Inhibition of PKC/MEK Pathway Suppresses β1-integrin and Mitigates Breast Cancer Cells Proliferation

Nahla E. EL-Ashmawy*, Enas A. EL-Zamarany, Naglaa F. Khedr*, Hend M. Selim* Eman G. Khedr***

*Biochemistry Department, Faculty of Pharmacy, Tanta University, Egypt

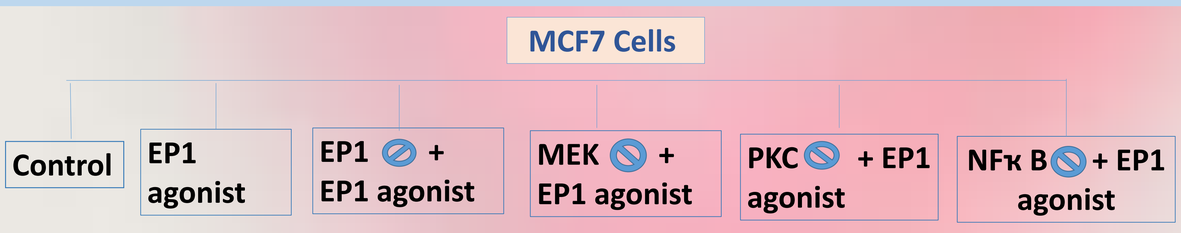
** Clinical Pathology Department, Faculty of Medicine, Tanta University, Egypt

Introduction

Cyclooxygenase-2-induced PGE2 exerts its action through coupling with four EP receptors among them EP1 that plays a crucial role in cancerous tissues including breast cancer (1). β1-integrin is reported to play a significant role in breast cancer *in vivo* and *in vitro* by mediating proliferation, invasion. Cancer cells might utilize inflammatory mediators like NF-κB and other tumor-propagating factors like MAPK and PKC to maintain growth, proliferation and migration (2). FOXC2 and E2F1 are transcription factors, that is known to be involved in the upregulation of β1-integrin expression. Another molecular protein is survivin which is overexpressed in many types of malignancies, where it inhibits apoptosis (3).

Our study was aimed to elucidate the ability of PGE2 to elicit β1-integrin over-expression in MCF7 breast cancer cells through EP1 receptor and the possible underlying molecular mechanistic pathway that may be involved.

Materials and Methods



Cells were treated with inhibitors for 1 h followed by EP1 agonist for 24 h.

At the end of experiment, MCF7 cells were harvested, some cells were kept intact for cell cycle analysis, PCR analysis (for B1-integrin) and others were handled to prepare cell lysate for ELISA measurement (for NFκB, FOXC2, E2F1 and survivin)

Discussion

The present study aimed to examine the effect of EP1 agonist along with EP1, MEK, PKC and NF-κB inhibitors on breast cancer cell line to explore the crosslink between PGE2 and β1-integrin expression.

Herein, pretreated MCF7 cell line with PKC, MEK, NF-κB inhibitors showed an increase in apoptosis and downregulation of EP1-mediated β1- integrin expression, cell cycle transition (G1/S) together with cell proliferation, when compared to EP1 agonist treated group. Our results were in consistence with others who reported that PKC and MEK inhibition decreased proliferation in triple negative breast cancer cells and colorectal cancer cell line HCT 116 (4, 5).

Previous reports showed that inhibition of NFκB reduced cells survival, induced apoptosis in T4-2 cancer cells (6, 7). Our data revealed a connection between EP1, MEK, PKC with NF-κB; as pretreatment with their inhibitors, decreased phosphorylated NF-κB P65 level. NF-κB was shown to upregulate FOXC2 expression and E2F1 was found to act on specific site on the promoter of FOXC2 (8). In the present study, EP1 agonist increased FOXC2, E2F1 protein levels together with increasing β1 integrin expression and cell proliferation, an effect that was reversed by EP1, MEK, NF-κB and PKC antagonist's pretreatments. Herein, EP1 agonist increased survivin level and inhibitors pretreatment antagonized the effect.

Conclusion

Altogether, in MCF-7 cancer cells, EP1 agonist increased β1-integrin expression, an effect reflected by enhanced cell proliferation, cell cycle transition and survivin level. Furthermore, EP1, PKC, MEK and NF-κB inhibitors, partially, mitigated EP1 agonist mediated effect on β1- integrin through inhibiting FOXC2 and E2F1 levels. Targeting these biochemical mediators may afford a new breast cancer therapy strategy.

