Reviewer's report

Title: Proliferation and survival molecules implicated in the inhibition of BRAF pathway in thyroid cancer cells harbouring different genetic mutations.

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Reviewer: Xiulong Xu

Reviewer's report:

Preto et al. studied the effect of BRAF suppression and Bay 43-9006, a non-specific inhibitor, on cell proliferation and apoptosis on three anaplastic thyroid tumor cell lines. These authors further examined the effect of BRAF suppression and Bay 43-9006 on the expression of several molecules involved in cell proliferation and apoptosis. They demonstrated that suppression of BRAF expression in all three cell line led to different levels of inhibition of cell proliferation, ERK phosphorylation, cyclin D expression, and induction of p27. In contrast, Bay 43-9006 inhibited cell proliferation of all three cell lines and induced apoptosis of a BRAF-mutated cell line. However, Bay 43-9006 had diverse effect on the expression of the signaling molecules in cell lines with different genetic background.

The overall impression by this reviewer is that the effect of both BRAF suppression and Bay 43-9006 on the expression of its downstream signaling molecules is not necessarily associated with cell proliferation. In addition, Bay 43-9006 is not a specific inhibitor of B-Raf kinase. It is very difficult to corroborate the findings made from these two experimental approaches. Also, the effect of BRAF knockdown and Bay 43-9006 have been extensively studies in thyroid cancer and other malignancies in vitro and in vivo. The findings in this manuscript only marginally advance the field.

Major weakness:

The authors stated that the BRAF signaling pathway provides important proliferation signals in thyroid cancer cells, regardless of the genetic background, through ERK1/2, p27kip1 and cyclin D1. The data presented in this paper do not support this conclusion. The BRAF-ERK pathway is more involved in the proliferation of BRAF-mutated 8505C cells but much less so in RAS-mutated C643 cells since suppression of BRAF in this cell line had minimal effect on cell proliferation and ERK phosphorylation, cyclin D and p27 expression. Suppression of BRAF in TPC1 cells had no effect on ERK phosphorylation but inhibited TPC1 cell proliferation, cyclin D1 expression, and increased p27 expression. It appears that RET/PTC1-mediated cell proliferation requires B-Raf kinase but not BRAF-MAP kinase pathway.

Bay 43-9006 achieved a good inhibitory effect on C643 cell proliferation (Fig. 3a). However, it increased ERK phosphorylation (Fig. 4a), inhibited cyclin D1
expression but did not increase p27 expression. It is understandable that Bay 43-9006 may inhibit cell proliferation by inhibiting other receptor tyrosine kinases. It appears that any of these molecules could be involved in regulating cell proliferation, but they are not interconnected.

Fig. 2: The statistical analysis does not appear to be correct. BrdU incorporation rate was significantly lower in BRAF-C2-transfected C643 cells than control cells, but not significantly lower in siRNA control-transfected TPC-1 cells than control cells? BRAF suppression significantly increased apoptosis in TPC1 cells but did not significantly increase apoptosis in other two cell lines. The authors need to provide some explanation or discussion.

The manuscript would be strengthened if a detailed, thorough discussion is given. For example, how Bay 43-9006 regulates MCL-1 and Bcl-2 expression, why it only selectively does so on BRAF-mutated cell line.

There are numerous typo and grammar errors. A careful editing and proofreading is needed.

Overall, using two loosely related experimental approaches on three cell lines with different genetic backgrounds, the data described in this manuscript are not integrated to draw a solid conclusion if the BRAF-ERK pathway and specifically its downstream molecules such as cyclin D1 and p27kip are implicated in thyroid tumor cell proliferation.

**Level of interest:** An article of limited interest

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests