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Brief Communication

Parkin Reduces Expression of Monocyte Chemotactic Protein-1 (MCP-1) in TNF-α-stimulated MCF7 Breast Cancer Cells

Kyunghong Lee[1], Min Ho Lee[1], In-Soo Lee[2], Ki-Jong Rhee[1,†] and Yoon Suk Kim[1,†]

[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Gangwon-do 220-710, Korea

[2]Department of Clinical Laboratory Science, Hyejeon College, Hongseong, Chungcheongnam-do 350-702, Korea

Parkin is a putative tumor suppressor protein and its expression is frequently reduced or absent in several types of tumors. In this study, we examined the role of Parkin in mRNA expression of monocyte chemotactic protein-1 (MCP-1) in the breast cancer cell line MCF7. Expression of MCP-1 mRNA increased after TNF-α treatment. However, overexpression of Parkin induced a decrease in expression of MCP-1 mRNA in TNF-α-stimulated MCF7. This decrease in MCP-1 mRNA by Parkin overexpression occurred in a dose- and time-dependent manner. Using a wound scratch assay, we found that Parkin overexpression in MCF7 cells also resulted in a decrease in cell migration. These results suggest that Parkin down-regulates MCP-1 synthesis leading to decreased migration of tumor cells. We suggest that one possible mechanism by which Parkin acts as a tumor suppressor is by inhibiting migration or metastasis of cancer cells.

Key Words: Parkin, MCP-1, Tumor suppressor, Cell migration

The protein Parkin is a ubiquitin E3 ligase (Ciechanover, 2001; Shimura et al., 2001) and mutations in the Parkin gene are known to be a predominant cause of inherited parkinsonism (Kitada et al., 1998). The Parkin gene is located on the common fragile site (CFS) FRA6E, genetic region that has a propensity to break when the cell is exposed to partial replication stress (Denison et al., 2003; Picchio et al., 2004; Poulogiannis et al., 2010; Tay et al., 2010; Veeriah et al., 2010; Wang et al., 2004). Recently, it has been reported that expression of Parkin was frequently diminished in several types of tumors including breast, ovarian, kidney, and liver cancers. Therefore, Parkin may have tumor suppression properties although the mechanism is still unclear.

Increase of migration and metastasis properties induced by tumor necrosis factor-alpha (TNF-α) was reported in breast cancer cells (Balkwill, 2006; Yin et al., 2009). TNF-α is a potent pleiotropic pro-inflammatory cytokine that plays an important role in regulation of the immune response and inflammation (Anderson et al., 2004). In malignant disease, however, it is reported that TNF-α does not cause tumor regression, but rather mediates tumor progression by inducing cancer cell growth, proliferation, angiogenesis, invasion, and metastasis (Mochizuki et al., 2004). TNF-α also induces expression of a variety of cytokines (Lu and Stark, 2004; Neumark et al., 2003). Monocyte chemotactic protein-1 (MCP-1), also called chemokine (C-C motif) ligand-2 (CCL2), recruits monocytes, T-cells and dendritic cells to sites of injury, infection and inflammation. MCP-1 is also implicated in the proliferation and metastasis of tumor cells (Conti and Rollins, 2004; Soria and Ben-Baruch, 2008) such as breast cancer (Nam et al., 2006; Soria et al., 2011). Moreover, studies in breast cancer cells showed that TNF-α increased MCP-1 expression and the subsequent induction of MCP-1 resulted in increased migration

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†Corresponding author: Yoon Suk Kim, Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University Wonju 220-710, Korea.
Tel: +82-33-760-2860, Fax: +82-33-760-2195
e-mail: yoonsukkim@yonsei.ac.kr

†Corresponding author: Ki-Jong Rhee, Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University Wonju 220-710, Korea.
Tel: +82-33-760-2445, Fax: +82-33-760-2195
e-mail: kjrhee@yonsei.ac.kr

