Reviewer’s report

Title: Sulindac Sulfide Inhibits Colon Cancer Cell Growth and Downregulates Specificity Protein Transcription Factors

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Reviewer: Carlos J. Ciudad

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In this manuscript Stephen Safe and collaborators continue with their research dealing with the mechanisms of action of Sulindac and metabolites with a special emphasis on the expression of Sp proteins. Specifically, they report that sulindac sulfide inhibits growth in the colon cancer cell lines SW480 and RKO downregulating the expression of Sp1, Sp3 and Sp4 proteins. Sulindac sulfide also decreased expression of survivin, bcl-2, epidermal growth factor receptor (EGFR), cyclin D1, p65 subunit of NFkB and VEGF. Sulindac sulfide also induced ROS and decreased miRNA-27a in colon cancer cells resulting in an upregulation of the Sp-repressor ZBTB10 and finally a downregulation of Sp proteins. The study is of interest and would gain substantially if the following comments are dealt with. That would strengthen the conclusions of the manuscript.

1. The three compounds used, Sulindac, sulindac sulfone and sulindac sulfide are able to inhibit proliferation with different potency but the expression of Sp proteins are decreased only with Sulindac sulfide. Therefore it is not completely clear if the mechanism for growth inhibition is due to the decrease of Sp proteins since the other two compounds also inhibit cell growth yet at higher concentrations.

2. It would be interesting to know whether or not the effects of sulindac and metabolites are also observed in non tumoral cells, for instance using HuVEC. This experiment would address the specificity of sulindac to tumoral or/and not tumoral cells.

3. While reporting luciferase experiments with sulindac sulfide, there is a lack of negative controls. All the graphs show decreases in expression: Sp1, Sp3, survivin and VEGF for both cell lines. However, there are no experiments controlling the specificity of that effect, for instance introducing a luciferase construct for a gene that is not decreased by sulindac (a non-related gene) (or even one that was activated). See point 6 for possible candidates. Are sulindac and sulindac sulfone also decreasing luciferase activity?

4. Are the levels of ROS also increased with sulindac or sulindac sulfone?
5. Test under the experimental conditions of this manuscript whether an antagonim against miR-27a reproduce the effect of sulindac sulfide on ZBTB10 and Sp. This information is not found in reference 44.

6. At the level of references, there are some important missing: For instance Nakamura and co-workers performed and published in BBRC 292, 498-512 (2002) a study entitled "Expression Profile Analysis of Colon Cancer Cells in Response to Sulindac or Aspirin" with the results of microarrays after incubating SW480 cells with Sulindac observing also that survivin expression was decreased. Therefore this article should be cited. At the same time, the information contained in this article may serve to find non-related genes or activated genes to be used as negative or positive controls.

7. 7) Also regarding missing references, when describing the luciferase reporters used in the experiments, it would be fair to include the reference of the article describing the generation of those reporters. For instance, reporter Sp1-luc was that generated in: Cloning and Characterization of the 5'-Flanking Region of the Human Transcription Factor Sp1 Gene* by Ciudad and co-workers published in J. Biol. Chem. Vol. 276, pp. 22126-22132, (2001); and the reporter Sp3-Luc was generated in: Characterization of the 5'-flanking region of the human transcription factor Sp3 gene by Noe and co-workers published in Biochim Biophys Acta. 1730(2):126-36 (2005). As a courtesy to the authors who generated and supplied the plasmids their references should also be included. This also probably applies to the reporters for survivin and VEGF.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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