SPANDIDOS PUBLICATIONS

Effects of protein kinase Cδ and phospholipase C-γ1 on monocyte chemoattractant protein-1 expression in taxol-induced breast cancer cell death

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Abstract. Monocyte chemoattractant protein-1 (MCP-1) is a CC chemokine that plays an important role in immune cell migration. It has been reported that chemokines, including MCP-1, are involved in angiogenesis and metastasis. However, the exact role of chemokines in cancer development is still obscure. We investigated the involvement of MCP-1 in taxol-induced breast cancer cell death. The anti-cancer drug taxol induced MCF-7 breast cancer cell death. Treatment with taxol increased the mRNA expression level of MCP-1 in a dose- and time-dependent manner. Up-regulation of MCP-1 by taxol was augmented in cells treated with rottlerin, a specific inhibitor of protein kinase Cδ (PKCδ). In addition, taxol-induced MCP-1 expression was reduced by the ectopic expression of PKCδ in a dose-dependent manner, indicating that PKCδ plays a negative role in taxol-induced MCP-1 expression in MCF-7 cells. On the other hand, taxol-induced up-regulation of MCP-1 was reduced in cells treated with U73122, an inhibitor of phospholipase C (PLC), and ectopic expression of PLC-γ1 increased the expression of MCP-1 in taxol-treated MCF-7 cells, indicating that PLC-γ1 functions as a positive regulator in taxol-induced MCP-1 expression. These results indicate that MCP-1 is involved in taxol-induced breast cancer cell death and we propose that taxol induces up-regulation of MCP-1 by affecting both positive and negative regulatory signaling pathways.

Introduction

Chemokines are a family of cytokines that are involved in migration and recruitment of leukocytes to sites of tissue injury and inflammatory lesions (1). Recent reports propose that chemokines are also involved in carcinogenesis (1). Chemokines are broadly classified into four distinct groups, CXC(α), CC(ß), C(γ), and CX_3C(δ), based on the number and location of N-terminal cysteine residues (2,3). A wide variety of cells, including leukocytes, muscle cells, endothelial cells, fibroblasts, and cancer cells produce chemokines in response to various stimuli, such as tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS), interferon-γ (IFN-γ), and phorbol 12-myristate 13-acetate (PMA) (4-6).

Monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) is a member of the CC chemokine family and binds to CC chemokine receptor (CCR) 2 (7). MCP-1 plays an important role in migration of leukocytes to inflammatory lesions, such as atherosclerotic plaques (8-10). Recent studies suggest that MCP-1 is involved in the development of cancers, including angiogenesis and metastasis (11,12). It was also reported that MCP-1 confers a protective advantage in prostate cancer (13-15). MCP-1 is induced and secreted from a variety of cell types, including cancer cells, due to various stimuli (16,17).

The protein kinase C (PKC) family is known to be involved in various cellular processes, including breast cancer development (18). It has been reported that breast cancer biopsies exhibit higher levels of total PKC activity compared to surrounding normal tissues (18,19). The PKC superfamily is classified into three subfamilies, conventional PKCs (PKCα, ßI, ßII and γ), novel PKCs (PKCδ, ε, θ and η), and atypical PKCs (PKCζ and PKCι/λ) (20). Each subfamily is involved in different and diverse cellular processes (21).

Taxol (paclitaxel) is a natural anti-cancer drug that is used for the treatment of ovary, non-small cell lung and breast cancers (22). Taxol plays an important role in regulation of the cell cycle by arresting the cycle in the G_2/M phase via stabilization of microtubule assembly and inhibition of cell proliferation, leading to programmed cell death (22,23). In this study, involvement of MCP-1 in taxol-induced breast cancer cells was investigated using the MCF-7 human breast cancer cell line. We report herein that MCP-1 is up-regulated in taxol-induced breast cancer cell death and the up-regulation is mediated by a combination of the effects of PKCδ and PLC-γ1.

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Materials and methods

Materials. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS), penicillin and streptomycin were purchased from Gibco-BRL Life Technologies Inc. (Grand Island, NY). Taxol, trypan blue and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO). Lipofectamine 2000 reagent was purchased from Invitrogen (Carlsbad, CA). PD98059, U73122, LY294002, rottlerin, Ro-31-8425, SB202190, and SP600125 were obtained from Calbiochem (San Diego, CA).