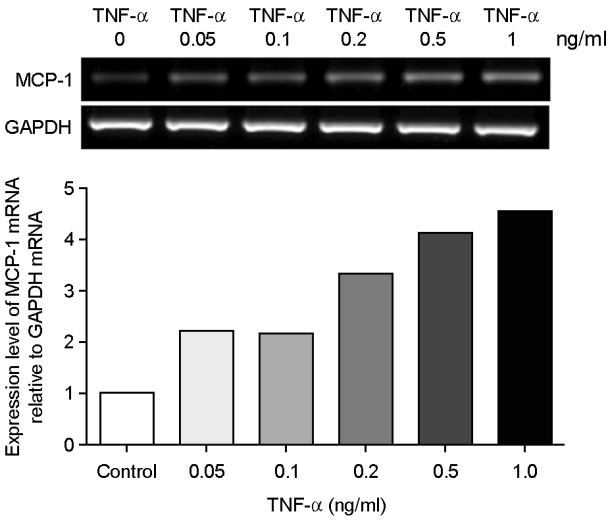

Fig. 1. TNF-α enhances expression of MCP-1 in MCF7 cells in a dose-dependent manner. MCF7 cells were treated with the indicated concentrations (0, 0.05, 0.1, 0.2, 0.5, 1 ng/ml) of human recombinant TNF-α for 24 h. Total RNA was extracted and cDNA was prepared. PCR analysis was performed using MCP-1-specific primers. The PCR products were resolved by 1.5% agarose gel to detect expression of MCP-1 and GAPDH (upper panel). Densitometric analysis was performed and data are presented as the expression levels of MCP-1 mRNA relative to GAPDH mRNA (lower panel). The expression level of MCP-1 relative to GAPDH in the absence of TNF-α treatment was set as 1.0.

(Neumark et al., 2003; Yin et al., 2009). In this study, we examined the effect of Parkin on TNF-α-induced expression of MCP-1 and cell migration in the breast cancer cell line MCF7.

Reports suggest that TNF-α promotes cellular migration and activates expression of various chemokines in breast cancer cells. First, we confirmed TNF-α-induced expression of MCP-1 mRNA in MCF7 cells. MCF7 (2×10^5 cells) were plated in a 6-well plate with DMEM containing 10% FBS and cultured for 24 hours (37℃, 5% CO_2). Cells were then treated with recombinant TNF-α (R&D Systems, Minneapolis, MI) in a dose-dependent manner (0, 0.05, 0.1, 0.2, 0.5, 1 ng/ml). Twenty-four hours later total RNA was extracted with Trizol (Invitrogen, Carlsbad, CA) and cDNA was prepared by incubating total RNA with random primers (Invitrogen, Carlsbad, CA) and MMLV-RT (Invitrogen, Carlsbad, CA) for 50 minutes at 37℃, then for 15 minutes at 70℃. PCR analysis was performed using 0.2 units of Taq polymerase (Cosmogenetech, Jeungpyung-gun, Korea), template cDNA and MCP-1 specific primers (sense 5'-CAGCCAGATGCAATCAATGC-3', antisense 5'-AAGT-CTTCGGAGTTTGGG-3'). GAPDH was analyzed as an internal control (GAPDH sense 5'-CGGGAAGCTTGTC-ATCAATGG-3', antisense 5'-GGCAGTGATGGCATGGA-CTG-3'). Reaction conditions were as follows. MCP-1: 94℃ for 30s, 60℃ for 30s, 72℃ for 30s, 40 cycles; GAPDH: 94℃ for 30s, 55℃ for 30s, 72℃ for 30s, 24 cycles. We found that expression of MCP-1 mRNA was increased with TNF-α treatment in a dose-dependent manner (Fig. 1).

Next, we examined whether Parkin affects TNF-α-stimulated expression of MCP-1 in MCF7 cells. Cells were infected with a recombinant adenoviral vector carrying the Parkin gene or adenovirus without the Parkin gene (Mock) and 24 hours later treated with TNF-α (5 ng/ml). Total RNA was prepared and level of MCP-1 mRNA was examined by RT-PCR. The data show that MCP-1 induction by TNF-α was attenuated by Parkin over-expression implying Parkin down-regulates MCP-1 (Fig. 2A). To confirm Parkin-induced down-regulation of MCP-1, Parkin was over-expressed in a dose-dependent manner in TNF-α-treated (0.2 ng/ml) MCF7. Total RNA was extracted and mRNA expression of MCP-1 was analyzed by RT-PCR and also quantified by densitometry. MCP-1 mRNA expression was decreased in a Parkin dose-dependent manner (Fig. 2B). MCP-1 level after Parkin overexpression was also measured in a time-dependent manner and the mRNA level of MCP-1 began to decrease at 48 hours after infection (Fig. 2C). Infection with mock adenovirus was used as a control. These results suggest that Parkin downregulates MCP-1 mRNA expression.