Results

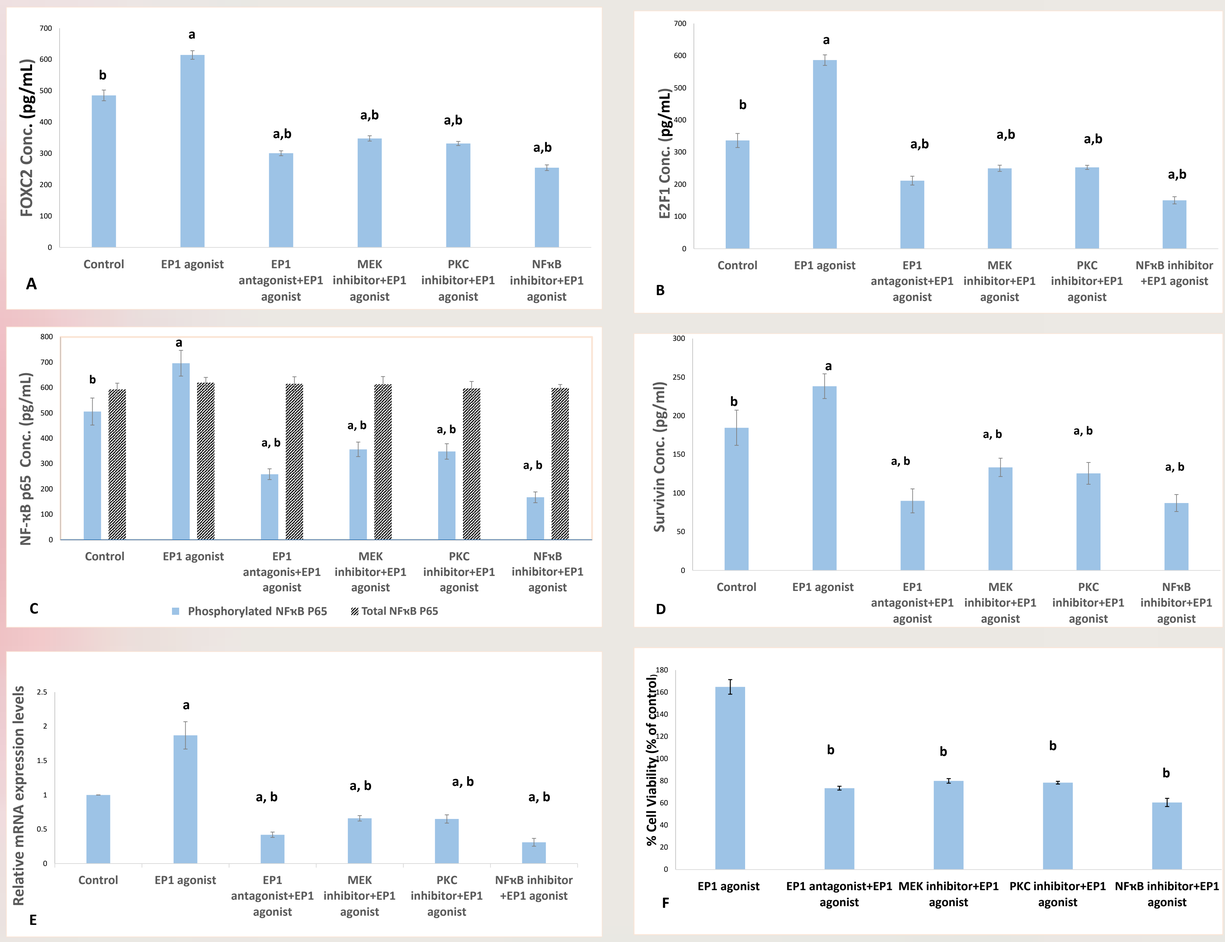


Figure 1: Effect different treatments on MCF7 content of FOXC2 (A), E2F1 level (B), NF-κB(total and phosphorylated) (C), Survivin level (D), B1-integtin (E) and cell proliferation rate (F). Data are presented as mean ±SD. a: Significant *versus* control group. b: Significant *versus* EP1 agonist group. n= 6 replicates. EP1: E prostanoid receptor 1, MEK: Mitogen activated protein kinase, PKC: Protein kinase C, NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells.