Cell culture and transient transfection. Human breast cancer cell line MCF-7 cells were obtained from ATCC. MCF-7 cells were maintained in DMEM supplemented with 10% heat-inactivated FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml). MCF-7 cells were seeded into 6-well plates at a density of $5x10^5$ cells/well. After incubation for 24 h, cells were transfected with 0.5-4 μg of DNA using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions.

MTT assay. MCF-7 cells were plated at a density of $5x10^4$ cells/well in 96-well plates, and cell survival was evaluated by MTT reduction assay. The reduction status of cells was measured by a colorimetric assay for cell survival. MTT was dissolved in DMEM. After 12-24 h exposure to 40 nM taxol, MTT was added to each well and incubated for 2 h at 37˚C in the dark. MTT was converted to a water-insoluble blue product (formazan) when absorbed by living cells. The formazan product was dissolved by adding 100 μl DMSO to each well. The absorption value at a wavelength of 570 nm was determined using an ELISA plate reader (Bio-Rad Laboratories, Inc.). Data were presented as the percentage of survival relative to vehicle-treated control culture. All measurements were performed in triplicate and each experiment was repeated at least three times.

Trypan blue exclusion assay. MCF-7 cells were seeded into 6-well plates at a density of $5x10^5$ cells/well. After incubation for 24 h, cells were treated with various concentrations of taxol. Trypan blue solution (0.4%) (10 μl) was added to the cell suspension and cells were counted on a hemocytometer under a microscope. The percentage of viable cells was expressed as the ratio of the number of trypan blue non-permeable cells to the total cell count (trypan blue non-permeable cell number/total cell number).

cDNA preparation and PCR amplification. Total cellular RNA was extracted from cells using Trizol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized from 2 μg of total RNA using AccuPower RT PreMix (BioNeer, Daejeon, Korea). The reaction was carried out for 60 min at 42˚C and for 5 min at 94˚C. The resulting cDNA samples were amplified by 30 cycles (denaturating at 96˚C for 40 sec, annealing at 55˚C for 40 sec, and elongating at 72˚C for 40 sec) using MCP-1 primers. GAPDH was amplified as an internal control. The PCR products were electrophoresed on a 1.5% (w/v) agarose gel containing 0.5 μg/ml ethidium bromide (EtBr), and sizes of the products were determined by comparison with a 1-kb DNA ladder marker (Invitrogen).

Table 1. Primer sequences used in this study.

Primer	Primer type	Primer sequences
MCP-1	Forward	CAGCCAGATGCAATCAATGC
	Reverse	AAG TCT TCG GAG TTT GGG
Lkn-1	Forward	ACTTTGCTGACTGCT
	Reverse	GTGGCTGGCCTCTTTTGT
TNF-α	Forward	CAGAGGGAAGAGTTCCCCAG
	Reverse	CCTTGGTCTGGTAGGAGACG
MIP-1α	Forward	CTGCCCTTGCTGTCCTCCTCTG
	Reverse	CTGCCGGCTTGCTTGGTTA
PKCδ	Forward	TTTCTCACCCACCTCATCTG
	Reverse	CGAAGAGTTCATCCTCATCATC
PLCß1	Forward	CAGAGTGTCTTAACAGAAGTGGAAG
	Reverse	GACCGGATCATCTCTGTCTTCTCC
PLCγ1	Forward	GAAGAGAAGATTGGCACAGAACGTG
	Reverse	GTCCACCACAAACTCTGTCTTCTGC
GAPDH	Forward	CACCACCATGGAGAAGGCTGG
	Reverse	TTGTCATGGATGACCTTGGCCAGG

The intensity of each band amplified by RT-PCR was analyzed using a UV Image analyzer (Vilber Lourmat, Germany), and normalized to that of GAPDH mRNA in corresponding samples. Primer sequences used in this study are summarized in Table I.

Results

Taxol induces breast cancer cell death and affects mRNA expression of MCP-1. Taxol is known to have a cytotoxic effect on a variety of cancer cells and is used as an anticancer drug for cancer therapy (22,24). We first confirmed the anticancer activity of taxol. As shown in Fig. 1, treatment with taxol induced MCF-7 breast cancer cell death. Cell morphology was determined microscopically in taxol-treated cells. Cells treated with taxol exhibited morphological changes that indicate cell damage or death (Fig. 1A). We also determined taxol-induced cell death by counting the number of viable cells after trypan blue staining and by performing an MTT assay. Taxol decreased cell viability in a dose-dependent manner (Fig. 1B and C). These results indicate that taxol effectively induces breast cancer cell death.

