Identification of Aldo-keto reductase AKR1B10 as a Selective Target for Modification and Inhibition by PGA_1: Implications for Anti-tumoral Activity

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Supplementary information

Supplementary figure legends

Fig. S1. Outline of the procedures for the identification of the PGA_1-selectively modified 37 kDa band. S100 fractions from NIH-3T3 fibroblasts treated with vehicle, 15d-PGJ_2-B or PGA_1-B, as in Fig. 1 were subjected to chromatography on Neutravidin beads and analyzed by SDS-PAGE and biotin detection or Coomassie staining. The biotin-positive 37 kDa band, marked by a rectangle, was selectively retained in the avidin-bound fractions from PGA_1-B-treated cells, but not from control or 15d-PGJ_2-B-treated cells. This band was excised from the gel and subjected to trypsin digestion and LC-MS/MS. The results from this analysis identified GAPDH as the major protein present in this band. To confirm whether GAPDH was the selectively modified protein, S100 fractions from NIH-3T3 fibroblasts treated as above were subjected to immunoprecipitation with anti-GAPDH and immunoprecipitates (IP) and non-retained fractions (NR) were analyzed by SDS-PAGE followed by blot with HRP-streptavidin or with anti-GAPDH antibody. In spite of quantitative GAPDH immunoprecipitation, the PGA_1-selectively modified band remained in the non retained fraction in this assay. Therefore, this GAPDH immunodepleted fraction was used for subsequent avidin chromatography, SDS-PAGE and biotin detection or Coomassie staining as above. The position of the 37k Da band is shown by the rectangle. Dotted lines indicate where lanes from the same gel have been cropped. After trypsin digestion and LC-MS-MS, the results obtained were compatible with the presence of aldose reductase in this band. Confirmation of these results by Western blot and 2D electrophoresis and MALDI-TOF MS, is shown in Fig. 1.

Fig. S2. Concentration and time-dependent effects of PGA_1 on AKR1B10 activity in COS-7 cells. COS-7 cells were transiently transfected with AU5-AKR1B10 wt. Transfected cells were treated with the indicated concentrations of PGA_1 for 24 h (A) or with 60 μM PGA_1 for the indicated times (B). Cells were lysed and ARK activity was

determined in S100 fractions as described in Methods. Results are average values ± SEM of three experiments (*p<0.05 vs vehicle by Student's *t*-test).

Fig. S3. Effects of PGA_1 on various lung cancer cell lines. (A) Levels of AKR1B10 and actin in A549, H1299 and Calu-3 lung cancer cells were assessed by Western blot. (B) Cells were treated with 30 μM PGA_1 for 16 h, and induction of apoptosis or necrosis was evaluated by flow cytometry analysis after propidium iodide and Annexin-V staining. The proportions of viable, apoptotic (early plus late apoptotic) and necrotic cells after PGA_1-treatment are shown. Results are average values of two experiments. (C) Cells were treated with PGA_1 as above and subjected to flow cytometry for analysis of cell cycle distribution. The proportions of cells in the G2/M phases are represented as average values ± SEM of three experiments. (*p< 0.05 vs control).

Fig. S4. Effect of BSO on PGA_1-B incorporation into proteins in A549 cells. A549 cells were incubated with vehicle or 50 μM buthionine sulfoximine (BSO) for 16 h, after which they were treated with vehicle or 60 μM PGA_1-B for 24 h. S100 fractions were subjected to pull-down on avidin-agarose and the biotin signal present in the S100 and in the avidin-bound fractions was assessed by Western blot and detection with HRP-streptavidin. Results are representative from four independent experiments.

Fig. S5. Effect of PGA_1 on intracellular accumulation of doxorubicin. A549 cells were treated with vehicle or 30 μM PGA_1 in the presence or absence of 250 nM doxorubicin for 16 h, after which, they were fixed and stained with DAPI. Intracellular doxorubicin was visualized by confocal fluorescence microscopy on a Leica TCS-SP5-AOBS confocal microscope. Fluorescent images shown are single sections taken at an intermediate z position. Bars, 50 μm.