PGE_2 induces epithelium-mesenchymal transition in colorectal cancer cells

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ABSTRACT

Background: The inflammatory process is one of the most important risk factors in colorrectal cancer (CRC) and the increased expression of COX-2 enzyme is associated with this process. It is known that prostaglandin E_2 (PGE_2), the main product of COX_2, is a potent inducer of tumor progression, increasing the metastasis ability of cancer cells. During initial steps of cancer progression the cells adquire an undifferentiated phenotype providing a more migratory and invasive potential, an event kowns as epithelial-mesenchymal transition (EMT). In this context, the role that PGE_2 plays during the EMT is not completely understood. Aims: To analyse the role of PGE_2 during the EMT development in CRC.

Methods: CRC cells, HT-29, were treated with different concentrations of PGE_2 and the proliferation rates were analyzed after 24, 48 and 72hs. Western blotting (WB) and immunofluorescense (IF) assays were employed to analyze the expression and subcellular localization of epihelial and mesenchymal markers. Additionally, analysis by polymerase chain reaction was conducted to evaluate the PGE_2 receptors profile.

Results: 1000nM PGE_2 increased the proliferation of HT-29 cells after 48h of treatment. We observed that PGE_2 in this time and concentration induced a decrease of E- cadherin and claudin 3 expression as well as protein redistribution from the cell-cell contacts. In addition, IF for beta-catenin sugests translocation of the protein to the nucleus in the treated cells. Conclusion: HT-29 cell treated with PGE_2 display events related with EMT development and these results are the basis to determine the mechanistic by which PGE2 induces EMT.

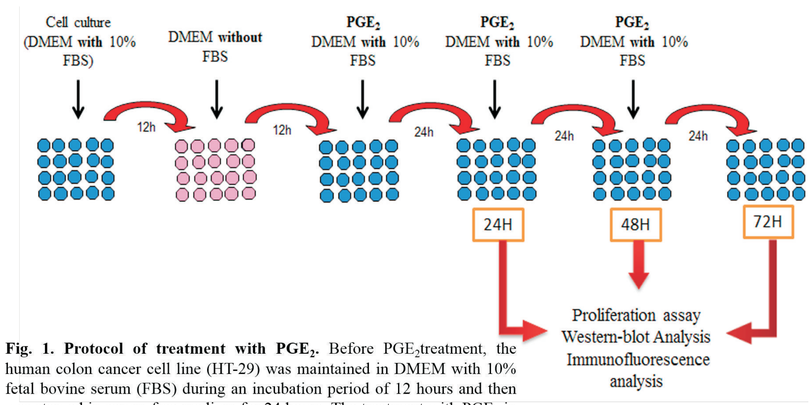


Fig. 1. Protocol of treatment with PGE_2. Before PGE_2treatment, the human colon cancer cell line (HT-29) was maintained in DMEM with 10% fetal bovine serum (FBS) during an incubation period of 12 hours and then was starved in serum-free medium for 24 hours. The treatment with PGE_2 in different concentrations (10nM , 100nM and 1000nM) was reapplied every day for each time of analyses (24, 48 and 72 hours).

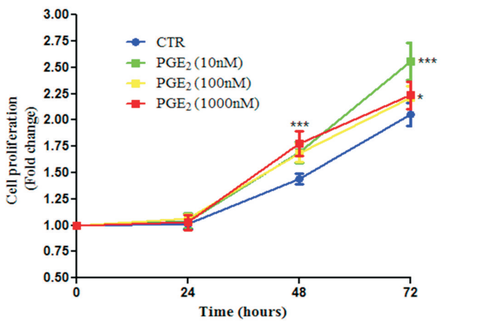


Fig. 2. The treatment with PGE_2 induces cell proliferation in HT-29 cell. HT-29 cells were treatated with PGE_2 (10nM, 100nM and 1000nM) and the proliferative effect was analysed by violet cristal assay. The results are expressed as mean ± s.e.m. of three independent experiments. *P<0.05; **P<0.005; *** P<0.001 compared with control (CTR).

