Γ-Ionizing radiation activated EGFR–p38/ERK–STAT3/CREB-1–EMT pathway for promotion of the migration/invasion of lung cancer cell and its inhibition by podophyllotoxin acetate.

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1. Introduction

Lung cancer is a major lethal cancer worldwide. In particular, non small-cell lung cancer (NSCLC) has an extremely low 5-year survival rate [1]. IR, chemotherapy and surgery are extensively used for the treatment of NSCLC [2]. However, the clinical usefulness of IR may be limited by problems with radioresistance and damage to normal adjacent tissues [3]. Moreover, recent *in vitro* studies have suggested that may increase the invasiveness of some cancer cells (e.g., glioma, hepatocellular carcinoma, and pancreatic cancer cells) by stimulating several intracellular signaling pathways and *in vivo* studies have found that radiotherapy of primary tumor sites may promote metastasis [4–6]. Thus, in addition to having therapeutic effects, IR might promote the malignant traits of surviving cancer cells. The existing efforts to develop radiosensitizing agents have focused on overcoming radioresistance and reducing damage to normal tissues. However, the evidence suggesting that IR could promote cancer cell invasion/metastasis prompted us to consider developing new candidate radiosensitizers that can also inhibit metastasis. In this study, we sought to identify the intracellular machinery responsible for IR-induced cancer invasion/migration. We report that IR activates the EGFR – p38/ERK – CREB-1/STAT3 pathway, which triggers EMT and increases invasion/migration of lung cancer. Moreover, we show that podophyllotoxin acetate (PA) inhibits IR-induced invasion/migration at least partly by blocking EGFR – p38/ERK – STAT3/ CREB-1signaling and thereby suppressing EMT.

2. Methods and Results

2.1 PA suppresses IR-induced invasion/migration by inhibiting EMT

We previously reported that PA (Fig. 1a). In our previous report, we found that 10 Gy IR could enhance the invasion of A549 cells, and that the IC_{50} value of PA against A549 cells was 16.1 nM. As shown in Figures 1b and 1c, IR treatment increased the invasion/migration of A549 cells by about 50 ~ 80%, whereas PA treatment decreased the invasion/migration of IR-treated cells by about 100 ~ 130 %. To elucidate the mechanism underlying the ability of PA to inhibit IR-induced invasion/migration, we used immunoblotting to examine the expression levels of MMP-2, MMP-9 and vimentin. IR treatment enhanced the expression levels of MMP-2, MMP-9 and vimentin, but these effects were decreased or reversed in the presence of PA (Fig. 1d). Similar results were obtained for the activity levels of MMP-2 and -9, which were detected with gelatin zymography (Fig. 1e). PA treatment following IR did not affect cell viability, as shown by PI uptake (Fig. 1f).

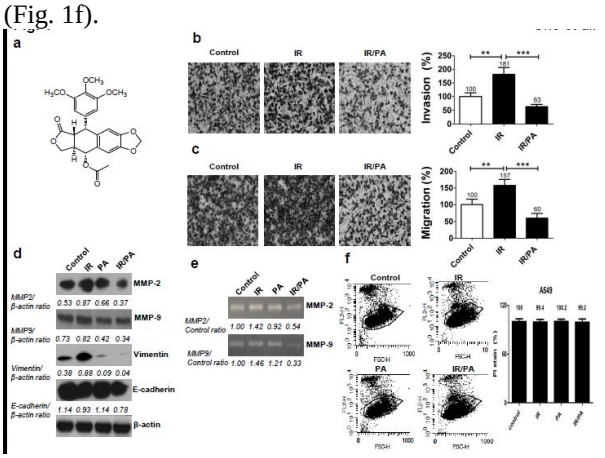

Fig. 1. IR induces invasion/migration in A549 cells, and PA inhibits this effect by blocking EMT.

2.2.PA inhibits IR-induced EGFR/MAPK-related invasion/migration.

We found that IR treatment increased the phosphorylation of EGFR and its downstream molecule, AKT, but PA treatment reversed the phosphorylations of both molecules (Fig. 2a). Gefinitib treatment suppressed the IR-induced phosphorylation of EGFR and increased the expression/activity levels of MMP-2, MMP-9 and vimentin, and co-treatment with Gefinitib plus PA synergistically inhibited the IR-induced expression/activity levels of these proteins (Fig. 2b). We also observed that IR increased the phosphorylations of p38 and p44/42 ERK, but not JNK, and that PA suppressed the phosphorylation of the former two MAPKs to below basal levels (Fig. 2 middle amd lower panels). Combined treatment with PA plus each inhibition synergistically suppressed the expressions and activities of MMP-2, MMP-9 and vimentin. These results show that PA appears to block IR-induced invasion/migration by inhibiting the EGFR and MAPK signaling pathways.