We next investigated whether chemokines are involved in taxol-induced breast cancer cell death. MCF-7 cells were treated with taxol and the mRNA expression levels of chemokines, including leukotactin-1 (Lkn-1), MCP-1, macrophage inflammatory protein-1α (MIP-1α), and TNF-α were determined using semi-quantitative RT-PCR. The mRNA levels of Lkn-1, MIP-1α, and TNF-α were not affected by the treatment with taxol (Fig. 1D). However, the mRNA expression of MCP-1 was increased ~1.8-fold in taxol-treated cells compared to control cells. These results indicate that MCP-1 is involved in breast cancer cell death induced by taxol treatment.

Taxol induces up-regulation of MCP-1 in a dose- and time-dependent manner. Since taxol affects MCP-1 expression in breast cancer cells, we examined whether taxol regulates

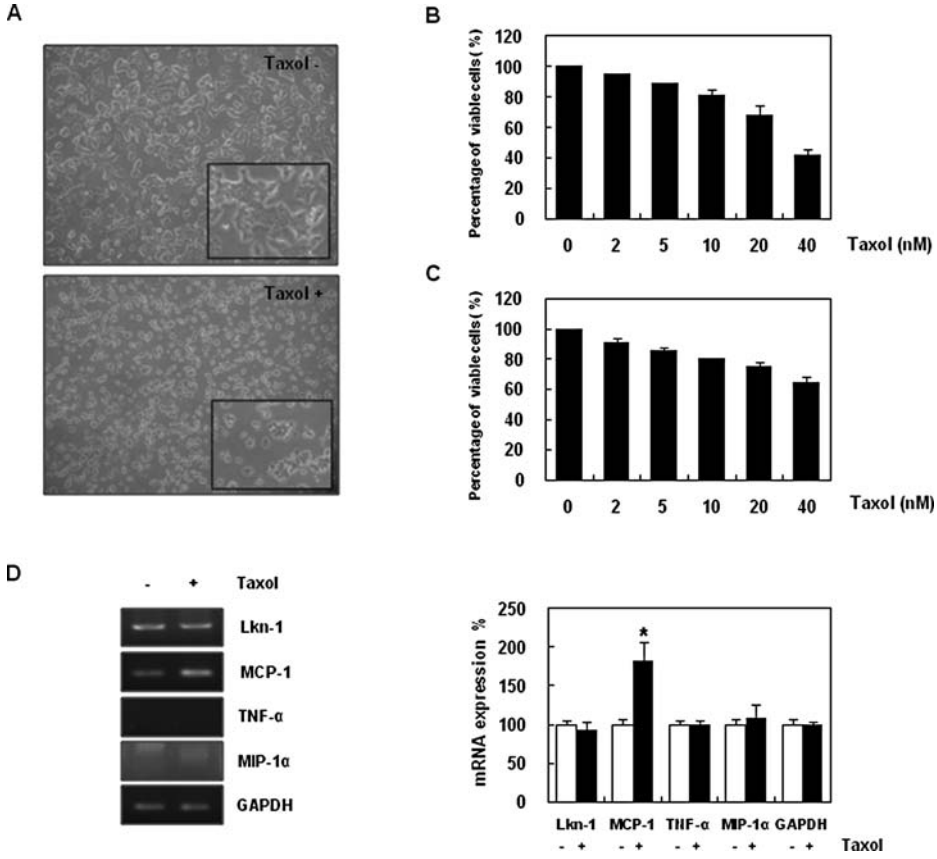

Figure 1. Taxol induces breast cancer cell death and increases mRNA expression of MCP-1. (A) MCF-7 cells were treated with or without 40 nM taxol for 24 h and morphological changes were examined microscopically. Cells were incubated with the indicated concentrations of taxol (0, 2, 5, 10, 20, and 40 nM) for 24 h and cell viability was determined by the cell counting after (B) trypan blue staining and (C) MTT assay. (D) MCF-7 cells were incubated with 40 nM taxol for 24 h and total RNA was extracted. The mRNA expression levels of chemokines (Lkn-1, MCP-1, TNF-α, and MIP-1α) were examined using semi-quantitative RT-PCR. GAPDH was used as an internal control. Densitometry analysis was performed using Quantity One software (Bio-Rad Laboratories, Hercules, CA). Data are presented as the percentage of chemokine expression level (each chemokine expression level in the absence of taxol is set to 100%).

expression of MCP-1 in MCF-7 cells. Cells were treated with various concentrations of taxol and the levels of MCP-1 mRNA were examined using semi-quantitative RT-PCR. As shown in Fig. 2A, MCP-1 expression was increased in a dose-dependent manner in taxol-treated cells. Taxol also induced expression of MCP-1 time-dependently. MCP-1 expression began to increase at 3 h after taxol treatment and the increased level of MCP-1 was sustained for 2 days after stimulation (Fig. 2B and C). These results indicate that induction of MCP-1 is a relatively early response to taxol treatment and is sustained for a long time in breast cancer cells.