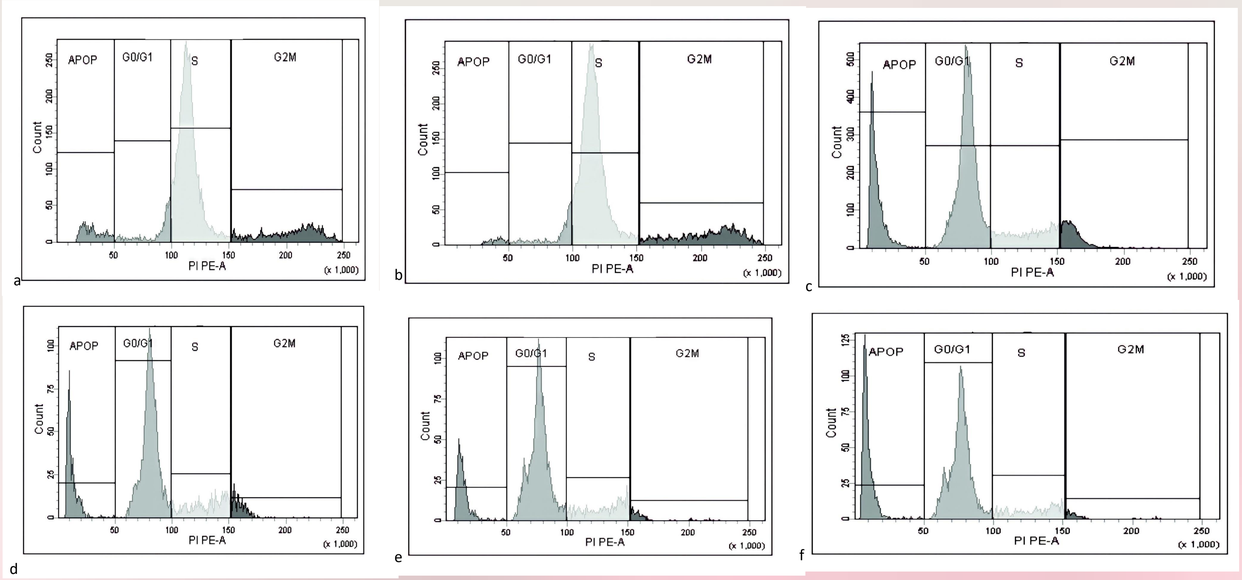


Figure 2: Cell cycle analysis of the studied groups: a: Control group, b: EP1 agonist, c: EP1 antagonist +EP1 agonist, d: MEK inhibitor+EP1 agonist, e: PKC inhibitor +EP1 agonist and f: NF-κB inhibitor+EP1 agonist. EP1: E prostanoid receptor 1, MEK: Mitogen activated protein kinase, PKC: Protein kinase C, NF-κB: Nuclear factor kappa -light-chain-enhancer of activated B cells. Apop: Apoptosis phase; G0/G1: Gap phase G0 and G1 phase; S: Synthesis phase, G2/M: Gap phase 2 and mitosis phase. PIPE-A: Propidium Iodide Phycoerythrin area.

References

(1) de Almeida V et al. (2018): Positive crosstalk between EGFR and the TF-PAR2 pathway mediates resistance to cisplatin and poor survival in cervical cancer. *Oncotarget.* 9: 30594-30609.

(2) O'Callaghan G and Houston A (2015): Prostaglandin E2 and the EP receptors in malignancy: possible therapeutic targets?. Br J Pharmacol.172(22):5239–5250.

(3) Lv YG, Yu F, Yao Q, Chen JH and Wang L (2010): The role of survivin in diagnosis, prognosis and treatment of breast cancer. J thorac dis, 2(2), 100–110.

(4) Gan L et al. (2015): Foxc2 enhances proliferation and inhibits apoptosis through activating Akt/mTORC1 signaling pathway in mouse preadipocytes. J Lipid Res. 56(8):1471–1480

(5) Chen X et al. (2016): Survivin and Tumorigenesis: Molecular Mechanisms and Therapeutic Strategies. J Cancer. 7(3):314-323.

(6) Ahmed et al. (2013): NF-κB regulates radioresistance mediated by β1-integrin in three-dimensional culture of breast cancer cells. Cancer res, 73(12), 3737–3748.

(7) Tang D et al. (2017): TNF-alpha promotes invasion and metastasis via NF-Kappa B pathway in oral squamous cell carcinoma. Med Sci Monit Basic Res. 23:141–149.

(8) Meng P and Ghosh R (2014): Transcription addiction: can we garner the Yin and Yang functions of E2F1 for cancer therapy? Cell Death Dis. 5:e1360. doi: 10.1038/cddis.2014.326

The 7th Euro-Med Conference &Expo Life Sciences, Pharma, and Biomedicine, Cairo, Egypt, 6-8 Oct 2021