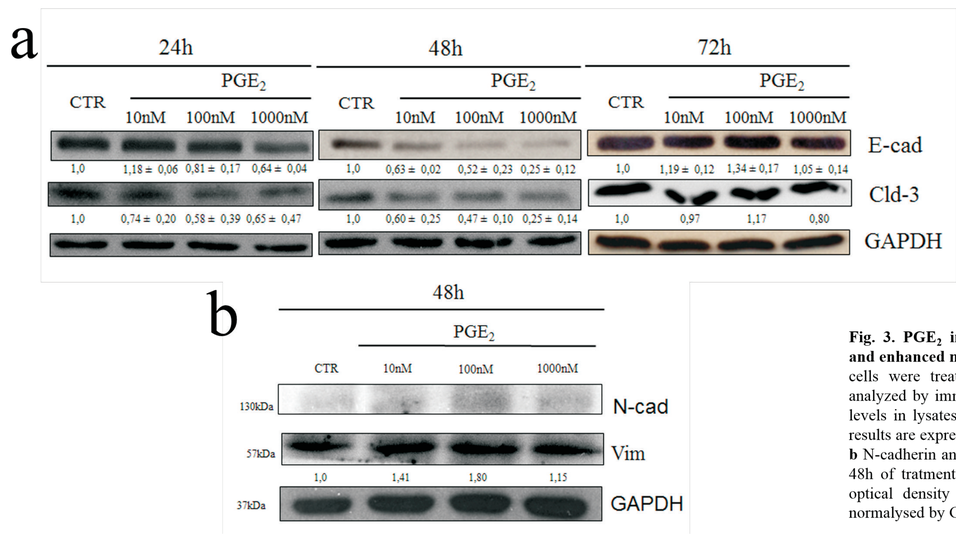


Fig. 3. PGE_2 induces decreased of epithelial proteins expression and enhanced mesenchymal proteins expression in HT-29 cell. HT-29 cells were treatated with PGE_2 (10nM, 100nM and 1000nM) and analyzed by immunoblotting assay. **a** E-cadherin and claudin 3 protein levels in lysates were analyzed after 24, 48 and 72h of tratment. The results are expressed as mean ± s.e.m. of three independent experiments. **b** N-cadherin and vimentin protein levels in lysates were analyzed after 48h of tratment. *Numbers below the figure* represent the ratio of the optical density of the bands as fold change of protein expression normalysed by GAPDH.

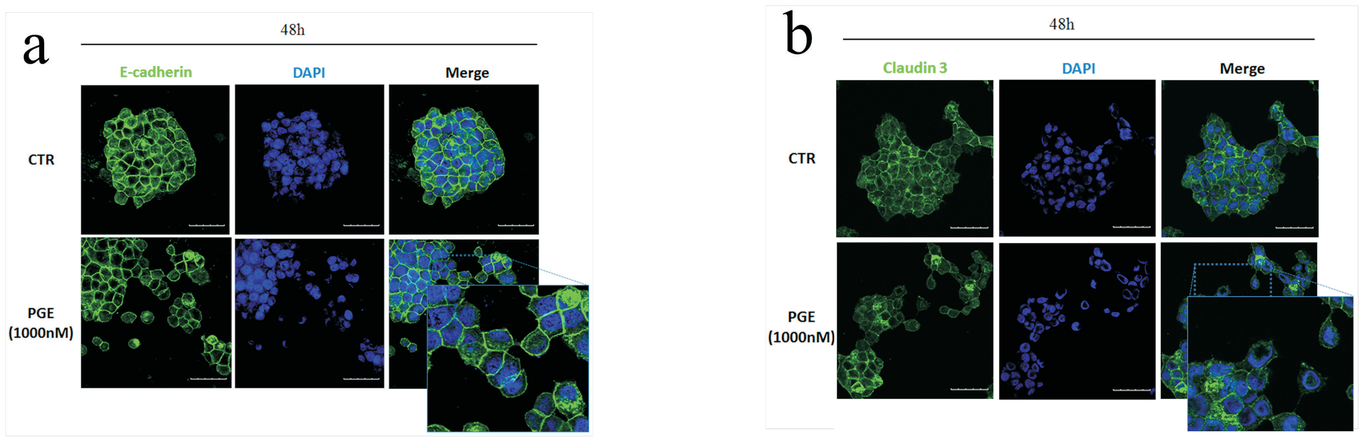
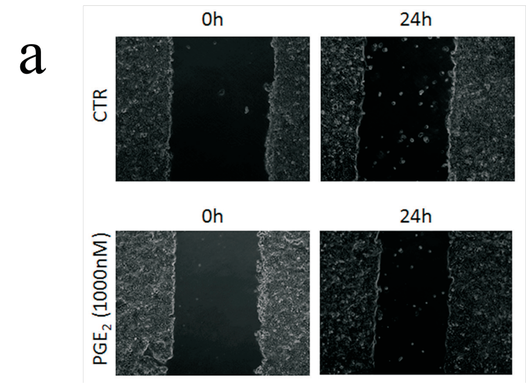
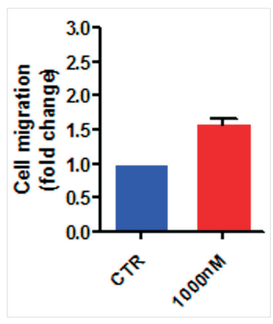
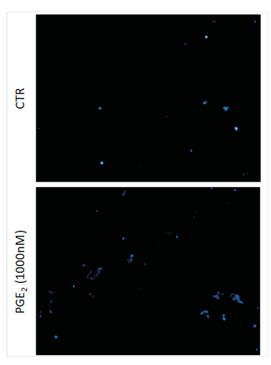


