Reviewer's report

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

Version: 3 Date: 14 March 2009

Reviewer: Mingzhao Xing

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In this manuscript, Preto et al in the group of Drs Paula Soares and Manuel Sobrinho-Simoes explored the effect of BRAF siRNA and RAF inhibitor sorafenib on the proliferation, apoptosis and expression of cyclin D1, p27 and several apoptosis-related proteins in three thyroid cancer cell lines that harbor RET/PTC1 rearrangement, Ras mutation and BRAF mutation, respectively. They found that both BRAF RNAi and sorafenib inhibit proliferation of all of the three thyroid cancer cell lines and caused alterations in p27Kip1 and/or cyclin D1 levels in some of the cells. They also found that sorafenib treatment down-regulated the expression of anti-apoptotic proteins Mcl-1 and Bcl-2. Although similar findings have been previously been reported in other cancer cells, their work further implicated the molecular mechanisms for the cellular effects of BRAF pathway inhibition in thyroid cancer cells. This an interesting piece of work from a highly reputable and respected group. Overall, the study is well performed and has important clinical and basic research implications.

Some discussions, if given in the manuscript, on the following several points may improve the manuscript:

1. Since sorafenib may not be a specific inhibitor of BRAF and may have effects on other kinases, it would be helpful to give some discussion on this point as some of the observations may not necessarily be from inhibition of the BRAF.

2. This study clearly showed the important involvement of p27 and/or cyclin D1 in the inhibition of thyroid cancer cells by inhibiting the BRAF. Other important molecules that were not examined, such as the classical molecules p21 and several cyclins involved in cell cycle other than cyclin D1 may well be involved. It may be helpful to discuss briefly on this point in the manuscript.

3. The data to support the conclusion that ‘The mechanism by which sorafenib induces apoptosis seems to be due to a balance of the anti-apoptotic proteins Mcl-1 and Bcl-2, which was more relevant in cells with BRAFV600E mutation' was only from one BRAF mutation-harboring cell line. It is therefore also worth giving a brief discussion on this somewhat weakness.

4. While the term ‘sorafenib’ is used most of the time, the term ‘Bay 43-9006’ is also used sometimes (e.g., Figure 4). It would look more consistent if only the former is uniformly used.
5. Since in the text of the manuscript, the western blotting data in Fig 5c is first described, it is advisable that the current Fig. 5c be changed to Fig. 5a, and other panels of Fig 5 be re-numbered correspondingly.

**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.