PKCδ and PLC are involved in taxol-induced up-regulation of MCP-1. Since taxol up-regulates expression of MCP-1 in breast cancer cells, we next investigated which signal pathways are associated with taxol-induced MCP-1 expression. MCF-7 cells were pre-incubated in the presence and absence of inhibitors specific for various signaling molecules and then cells were treated with taxol. As shown in Fig. 3, PD98059 (inhibitor of MEK), Ro-31-8425 (inhibitor of pan-PKCs), LY294002 (inhibitor of PI3K), SP600125 (inhibitor of JNK), and SB202190 (inhibitor of p38 MAPK) showed only a small inhibitory effect on taxol-induced up-regulation of MCP-1. However, the mRNA level of MCP-1 in taxol-treated cells was increased ~1.9-fold by pre-treatment with rottlerin, a specific inhibitor of PKCδ (Fig. 3). This result indicates that PKCδ acts as a negative regulator in taxol-induced MCP-1 expression. However, pre-treatment with U73122, a PLC inhibitor, completely abolished induction of MCP-1 in response to taxol (Fig. 3). This result indicates that PLC functions as a positive regulator of taxol-stimulated MCP-1 induction. Taken together, these data suggest that up-regulation of MCP-1 in taxol-treated breast cancer cells is regulated via PKCδ and PLC-mediated signal pathways.

PKCδ has a negative effect on MCP-1 expression in taxol-treated breast cancer cells. Since MCP-1 expression is increased by a specific PKCδ inhibitor, we confirmed the effect of rottlerin on taxol-induced expression of MCP-1. Pre-treatment with rottlerin increased MCP-1 expression in a dose-dependent manner in taxol-treated MCF-7 cells (Fig. 4A). We next investigated whether ectopic expression of PKCδ affects MCP-1 expression induced by taxol in breast cancer cells. MCF-7 cells were transfected with the PKCδ expression vector, and then transfected cells were treated with taxol. Ectopic expression of PKCδ down-regulated MCP-1 expression in control cells and PKCδ decreased the expression level of MCP-1 in a dose-dependent manner in taxol-treated cells (Fig. 4B). These results indicate that taxol has an inhibitory effect on PKCδ that is involved in down-regulation of MCP-1 in breast cancer cells.

