Autocrine Fibroblast Growth Factor 18 Signaling Mediates Wnt-dependent Stimulation of CD44-positive Human Colorectal Adenoma Cells

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Supporting information

Wnt-inhibition by sulindac sufide amide (SSA)

The impact of SSA on wnt-signaling, growth and CD44(+) colony formation is part of another manuscript currently in preparation. While we feel that reference to published literature describing the impact of sulindac derivatives on wnt-signaling is sufficient to support our conclusions in this manuscript, we want to offer additional results on the impact of the drug on wnt-signaling, tumor cell growth and $CD44^{(+)}$ colony formation solely for review purposes.

The impact of SSA on wnt-activity was analyzed by luciferase assay as described for ΔN-Tcf4 (shown in figure 6 of the main manuscript) and was inhibited by about 60% as compared to the controls by 5µM SSA (figure 1s a). The dose response relationship for SSA-induced growth inhibition was determined by MTT assay and the IC_{50} determination for both HT29 and LT97 cultures is shown in figure 1s b. Finally, addition of SSA to colony formation assays of $CD44^{(+)}$ cells caused a concentration-dependent loss of colony formation (figure 1s c). For methodology, please see the methods section of the main manuscript.

The LT97 adenoma cells were less sensitive to SSA than HT29 carcinoma cells. Colony formation was reduced already at 5µM, but 20µM were needed to achieve a 90% inhibition.

Figure 1s:

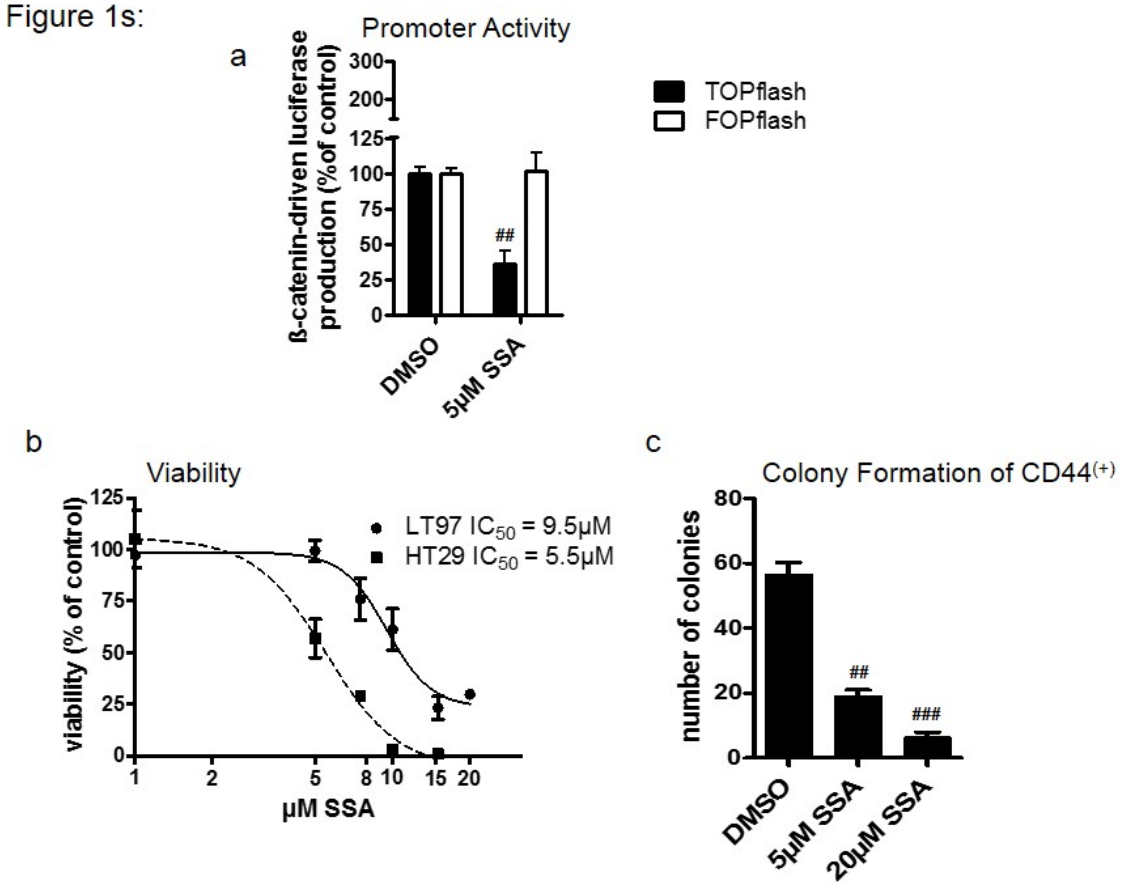

Figure 1s: Impact of SSA on wnt-activity, growth and colony formation:

(a) TOPflash or FOPflash vectors were introduced into LT97 cells by lipofection 24 hours before exposure to 5µM SSA. CMV/renilla was co-transfected for standardization. Cultures were lysed for luciferase assays 24 hours later.

(b) Semi-confluent cultures were exposed to increasing concentration of SSA for 48 hours and cell viability determined using MTT assay. IC_{50} was calculated using GraphPad Prism software.

(c) $CD44^{(+)}$-LT97 cells were isolated by flow cytometry and plated at 3000 cells / 24 well. Colony number was determined after 14 days.

and ### indicate a decrease at $p \leq 0.01$ and $p \leq 0.001$ respectively as compared to controls calculated by students‘ t-test.