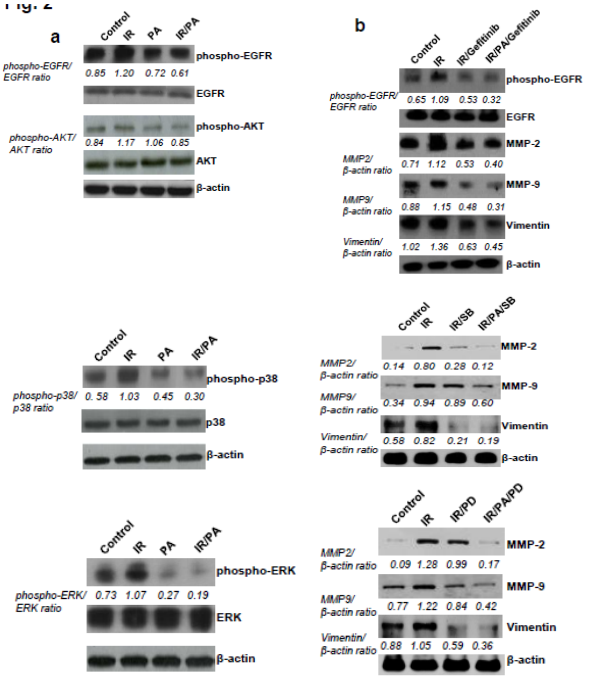

Fig. 2. IR induces invasion/migration by modulating EMT via EGFR-Akt and MAPKs, and this effect is inhibited by PA

2.3 The transcription factors, STAT3 and CREB-1, are involved with IR-induced invasion and are inhibited by PA.

Here, we confirmed that IR induced the phosphorylation (activation) of STAT3, and further found that PA treatment reversed this effect (Fig. 3a). Treatment with a specific pharmaceutical inhibitor of STAT3 (C188-9) eliminated the IR-induced expression/activity changes of MMP-2, MMP-9 and vimentin (Fig. 3b and 3c). We also identified CREB-1 as a major transcription factor involved in IR-induced invasion/migration in our system. Moreover, we found that PA treatment decreased the IR-induced activation of CREB-1 (Fig. 4c). Blockage of this interaction (and thus CREB-1 activity) with a specific inhibitor was found to suppress the IR-induced enhancement of MMP activity and EMT, and combined treatment with PA and the CBP-CREB inhibitor synergistically suppressed IR-induced EMT (Fig. 4b and 4c). Collectively, these results reveal the presence of an EGFR – p38/ERK – STAT3/CREB-1 – EMT signaling pathway that regulates IR-induced invasion/migration and is inhibited by PA.

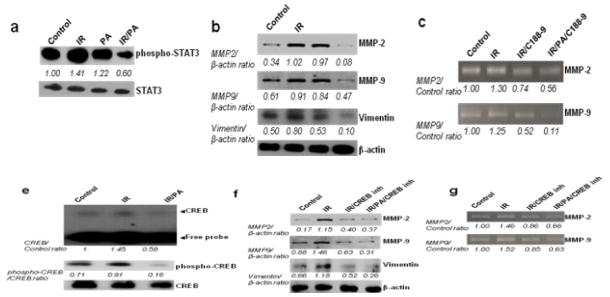

Fig. 3. IR induces invasion/migration by modulating EMT via transcription factors, STAT-3 and CREB-1 and this effect is inhibited by PA

3. Conclusions

We report a new intracellular signaling pathway involved in γ-ionizing radiation (IR)-induced migration/invasion, and show that podophyllotoxin acetate (PA) inhibits the IR-induced invasion and migration of A549 cells. Our results revealed that IR increased the invasion/migration of A549 cells, and this effect was decreased by 10 nM PA treatment. PA also inhibited the expressions/activities of matrix metalloprotase (MMP) -2, MMP-9, and vimentin, suggesting that PA could block the IR-induced epithelial-mesenchymal transition (EMT). The IR-induced increases in invasion/migration were associated with the activation of EGFR-AKT, and PA inhibited this effect. P38 and p44/42 ERK were also involved in IR-induced invasion/migration, and combined treatments with PA plus inhibitors of each MAPK synergistically blocked this invasion/migration. In terms of transcription factors (TFs), IR-induced increases in cyclic AMP response element-binding protein-1 (CREB-1) and signal transducer and activator of transcription 3 (STAT3) increased invasion/migration and EMT. PA also inhibited these transcription factors and then blocked IR-induced invasion/migration. Collectively, these results indicate that IR induces cancer cell invasion/migration by activating the EGFR – p38/ERK – CREB-1/STAT3 – EMT pathway, and that PA blocks this pathway to inhibit IR-induced invasion/migration.

REFERENCES

[1] Siegel R, Ma J, Zou Z, Jemal A. Cancer Statistics, 2014. CA Cancer J Clin 2014;64:9–29.

[2] B. Martini, Lung cancer–epidemiology, prognosis and therapy. Med Monatsschr Pharm 2006;29: 217–21.

[3] Begg AC, Stewart FA, Vens C. Strategies to improve radiotherapy with targeted drugs. Nat Rev Cancer 2011;11: 239–53.

[4] Park CM, Park MJ, Kwak HJ, Lee HC, Kim MS, Lee SH et al. Ionizing radiation enhances matrix metalloproteinase-2 secretion and invasion of glioma cells through Src/epidermal growth factor receptor-mediated p38/Akt and phosphatidylinositol 3-kinase/Akt signaling pathways. Cancer Res 2006;66: 8511–9.

[5] Cheng JC, Chou CH, Kuo ML, Hsieh CY. Radiation-enhanced hepatocellular carcinoma cell invasion with MMP-9 expression through PI3K/Akt/NF-kappaB signal transduction pathway. Oncogene 2006;25:7009–18.

[6] Ho JN, Kang GY, Lee SS, Kim J, Bae IH, Hwang SG et al. Bcl-XL and STAT3 mediate malignant actions of gamma-irradiation in lung cancer cells. Cancer Sci 2010;101: 1417–23.