MCP-1 is known to play an important role in migration of breast cancer cells (Nam et al., 2006). Therefore, we examined whether Parkin affects migration of MCF7 using the wound scratch assay as described by Tay *et al*. (Tay et al., 2010). MCF7 cells (2×10^5) were plated in a six-well plate and incubated for 24 h. Then, a wound line was made in the cell layer using a 200 μl micropipette tip. The cell layer was washed three times with PBS to remove debris before adding fresh medium. The migration of cells into the wound area was monitored at regular intervals under a phase contrast microscope for a period of 108 hours. The distance of cell moved was measured and expressed as an

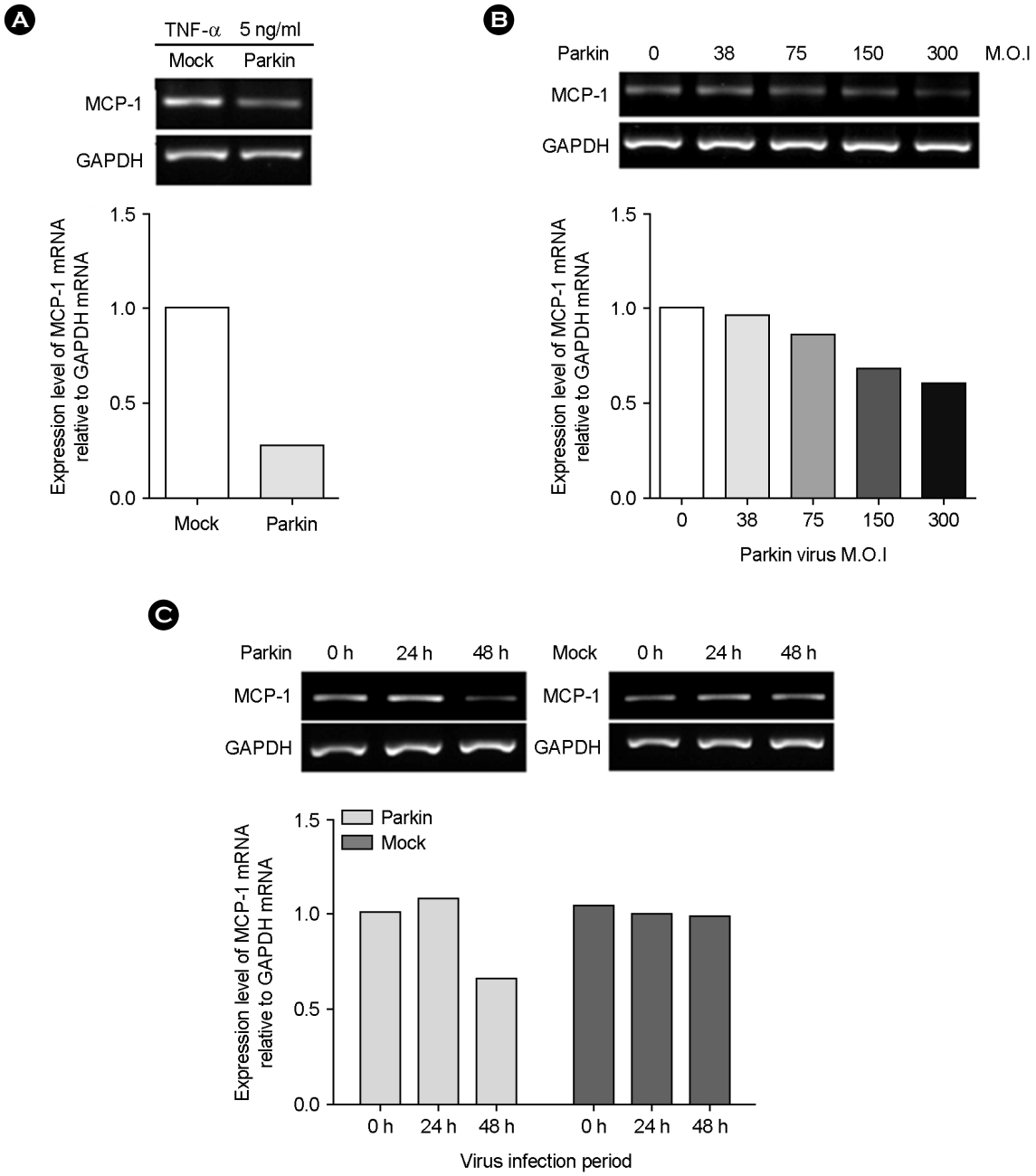

Fig. 2. Parkin reduces expression of MCP-1 in TNF-α-stimulated MCF7 cells in a dose- and time- dependent manner. (A) MCF7 cells were infected with either Parkin-expressing adenovirus or mock virus (150 M.O.I. respectively) and then treated with TNF-α (5 ng/ml) for 48 h. Total RNA was extracted and cDNA was prepared. PCR analysis was performed using MCP-1 specific primers. The PCR products were resolved by 1.5% agarose gel to detect expression of MCP-1. Densitometric analysis was performed and data are presented as the expression levels of MCP-1 mRNA relative to GAPDH mRNA. The expression level of MCP-1 relative to GAPDH in 0 M.O.I. of Parkin-expressing virus infection was set to 1.0. **(B)** MCF7 cells were infected with indicated concentrations (0, 38, 75, 150, 300 M.O.I) of Parkin-expressing virus and then treated with TNF-α (0.2 ng/ml) for 48 h. PCR and densitometric analysis was performed as described above. The expression level of MCP-1 relative to GAPDH in 0 M.O.I. of Parkin-expressing virus infection was set to 1.0. **(C)** MCF7 cells were infected with Parkin-expressing virus or mock virus (300 M.O.I respectively) and then treated with TNF-α (0.2 ng/ml) for 0, 24 h and 48 h. PCR and densitometric analysis was performed as described above. The expression level of MCP-1 relative to GAPDH in 0 h after infection with Parkin-expressing virus was set to 1.