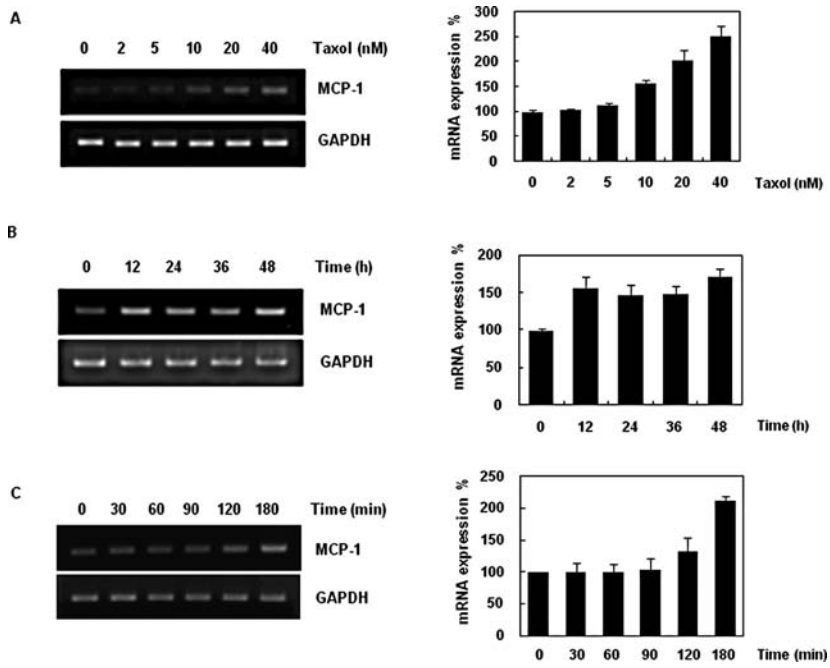

Figure 2. Taxol induces up-regulation of MCP-1 in a dose- and time-dependent manner. (A) MCF-7 cells were treated with the indicated concentrations of taxol (0, 2, 5, 10, 20, and 40 nM) for 24 h and total RNA was extracted. Semi-quantitative RT-PCR was performed using primers specific for MCP-1. The PCR products were separated on a 1.5% agarose gel. MCF-7 cells were incubated with 40 nM taxol for (B) a long time (0, 12, 24, 36 and 48 h) and for (C) a short time (0, 30, 60, 90, 120, and 180 min), and mRNA expression of MCP-1 was examined using semi-quantitative RT-PCR. GAPDH was used as an internal control. Densitometry analysis was performed using Quantity One software. Data are expressed as the means ±SD and presented as the percentage of MCP-1 (the MCP-1 level in the absence of taxol is set to 100%). The data represent three independent experiments.

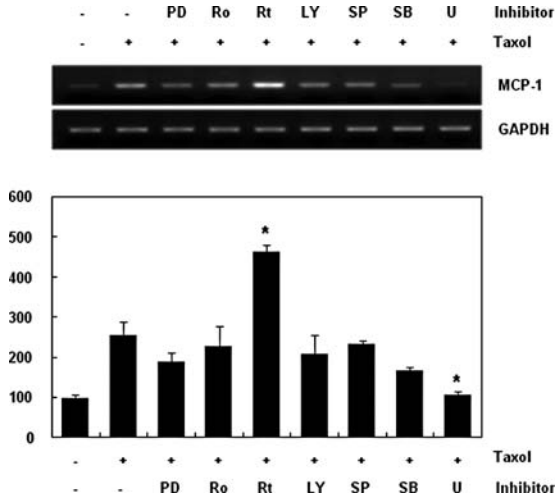

Figure 3. PKCδ and PLC are involved in taxol-induced up-regulation of MCP-1. MCF-7 cells were pre-incubated with various signaling molecule inhibitors including PD98059 (PD, 50 μg/ml), Ro-31-8425 (Ro, 50 ng/ml), rottlerin (Rt, 50 μg/ml), LY294002 (LY, 10 μg/ml), SP600125 (SP, 10 μg/ml), SB202190 (SB, 20 μg/ml) and U73122 (U, 40 μg/ml) for 30 min and were treated with 40 nM taxol for 3 h. Total RNA was extracted and MCP-1 mRNA expression was determined using semi-quantitative RT-PCR. GAPDH was used as an internal control. Densitometry analysis was performed using Quantity One software. Data are expressed as the means ±SD and presented as the percentage of MCP-1 (the MCP-1 level in the absence of taxol is set to 100%). The data represent three independent experiments.

PLC-γ1 is involved in up-regulation of MCP-1 in taxol-treated breast cancer cells. Since the PLC inhibitor U73122 depresses the taxol-induced increase of MCP-1 expression in MCF-7 cells, we confirmed the effect of U73122 on MCP-1 expression in taxol-stimulated breast cancer cells. As shown in Fig. 5A, MCP-1 mRNA expression was decreased in taxol-treated cells by pre-treatment with U73122 in a dose-dependent manner. We next determined whether the PLC isoforms PLC-ß and PLC-γ1 affect taxol-induced MCP-1 expression in breast cancer cells. MCF-7 cells were transfected with plasmids expressing PLC-ß and PLC-γ1, and then cells were incubated in the presence and absence of taxol. Similar amounts of PLC-ß and PLC-γ1 were expressed in both the taxol-free and taxol-treated MCF-7 cells (Fig. 5B, upper panel). Ectopic expression of PLC-ß did not affect MCP-1 expression, however ectopic expression of PLC-γ1 induced up-regulation of MCP-1 in breast cancer cells (Fig. 5B, lower panel). These results indicate that PLC-γ1 functions as a positive regulator of MCP-1 expression in breast cancer cells and that treatment with taxol induces activation of PLC-γ1, leading to up-regulation of MCP-1 in breast cancer cells.

Discussion

MCP-1 is a CC chemokine and a ligand for CCR2. Chemokines are known to play an important role in migration of leukocytes to inflammatory lesions (1). Recent reports propose that a variety of chemokines are involved in development of cancer including angiogenesis and metastasis (11,12). However, the exact roles of chemokines in cancer development are still unknown. In this study, we determined that MCP-1 is involved in taxol-induced breast cancer cell death. Results from this study show that (i) MCP-1 is up-regulated in taxol-treated breast cancer cells, (ii) taxol increases MCP-1 expression by enhancing activation of PLC-γ1 and (iii) by reducing the activity of PKCδ.

