**Reviewer's report**

**Title:** Regulation of Early Growth Response 2 expression by Secreted Frizzled Related Protein 1

**Version:** 0  **Date:** 24 Feb 2017

**Reviewer:** Gabriella Castoria

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Secreted frizzled-related protein (SFRP1) is down regulated in breast cancer and its re-expression limits the invasiveness and proliferation, suggesting an onco-suppressor role for SFRP1. Additionally, a role for SFRP1 in inflammatory events has been also described.

In this manuscript, the Authors aim to assess whether SFRP1 modulation in human mammary tissues affects inflammatory cytokine expression, EGR2 expression and macrophage polarization. SFRP1 knockdown increases the expression of IL-6 and EGR2 mRNA in human mammary epithelial cells. Chemical inhibition of TGF-β and MAPK signaling in Sfrp1−/− or knockdown mammary epithelial cells results in decreased expression of EGR2. Stimulated murine macrophages obtained from Sfrp1−/− mice and treated with rSFRP1 exhibit a reduction in Egr2 expression. Lastly, human mammary explant tissue treated with rSFRP1 shows a decreases in CD163 protein expression. Based on these findings, the Authors claim that SFRP1 controls the pro-tumorigenic niche and its loss contributes to tumor progression by affecting signaling from epithelium to the immune system.

Although interesting, the manuscript should be significantly improved with additional data and controls.

Fig. 1- Middle panel in B, shows the that siSFRP1 significantly decreases EGR2 mRNA in MCF-7 cells. What happens in different breast cancer-derived cells (i.e. T47D or MDA-MB231 cells)? The paucity of cell system here employed limits the value of data. In this regard, the manuscript would also benefit from an extensive discussion about the putative link between SFRP1 and steroid receptor action in different breast cancer cells.

Fig. 3- It shows that chemical inhibition of TGF-βR and MEK1/2 reduces Egr2 expression in both human mammary epithelial SFRP1 knockdown cells as well as Sfrp1−/− MMECs. Experiments showing that MEK activation actually depends on TGF beta signaling are needed. I would like to see in the revised paper that Erk1/2 activation is induced by TGF beta in both cell types (TERT-siSFRP1 and Sfrp1−/− cells) and that TGFbeta-R inhibition blocks the MAPK activation.

Again, since UO126 also affects other intracellular target (MKK6/p38 MAPK,MKK3/p38 MAPK), it would be interesting to use other, more potent and specific MEK 1/2 inhibitors.
It would be of value to show the role of other pathways activated by TGF beta in EGFR2 mRNA regulation (i.e., Smad, RhoA, PI3K). For instance, what happens in the two cell types (siSFRP1 and Sfrp1 -/-) upon inhibition of the PI3K? Again, the authors should also test the phosphorylation status of AKT upon TGFb-R inhibition.

Figs. 4 and 5—Here again, the role of TGF-β/MAPK signaling should be demonstrated.

In sum, data in the entire manuscript need to be further supported by new experiments before to drive any conclusion.

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

No

**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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