Prostaglandin E2 enhance hepatocellular carcinoma HA22T cells survival and metastasis effects via EP2/EP4 signaling pathways

Yen-Hong Chou[1], Wei-Wen Kuo[2], Tian-Shung Wu[4], Chih-Yang Huang[1, 3,5]

[1]Graduate Institute of Basic Medical Science, China Medical University, Taichung
[2]Department of Biologic Since and Technology, China Medical University, Taichung
[3]Graduate Institute of Chinese Medical Science, China Medical University, Taichung
[4]Department of Pharmacy, China Medical University, Taichung
[5]Department of Biotechnology, Asia University, Taichung

JACBS
2013 The 28th Joint Annual Conference of Biomedical Science
第28屆生物醫學聯合學術年會
March 23-24,2013 國防醫學院

Abstract

Backgrounds:

Earlier studies have shown that, Prostaglandin E2 (PGE2) enhances the growth of various cancers (breast, stomach, pancreas, lung and prostate) by activating various proteins that are involved in controlling proliferation and survival pathway. However, the mechanisms of PGE2 regulate HA22T hepatocellular carcinoma cell survival and metastasis is still unknown, roles of EP receptor and related signaling pathways are needed to be investigated. Furthermore, using pure compounds from Danshen extract, we will investigate its efficiency in PGE2 treated HA22T cells survival and metastasis ability.

Materials and Methods:

Firstly, we investigated the Prostaglandin E Receptor 2(EP2) and Prostaglandin E Receptor 4(EP4) protein level in liver cancer tissues via Immunoblotting. Then, we used HA22T cells to investigate PGE2 induced EP2/EP4 receptor expression and also β-catenin, pEGFR, PI3K, Akt and their downstream anti-apoptotic protein levels by western blot analysis. Moreover, the role of PGE2 in regulating HA22T cell migration was investigated by Boyden chamber.

Results:

High levels of EP2 and EP4 proteins were expressed in human hepatocellular carcinoma than normal liver tissue. Using HA22T cell line we observed PGE2 induced survival pathway by either activating EP2/ EP4 expression or EGFR expression. Furthermore, PGE2 induced HA22T cell migration by up-regulating GSK3-β and β-catenin expression in a dose dependent manner. MTT assay showed that out of 35 pure compounds, 16 compounds showed better inhibitory effect in controlling Apicidin-Resistance HA22T cells proliferation in a dose-dependent manner. We also found that Dsh003 inhibited HA22T cells proliferation, survival, and migration ability and finally induced apoptosis.

Conclusion:

EP2 and EP4 could be used as novel prognostic markers in hepatocellular carcinoma and activation of these two receptors by PGE2 enhanced HA22T cells survival and migration abilities, finally we found Dsh003 could inhibit PGE2-induce cell survival and metastasis effects in HA22T
Cells.