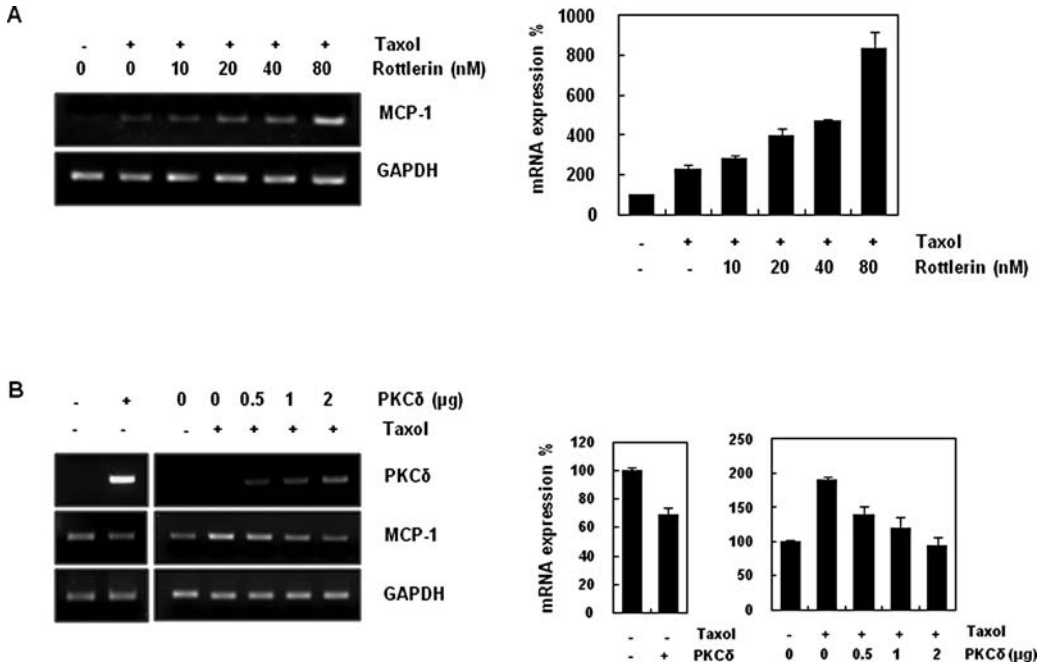

Figure 4. PKCδ down-regulates taxol-induced MCP-1 expression. (A) MCF-7 cells were pre-incubated with the indicated concentrations of rottlerin (0, 10, 20, 40, and 80 nM) for 30 min and treated with 40 nM taxol for 3 h. Total RNA was obtained and the MCP-1 mRNA levels were examined using semi-quantitative RT-PCR. (B) MCF-7 cells were transfected with the indicated amounts of the PKCδ expression vector (0, 0.5, 1, and 2 μg) for 24 h. The total amount of DNA (2 μg) was kept constant using the mock vector. The transfected cells were treatead with 40 nM taxol for 3 h and total RNA was extracted. The mRNA level of MCP-1 was determined using semi-quantitative RT-PCR. GAPDH was used as an internal control. Densitometry analysis was performed using Quantity One software. Data are expressed as the means ±SD and presented as the percentage of MCP-1 (the MCP-1 level in control is set to 100%). The data represent three independent experiments.

