Reviewer's report

Title: Induction of p21 WAF1/CIP1 via the EP4 receptor mediates the regulation of cell cycle progression by cyclooxygenase-2 in colorectal cancer

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Reviewer: F.M. M Robertson

Reviewer's report:

The title and the abstract could be more reflective of the studies described in this manuscript which are focused on validating the transactivation of the EGFR/amphiregulin pathways through Cox-2/EP4. The writing is clear and the manuscript does an excellent job at describing the studies and their importance in the context of the literature in colon cancer. The question posted by the manuscript is very well defined. This manuscript focuses on evaluation of the role of Cox-2 and EP4 in regulating proliferation of colon carcinoma cells using colon tumor cell lines.

The methods are appropriate and are well described, the data are sound and the manuscript adheres to the standards for reporting and data deposition. The data set are matched by immunochemical documentation that the proteins being evaluated are clinically relevant. This manuscript is of interest since it provides evidence for a potential transactivation of EGFR/amphiregulin pathway by the EP4/COX-2/PGE2 axis.

With respect to whether the discussion and conclusions are well balanced and supported by the data, there are three main comments about suggested changes to enhance the impact of the discussion and conclusions of this manuscript.

Minor Essential Revisions:

Comments 1 and 2 are with respect to the need to add references or to rewrite a few sections of the background and discussion. Although the authors are clearly well versed in the area, with the exception of 1 reference, all of the references are specific to Cox-2/EP4/EGFR/amphiregulin signaling axis in colon cancer. There are clearly multiple other tumor types in which important and relevant work has been done in this area. Citing these relevant studies would acknowledge the work that has been done previously.

Comment 3 is the suggestion to either add pertinent data or suggest why this should not be added. Otherwise, this manuscript is well written, focused and the data are clearly presented.

1. While these studies are of interest, the rationale for the studies, as described in the first page [last sentences] of the background are not strong. This should include a statement of why there...[ is a renewed attention of altered signaling occurring downstream of Cox-2 in cancers as a source of refined therapeutic
targets. While there is a strong rationale for searching for alternatives to inhibiting Cox-2 in tumor cells, there is no mention of the cardiovascular side effects observed with the clinical administration of selective Cox-2 inhibitors celecoxib and rofecoxib, which has decreased [in the case of celecoxib] or in the case of rofecoxib, blocked the use of selective Cox-2 blockers.

2. With the exception of a single reference, the remaining papers cited are all focused on the role of Cox-2 and EP receptors in colon cancer. There are multiple papers describing the roles of EP receptors in other tumor types, for example breast tumors, with EP4 being shown to mediate migration, invasion and metastasis. In addition, there are multiple papers in systems other than colon tumors that describe that EGF regulates Cox-2, a link which should be considered in any manuscript such as this that is focused conversely to illuminate the transactivation of the EGFR pathway through PGE2. Additionally, the impact of the present observations could be expanded with mention of the global ability of Cox-2/PGE2 transactivation of EGFR through EP4 with at least a mention of the multiple systems in which this transactivation has been described [hepatic organoid, squamous cell carcinoma, epidermal keratinocytes, ovarian cancer, kidney tumor cells] and that the present studies demonstrate that this occurs through the EP4 receptor. This manuscript does not address the converse issue that has been documented about the EGFR transactivation of the Cox-2 pathway and should at least address this.

3. With respect to studies in Figure 4a. The authors state that the data demonstrating that p21 is induced following exposure of colon tumor cells to either an EGFR tyrosine kinase inhibitor or EP4 antagonist suggests EGFR transactivation by EP4. One other means to demonstrate this is to determine the dose and time dependent changes in p21 observed with the combination of treatment of PD153035 and EP4a.

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

**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.