arbitrary unit. We found that MCF7 cells over-expressing Parkin migrate significantly slower into the scratched area compared with Mock virus infected cells (Fig. 3).

In this study, we found that Parkin decreases TNF-α-

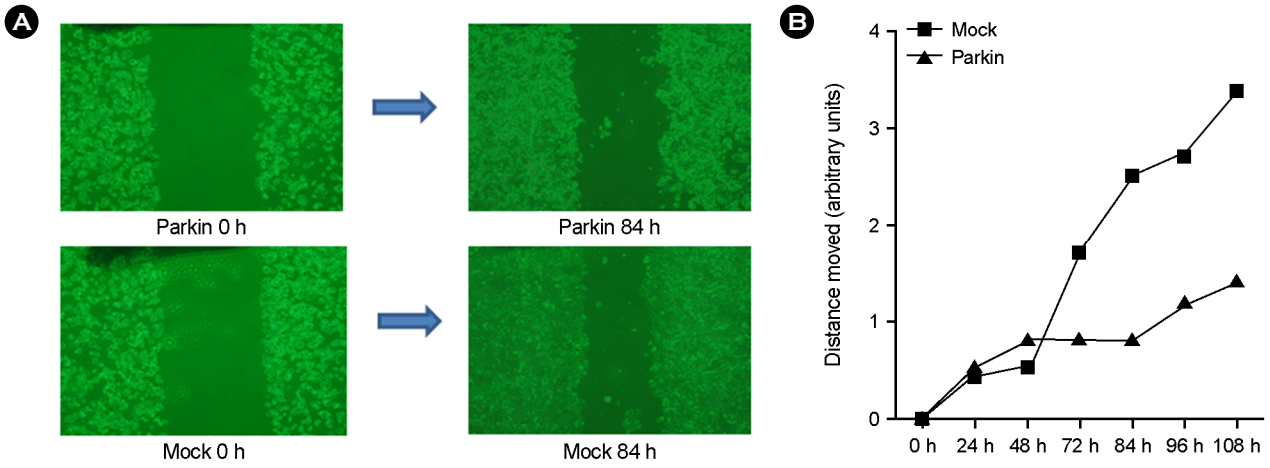

Fig. 3. Parkin diminishes migration rate of MCF7 cells. Wound scratch assay was performed as described by Tay *et al*. (Tay et al., 2010). Briefly, MCF7 cells (2×10^5) were plated into six-well plates and infected with Parkin-expressing virus or mock virus. A wound line was made by scratching the cell layer with a 200 μl micropipette tip. The migration of cells into the wound area was monitored at regular intervals for up to 108 hours using an inverted microscope. **(A)** Image of cells migrating into wound area at 84 h. **(B)** Migration distance of cells into wound area shown as arbitrary units (AU).

induced MCP-1 expression and reduces migration of the breast cancer cell line MCF7. We propose that the decreased migration of MCF7 cells is due to the downregulation of MCP-1 by Parkin. Consistent with this hypothesis, Jeong-Seok Nam *et al*. (Nam et al., 2006) demonstrated that down-regulation of MCP-1 in breast cancer cells decreased metastasis to lungs *in vivo*. Moreover, in a variety of metastatic cancers, including breast cancer, MCP-1 expression is generally high and positively correlates with the metastatic potential of the cancer cells (Soria and Ben-Baruch, 2008). In conclusion, we report that Parkin decreases MCP-1 expresssion which consequently results in decreased cell migration and metastasis. We propose that this pathway may be one of several pathways by which Parkin can act as a tumor suppressor *in vivo*.

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