of the human prostate cancer cell lines LNCaP and DU145. Reporter assays revealed c-Myc transcriptional activity in Pim1-expressing cells and mRNA expression profiling demonstrated that a large fraction of c-Myc target genes were also regulated by Pim1 expression.

Overall, the experimental design and conclusions are sound. However, there are some issues with data presentation and interpretation:

**Major compulsory Revisions**

1. Fig. 1A: The authors make a general claim that Pim1 increase c-myc expression LNCaP and DU145. Increased cMyc expression is evident in Pim1 expressing DU145 cells but not in LNCaP cells. It is possible that the LNCaP western blot is over-exposed complication the quantitation. The authors are requested to provide clear evidence in support of their claim.

2. The authors did not indicate whether they use male or female nude mice for their xenograft experiments. The use of either of these mice will be significant for interpreting the results in context of androgen dependent/ independent regulation.

3. In the context of androgen receptor signaling (Figure 5), did the authors determine the level of androgen receptor expression following Pim1 or its mutant (K67M) over-expression? The results shown in Figure 5 could very well be due to change in AR expression in Pim1-LNCaP cells.

**Minor Revisions:**
4. In Fig. 1B the FACS analysis demonstrates the absence of polyploidy. The authors also state that Pim1 induces polyploidy in a passage-dependent manner. In this context it will be better to mention the passage # following selection of Pim1 over-expressing cells used for FACS analysis.

5. In the second section of results (Page 9: Pim1 promotes proliferation and attenuates apoptosis of RWPE1…): In lines 4-6, it is not clear what the authors are trying to say: verifying that in vitro tumorigenicity of Pim1-expressing RWPE1 cells is due to polyploid cells driven by chromosomal instability as shown previously? This sentence contradicts their experimental observations.

6. Page 9 (section: Pim1 promotes in vitro and in vivo tumorigenicity of LNCaP and DU145 cells): The sentence “We next asked whether Pim1 can enhance the tumorigenecity established malignant prostate cancer cells” should be changed to “We next asked whether Pim1 can enhance the tumorigenecity of established malignant prostate cancer cells”.

**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