SUPPLEMENTAL TEXT MATERIAL

MATERIALS AND METHODS

Immunohistochemical detection and analysis of COX-2 and PCNA

Tissue samples that were embedded in paraffin were sectioned (5μm thick), deparaffinized and rehydrated in a graded series of alcohols. Following rehydration, an antigen retrieval process was performed by placing the slides in 10 mM sodium citrate buffer, pH 6.0 at 95°C for 20 minutes followed by 20-minutes cooling. The sections were washed in PBS and non-specific binding sites were blocked with 1% BSA with 2% goat serum in PBS. The sections were incubated with an anti-COX-2 or anti-PCNA antibodies for 2 h at room temperature. The sections were washed and then incubated with biotinylated secondary antibody for 45 min followed by horseradish peroxidase (HRP)-conjugated streptavidin. After washing in PBS, sections were incubated with diaminobenzidine substrate and counterstained with hematoxylin. Representative pictures were taken using a Nikon Eclipse E400 inverted microscope and DXM1200 digital camera.

RESULTS

Administration of GTPs inhibits UVB-induced enhancement of COX-2 and PCNA expressions in wild type mice skin but less effective in IL-12 KO mice

Immunohistochemical data revealed that in general, the staining of UVB-induced COX-2 was more intense in the epidermis of IL-12-KO mice than wild-type mice (Fig. 1A). Administration of GTPs in the drinking water reduced the UVB-induced expression of COX-2 in the skin of the wild-type mice but was less effective in reducing the levels of COX-2 expression in the IL-12 KO mouse skin (Fig. 1A).

The immunostaining pattern of PCNA-positive cells was more intense in the UVB-exposed skin of both the IL-12 KO and wild-type mice than in the non-UVB-irradiated mouse skin. The intensity of staining, however, was greater in the UVB-exposed skin of IL-12 KO mice than the UVB-exposed skin of wild-type mice suggesting that the epidermal cells in IL-12 KO mice skin have a higher proliferation potential after UVB irradiation. Administration of GTPs in drinking water reduced UVB-induced expression level of PCNA more efficiently in wild type mouse skin than in the skin of IL-12 KO mouse, as shown in Figure 1B.

Administration of GTPs inhibits the UVB-induced enhancement of expression of COX-2 and PCNA in the tumors of wild-type mice but is less effective in tumors of IL-12 KO mice

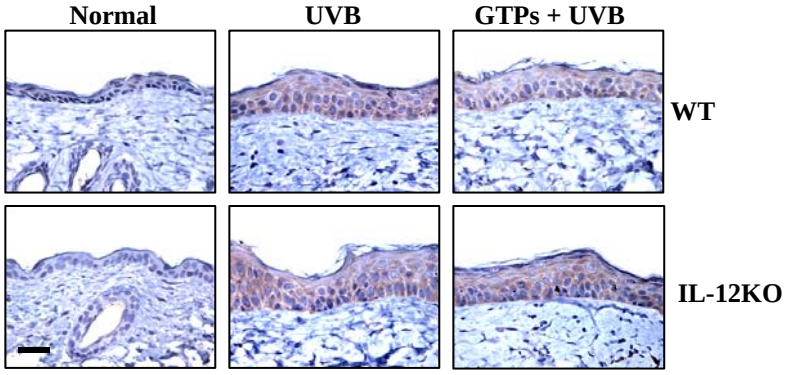
We determined the expression levels of COX-2 and PCNA in tumor samples of wild-type mice and IL-10 KO mice. Immunostaining clearly indicated that the level of COX-2 was higher in the tumors of IL-12 KO mice than in the tumors of wild-type mice both in terms of the intensity of staining and the number of positive cells (Fig. 2A). Administration of GTPs in drinking water resulted in greater inhibition of the expression levels of COX-2 in tumors of wild type mice than in the tumors of IL-12 KO mice. Similarly, the staining of PCNA was more intense and the numbers of $PCNA^+$ cells were higher in the tumors of IL-12 KO mice than in the tumors of wild-type mice (Fig. 2B). Administration of GTPs in the drinking water was associated with a greater reduction in the levels of UVB-induced expression of PCNA in the tumors of wild-type mice compared to the tumors of IL-12 KO mice (Fig. 2B).

Supplementary Figure legends

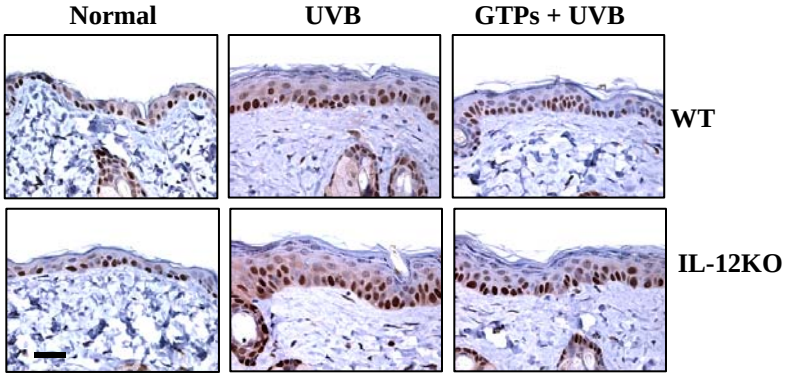
Supplementary Figure 1: Immunohistochemical detection of COX-2 and PCNA expressions in chronically UVB-exposed skin of IL-12 KO mice and their wild types (C3H/HeN). Representative examples of micrographs of staining for COX-2 and PCNA were presented from the skin of at least six mice per group which showed identical patterns.

Supplementary Figure 2: Immunohistochemical detection of COX-2 and PCNA expressions in UVB-induced skin tumors of IL-12 KO mice and their wild types (C3H/HeN). Representative examples of micrographs of staining for COX-2 and PCNA were presented from tumors at least from six mice per group which showed identical patterns.

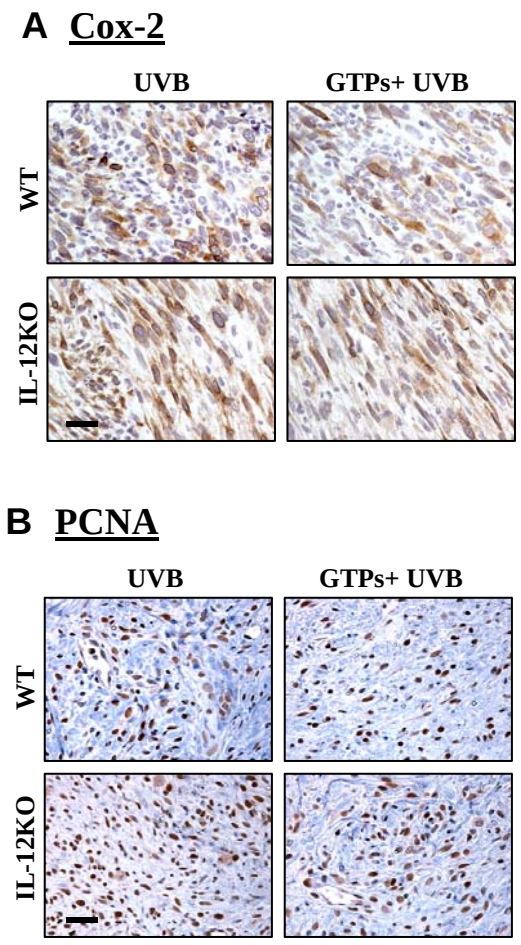
A **Cox-2**



B **PCNA**



Supplementary Figure 1S: Meeran *et al.*



Supplementary Figure S2: Meeran *et al*.