Result

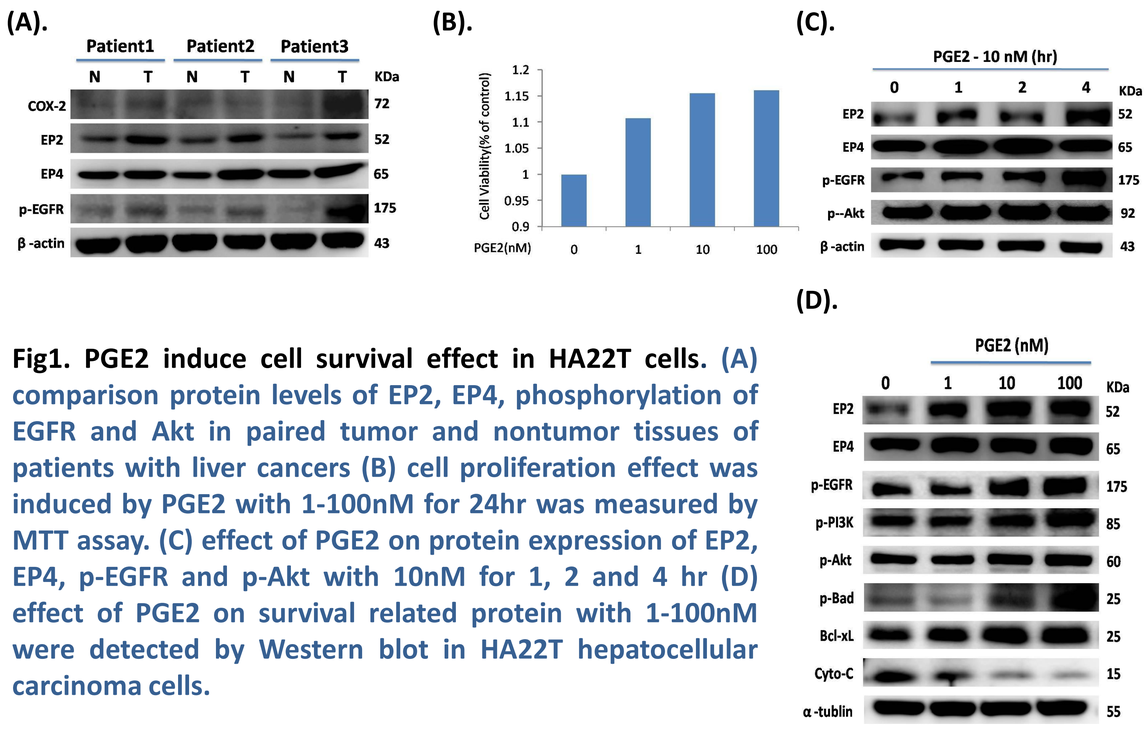


Fig1. PGE2 induce cell survival effect in HA22T cells. (A) comparison protein levels of EP2, EP4, phosphorylation of EGFR and Akt in paired tumor and nontumor tissues of patients with liver cancers (B) cell proliferation effect was induced by PGE2 with 1-100nM for 24hr was measured by MTT assay. (C) effect of PGE2 on protein expression of EP2, EP4, p-EGFR and p-Akt with 10nM for 1, 2 and 4 hr (D) effect of PGE2 on survival related protein with 1-100nM were detected by Western blot in HA22T hepatocellular carcinoma cells.

Fig2. PGE2 induce cell metastasis effect in HA22T cells. (A) cell migration effect was induced by PGE2 with 1-100nM for 4hr was measured by Boyden chamber. (B) effect of PGE2 on protein expression of β-catenin with 10nM for 1, 2, and 4 hr (C) effect of PGE2 on protein expression of snail, β-catenin, E-cadherin and GSK3β with 1-100nM for 4 hr in HA22T were detected by Western blot in HA22T hepatocellular carcinoma Cells.

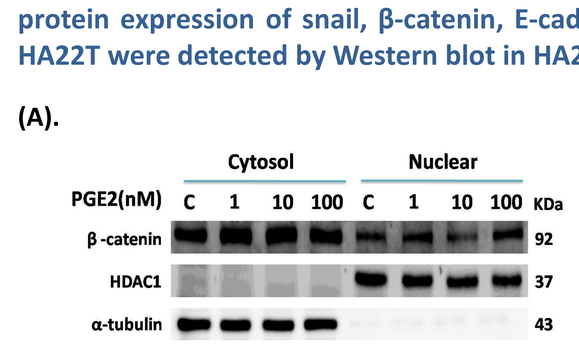
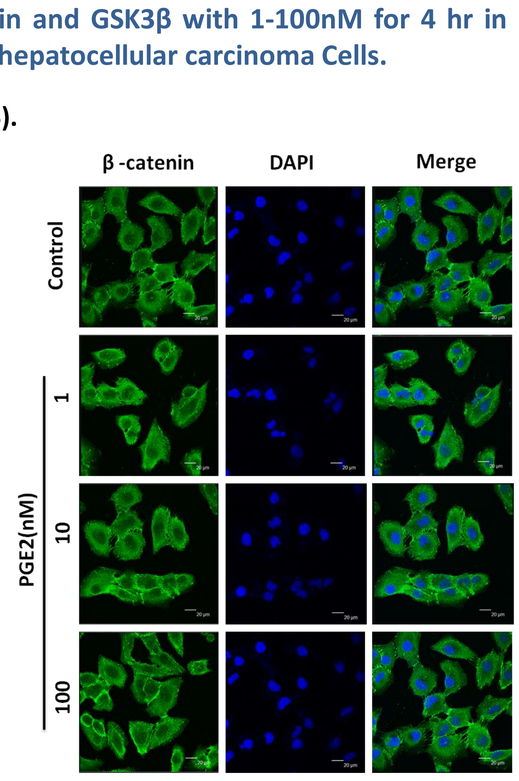
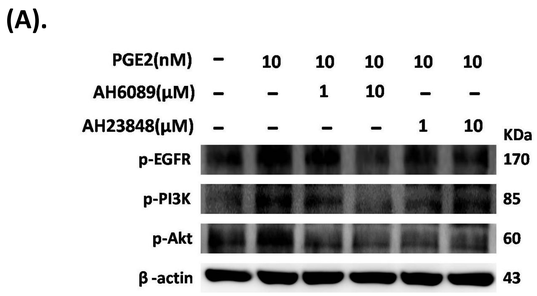


Fig3. PGE2 promoter β-catenin nuclear translocation. (A and B) role of PGE2 on β-catenin nuclear translocation was measured by nuclear cytosol fractionation kit and immunofluorescent assay. HA22T hepatocellular carcinoma cells were stimulated by PGE2 with 1 - 100 nM for 4 hr in HA22T hepatocellular carcinoma Cells.