Fig. 4. Ssubcellular localization of E-cadherin and claudin 3 and nuclear translocation of beta-catenin upon 48h of treatment with 1000nM PGE_2 in HT-29 cell. HT-29 cells were grown in glass coverslips until colony formation and treatated or not with 1000nM PGE_2 for 48h, after 24h of starvation . Then, cells were subjects to immunofluorescence analysis of : **a** E-cadherin. **b** Claudin 3. **c** beta catenin. The nucleus are stained with DAPI. Scale bar , 50μm.







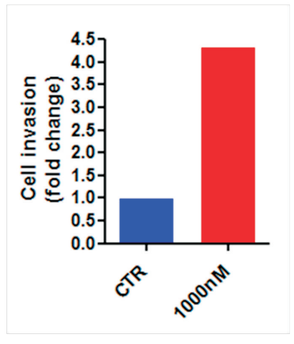


Fig. 5. Treatment with 1000nM PGE_2 enhanced HT-29 cell migration and cell invasion in 24 hours and 48h, respectively. a HT-29 cells were treatated or not with 1000nM PGE_2 and analyzed by wound healling assay at 0h and 24h of treatment. Bar grath are plotted as a fold change of cell migration (where non-treated cells = 1). Data are presented as the mean ± s.e.m. of two independent experiments. **b** HT-29 cells were treatated or not with 1000nM PGE_2 and the invasiviness was analyzed by Transwell assay at 48h of treatment. Bar grath are plotted as a fold change of cell invasion (where non-treated cells = 1).

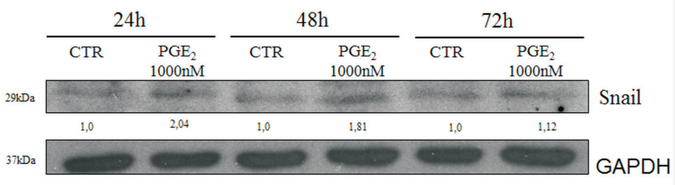


Fig. 6. PGE_2 induces increased of the transcriptional factor Snail expression in HT-29 cell. HT-29 cells were treatated with PGE_2 (1000nM) for 24, 48 and 72 hours and Snail protein levels in lysates were analyzed analyzed by immunoblotting assay. *Numbers below the figure* represent the ratio of the optical density of the bands as fold change of protein expression normalysed by GAPDH.

Fig. 7. HT-29 cells express mRNA of three PGE_2 receptors (EP1, EP2 and EP4) and COX_2 enzyme. HT-29 cells were cultured following the protocol with starvation for 24h and after maintained just in DMEM with 10% fetal bovine serum (FBS) for 48h. Conventinal PCR of mRNA for all PGE_2 receptors and COX_2 enzyme are represented in the figure.

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