Aspirin and salicylic acid induce REDD1 expression and dephosphorylate 4E-BP1 in breast cancer cells

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Objective

While the anticancer activity of aspirin (acetylsalicylic acid) is strongly supported by epidemiological data, the underlying molecular mechanism remains uncertain. Inhibition of mTORC1 (mechanistic target of rapamycin complex 1) signaling has been proposed to contribute to the effect. Although REDD1 (protein regulated in development and DNA damage response 1), encoded by the *DDIT4* gene, is known to function as an mTORC1 inhibitor, the involvement of REDD1 in aspirin anticancer action has not been reported in the literature. Since we have previously found that *DDIT4* mRNA is elevated upon aspirin treatment in breast cancer cells, here, we seek to determine whether this increase is concomitant with the induction of REDD1 protein. While aspirin has been shown to suppress mTORC1 signaling in *PIK3CA*-mutant breast cancer cells, we investigate the activity of mTORC1 signaling following aspirin exposure in *PTEN*-mutant breast cancer cells.

Methods

- MDA-MB-468 (*PTEN*-mutant) and MCF-7 (*PIK3CA*-mutant) breast cancer cell lines were used for the analysis.
- Cells were treated with 2 mM of aspirin (ASA) or its metabolite salicylic acid (SA) for 24 hours before cell lysates were prepared using RIPA (radioimmunoprecipitation assay) buffer.
- Protein concentration was determined with bicinchonic acid (BCA) assay.
- Protein expression and phosphorylation was assessed by western blotting.
- mTORC1 activity was evaluated by phosphorylation levels of its downstream substrates 4E-BP1 and S6K1.
- Statistical analysis was performed with SPSS (SPSS Inc, Chicago, Ill, USA). Differences were considered significant if $p<0.05$ by Student's *t* test.

Results

•We demonstrated that aspirin and salicylic acid increase REDD1 protein levels in MDA-MB-468 (Fig. 1A) and MCF-7 cells (Fig. 1B).

•In order to investigate the effect of aspirin on mTORC1 activity in MDA-MB-468 cell line we probed for phosphorylation levels of S6K1 and 4E-BP1 which are the main known targets of mTORC1. However, we found no detectable baseline S6K1 phosphorylation and therefore only assessed the phosphorylation of 4E-BP1. We observed that aspirin and salicylic acid dephosphorylate 4E-BP1 (Fig. 2).

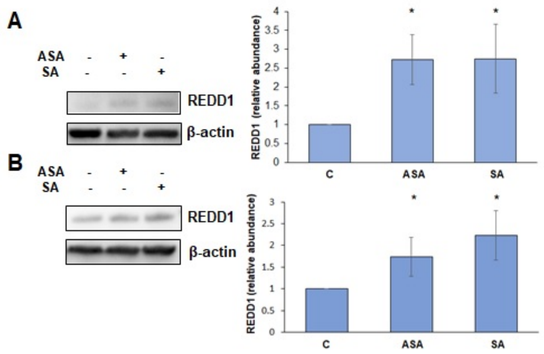

*Fig.1. Induction of REDD1 expression by aspirin and salicylic acid in breast cancer cells. MDA-MB-468 (A) and MCF-7 (B) cells were treated with vehicle control (C), 2 mM of aspirin (ASA) or 2 mM of salicylic acid (SA) for 24 hours before cell lysis and western blot analysis. Densitometric quantifications of REDD1 levels were normalized to β-actin for fold change calculations. Data are expressed as means ± S.D from three independent experiments. * P<0.05 (vs vehicle control) by Student's t-test.*

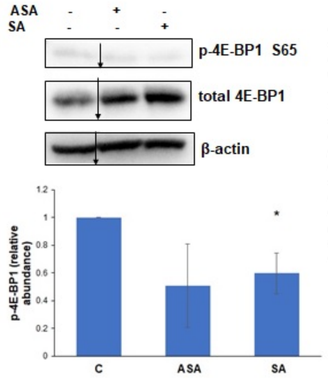

*Fig. 2. Aspirin and salicylic acid dephosphorylate mTORC1 target 4E-BP1 in MDA-MB-468 cells. Cells were exposed to vehicle control (C), 2 mM of aspirin (ASA) or 2 mM of salicylic acid (SA) for 24 hours before cell lysis and western blot analysis. Densitometric quantifications of phospho-4E-BP1 levels were normalized to β-actin for fold change calculations . Data are expressed as means ± S.D from three independent experiments. * P<0.05 (vs vehicle control) by Student's t-test. ↓indicates cropped irrelevant lane.*

Conclusions

Our observations show an increase in REDD1 protein level and decrease in phosphorylation of mTORC1 target 4E-BP1. Further research aimed at unraveling whether the aspirin-mediated dephosphorylation of 4E-BP1 is dependent on REDD1, is warranted.

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Key words

Aspirin, breast cancer cells, REDD1, mTORC1, 4E-BP1 dephosphorylation