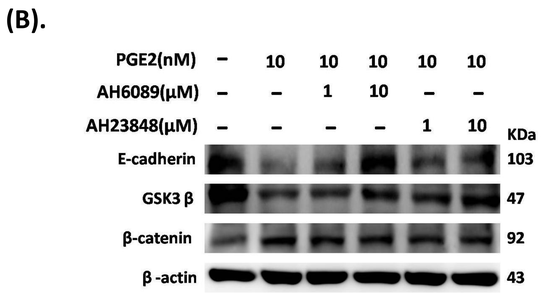


Fig4. EP2 and EP4 mediates PGE2 induce cell survival and metastasis effects. (A,B) role of EP2 antagonists AH6089 and EP4 antagonists AH23848 on PGE2 induce survival and metastasis pathway in HA22T cells. Western blot assay were done using cells pre-treated for 1hr, or not,with AH6089(1 or 10 μM) or AH23848 (1 or 10 μM) and stimulated with PGE2 (10nM).

Table 1 . Effect of 35 compounds of Danshen (*Savia miltiorrhiza*) extracts to inhibit cell viability in Apicidin-resistant HA22T Hepatocellular Carcinoma Cells

Inhibit Cell Viability (dose-dependent manner)			Inhibit Cell Viability (in high dose)			No Effect		
Number of Danshen Extract	Molecular Weight	Chemical Formula	Number of Danshen Extract	Molecular Weight	Chemical Formula	Number of Danshen Extract	Molecular Weight	Chemical Formula
Dsh-003	269.14	C19H20O3	Dsh-111	287.09	C19H12NO2	Dsh-281	335.15	C21H21NO3
Dsh-431	411.18	C27H25NO3	Dsh-242	351.18	C22H25NO3	Dsh-231	333.17	C22H23NO2
Dsh-451	316.16	C21H22N2O	Dsh-121	301.11	C20H15NO3	Dsh-321	321.17	C21H23NO2
Dsh-311	307.17	C20H21NO2	Dsh-421	395.19	C27H25NO2	Dsh-272	395.17	C22H25NO3
Dsh-411	388.17	C26H23NO2	Dsh-222	323.15	C20H21NO3	Dsh-232	337.17	C21H23NO3
Dsh-452	320.15	C20H20N2O2	Dsh-441	434.20	C29H26N2O2			
Dsh-412	385.17	C25H23NO3	Dsh-132	359.12	C22H17NO4			
Dsh-432	415.18	C26H25NO4	Dsh-124	305.11	C19H15NO3			
Dsh-261	377.16	C23H23NO4	Dsh-002	294.13	C19H18O3			
Dsh-271	391.18	C24H25NO4	Dsh-112	287.09	C19H12NO2			
Dsh-211	305.14	C20H19NO2	Dsh-114	291.09	C18H13N03			
Dsh-221	319.16	C21H21NO2	Dsh-001	276.08	C18H1203			
Dsh-422	399.18	C26H25NO3	Dsh-122	301.11	C20H15NO3			
Dsh-251	347.19	C23H25NO2	Dsh-252	351.18	C22H25NO3			
			Dsh-212	309.14	C19H19NO3			
			Dsh-241	347.19	C23H25NO2			