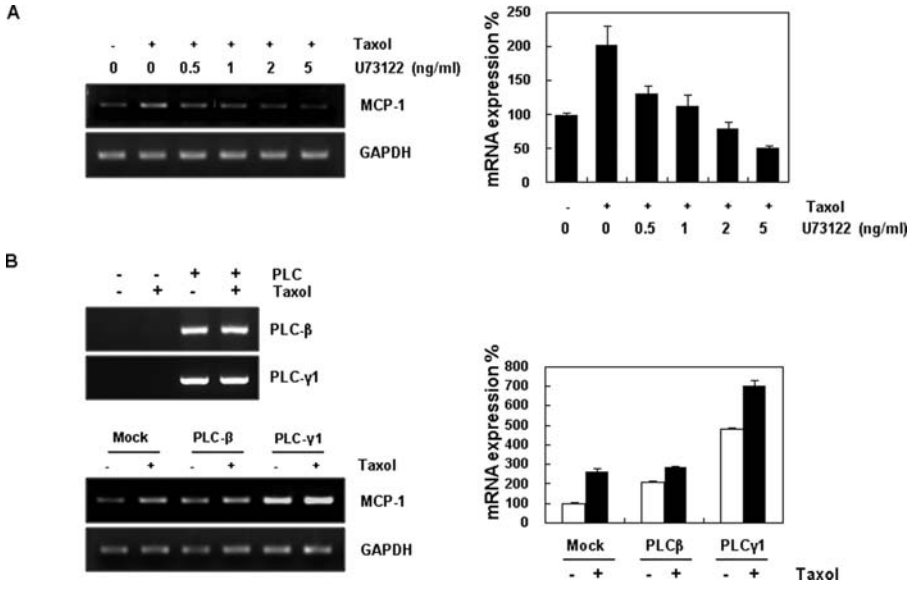

Figure 5. PLC-γ1 up-regulates taxol-induced expression of MCP-1. (A) MCF-7 cells were pre-incubated with the indicated concentrations of U73122 (0, 0.5, 1, 2, and 5 ng/ml) for 30 min and treated with 40 nM taxol. Total RNA was extracted and MCP-1 expression was determined using semi-quantitative RT-PCR. (B) MCF-7 cells were transfected with the plasmids expressing PLC-ß and PLC-γ1 (4 μg). After 24 h, cells were treated with 40 nM taxol for 3 h and total RNA was extracted. The expression level of MCP-1 was determined using semi-quantitative RT-PCR. GAPDH was used as an internal control. Densitometry analysis was performed using Quantity One software. Data are expressed as the means ±SD and presented as the percentage of MCP-1 (the MCP-1 level in the absence of taxol is set to 100%). The data represent three independent experiments.

Various stimuli are known to induce expression and secretion of MCP-1 in inflammatory diseases and cancer development (12,16,17,25,26). Herein, we determined that MCP-1 is up-regulated in taxol-induced breast cancer cell death. Taxol, a potent anti-cancer drug, causes cell cycle arrest in the G_2/M phase by stabilizing microtubule assembly, resulting in apoptosis (22,23). During programmed cell death, surrounding immune cells, especially macrophages, remove apoptotic cells by phagocytosis. In taxol-induced cell death, the affected breast cancer cells probably secrete the chemokine MCP-1 for recruitment

of immune cells that destroy damaged cancer cells. Recent reports indicate that chemokines are involved in proliferation of a variety of cells and MCP-1 confers a protective advantage in prostate cancer (13-15). Therefore, it is also possible that cancer cells release MCP-1 for proliferation and survival as a protective mechanism against taxol-induced cell death.

We also investigated the signal pathways involved in taxol-induced MCP-1 expression. Taxol-induced expression of MCP-1 was blocked due to inhibition of PLC activity, and overexpression of PLC increased MCP-1 expression. PLC-γ1 expression also induced MCP-1 expression in untreated cells, indicating that PLC-γ1 is a positive regulator of MCP-1 expression in breast cancer cells. Taxol apparently up-regulates MCP-1 expression by activation of this positive regulator. The treatment with rottlerin enhanced the taxol-induced expression of MCP-1, and overexpression of PKCδ reduced taxol-stimulated MCP-1 expression, indicating that PKCδ plays a negative role in MCP-1 expression in breast cancer cells. Taxol apparently induces MCP-1 expression by inhibition of this negative regulator.

In conclusion, we have demonstrated that taxol induces up-regulation of MCP-1 in MCF-7 breast cancer cells. This process is mediated both by positive and negative regulatory signaling pathways. Although further investigation is necessary to understand the exact role of MCP-1 in this event, for the first time we propose that MCP-1 is involved in taxol-induced breast cancer cell death.

Acknowledgements

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References

1. Gerard C and Rollins BJ: Chemokines and disease. Nat Immunol 2: 108-115, 2001.
2. Baggiolini M: Chemokines and leukocyte traffic. Nature 392: 565-568, 1998.
3. Rollins BJ: Chemokines. Blood 90: 909-928, 1997.
4. Baggiolini M and Dahinden CA: CC chemokines in allergic inflammation. Immunol Today 15: 127-133, 1994.
5. Kautz K, Schwarz M and Radeke HH: Interference with MCP-1 gene expression by vector generated triple helix-forming RNA oligonucleotides. Cell Mol Life Sci 62: 362-376, 2005.
6. Oppenheim JJ, Zachariae CO, Mukaida N and Matsushima K: Properties of the novel proinflammatory supergene 'intercrine' cytokine family. Annu Rev Immunol 9: 617-648, 1991.
7. Rollins BJ, Walz A and Baggiolini M: Recombinant human MCP-1/JE induces chemotaxis, calcium flux, and the respiratory burst in human monocytes. Blood 78: 1112-1116, 1991.
8. Fuentes ME, Durham SK, Swerdel MR, Lewin AC, Barton DS, Megill JR, Bravo R and Lira SA: Controlled recruitment of monocytes and macrophages to specific organs through transgenic expression of monocyte chemoattractant protein-1. J Immunol 155: 5769-5776, 1995.
9. Takeya M, Yoshimura T, Leonard EJ and Takahashi K: Detection of monocyte chemoattractant protein-1 in human atherosclerotic lesions by an anti-monocyte chemoattractant protein-1 monoclonal antibody. Hum Pathol 24: 534-539, 1993.
10. Nelken NA, Coughlin SR, Gordon D and Wilcox JN: Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest 88: 1121-1127, 1991.
11. Yla-Herttuala S, Lipton BA, Rosenfeld ME, Sarkioja T, Yoshimura T, Leonard EJ, Witztum JL and Steinberg D: Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. Proc Natl Acad Sci USA 88: 5252-5256, 1991.
12. Ueno T, Toi M, Saji H, Muta M, Bando H, Kuroi K, Koike M, Inadera H and Matsushima K: Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. Clin Cancer Res 6: 3282-3289, 2000.
13. Loberg RD, Day LL, Harwood J, Ying C, St. John LN, Giles R, Neeley CK and Pienta KJ: CCL2 is a potent regulator of prostate cancer cell migration and proliferation. Neoplasia 8: 578-586, 2006.
14. Roca H, Varsos Z and Pienta KJ: CCL2 protects prostate cancer PC3 cells from autophagic death via phosphatidylinositol 3-kinase/AKT-dependent survivin up-regulation. J Biol Chem 283: 25057-25073, 2008.
15. Loberg RD, Ying C, Craig M, Day LL, Sargent E, Neeley C, Wojno K, Snyder LA, Yan L and Pienta KJ: Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor regression in vivo. Cancer Res 67: 9417-9424, 2007.
16. Kuroda T, Kitadai Y, Tanaka S, Yang X, Mukaida N, Yoshihara M and Chayama K: Monocyte chemoattractant protein-1 transfection induces angiogenesis and tumorigenesis of gastric carcinoma in nude mice via macrophage recruitment. Clin Cancer Res 11: 7629-7636, 2005.
17. Neumark E, Sagi-Assif O, Shalmon B, Ben-Baruch A and Witz IP: Progression of mouse mammary tumors: MCP-1-TNFalpha cross-regulatory pathway and clonal expression of promalignancy and antimalignancy factors. Int J Cancer 106: 879-886, 2003.
18. O'Brian CA and Ward NE: Biology of the protein kinase C family. Cancer Metastasis Rev 8: 199-214, 1989.
19. Borner C, Wyss R, Regazzi R, Eppenberger U and Fabbro D: Immunological quantitation of phospholipid/Ca^{2+}-dependent protein kinase of human mammary carcinoma cells: inverse relationship to estrogen receptors. Int J Cancer 40: 344-348, 1987.
20. Newton AC: Regulation of protein kinase C. Curr Opin Cell Biol 9: 161-167, 1997.
21. Nabha SM, Glaros S, Hong M, Lykkesfeldt AE, Schiff R, Osborne K and Reddy KB: Upregulation of PKC-[delta] contributes to antiestrogen resistance in mammary tumor cells. Oncogene 24: 3166-3176, 2005.
22. He L, Orr GA and Horwitz SB: Novel molecules that interact with microtubules and have functional activity similar to Taxol. Drug Discov Today 6: 1153-1164, 2001.
23. Wani MC, Taylor HL, Wall ME, Coggon P and McPhail AT: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J Am Chem Soc 93: 2325-2327, 1971.
24. Pazdur R, Kudelka AP, Kavanagh JJ, Cohen PR and Raber MN: The taxoids: paclitaxel (Taxol) and docetaxel (Taxotere). Cancer Treat Rev 19: 351-386, 1993.
25. Rovin BH, Yoshiumura T and Tan L: Cytokine-induced production of monocyte chemoattractant protein-1 by cultured human mesangial cells. J Immunol 148: 2148-2153, 1992.
26. Friedland JS, Shattock RJ and Griffin GE: Phagocytosis of mycobacterium tuberculosis or particulate stimuli by human monocytic cells induces equivalent monocyte chemotactic protein-1 gene expression. Cytokine 5: 150-156, 1993.