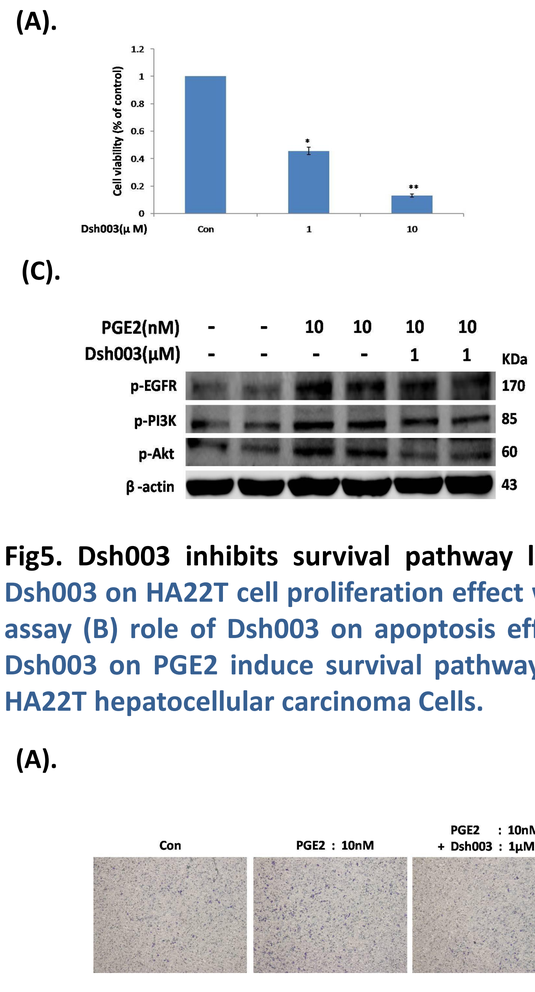


Fig5. Dsh003 inhibits survival pathway leading to enhance cell apoptosis. (A) role of Dsh003 on HA22T cell proliferation effect with 1 and 10μM for 24hr was detected by MTT assay (B) role of Dsh003 on apoptosis effect was measured by TUNEL assay. (C) role of Dsh003 on PGE2 induce survival pathway cells was measured by western blot assay in HA22T hepatocellular carcinoma Cells.

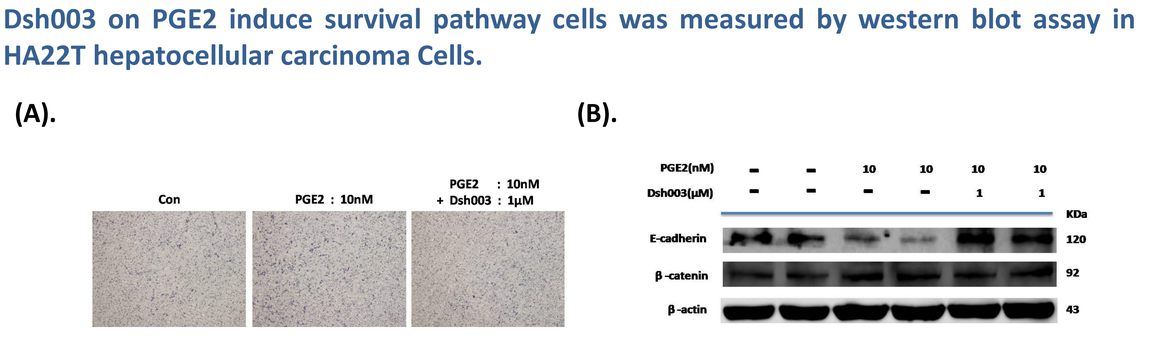


Fig6. Dsh-003 inhibits PGE2 enhance cell migration effect is through β –catenin pathway. (A) role of Dsh003 on PGE2 induce cell migration effect with 1 μM for 24hr was detected by boyden chamber (B) role of Dsh003 on PGE2 induce β –catenin pathway with 1 μM for 24hr in HA22T hepatocellular carcinoma Cells.

Summary

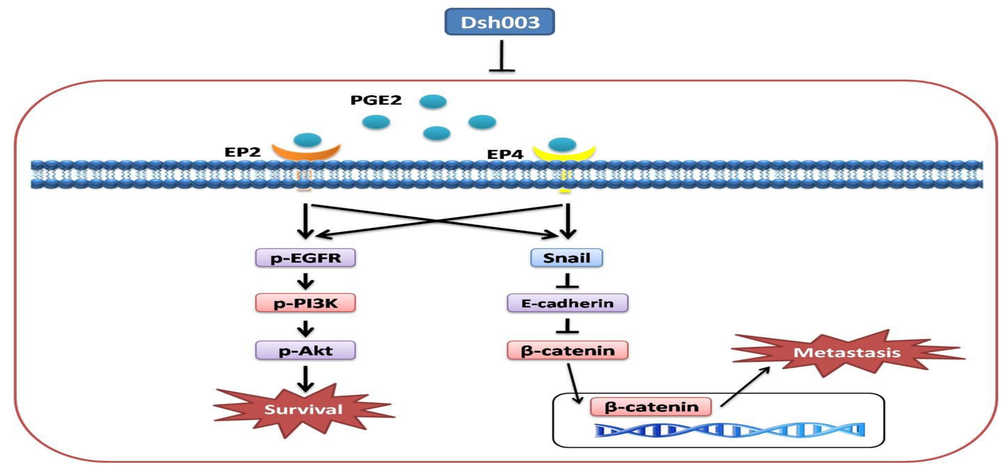


Fig7. PGE2 could induce cell survival and metastasis effects is through EP2 and EP4 receptor and their down-stream effectors, such as EGFR, PI3K, Akt, E-cadherin and β-catenin. Furthermore, its could promoter β-catenin nuclear translocation leading to enhance cell migration effect. However, the pure compound from Danshen: Dsh003 could total reveresal this effects in HA22T hepatocellular carcinoma cells .