Int J Clin Exp Pathol 2017;10(9):9400-9408
www.ijcep.com /ISSN:1936-2625/IJCEP0056800

Original Article

UHMWPE wear particles and dendritic cells promote osteoclastogenesis of RAW264.7 cells through RANK-activated NF-κB/MAPK/AKT pathways

Jianzhi Zhang[1], Xin Zheng[2], Fengchao Zhao[2], Xiaoyun Liu[1], Yong Pang[2], Qi Cheng[2], Yi Wang[2], Yi Zhu[2], Yan Zhang[2]

[1]*Central Laboratory,* [2]*Department of Orthopedics, Affiliated Hospital of Xuzhou Medical University, Xuzhou, Jiangsu, China*

Received May 4, 2017; Accepted July 19, 2017; Epub September 1, 2017; Published September 15, 2017

Abstract: Dendritic cells (DCs) were shown to enhance UHMWPE particle promoted osteoclastogenesis in RAW264.7 macrophages. This study aimed to elucidate the signaling network mediating the osteoclastogenic effects of UHMWPE particles and DCs. RAW264.7 cells were induced with UHMWPE particles and /or DC2.4 cells. The expression of Rank was silenced by shRNA. The activation of p38MAPK, AKT, and NF-κB was inhibited specific inhibitors. The osteoclasts were assessed by TRAP staining. The expression of osteoclastogenic genes and activation of p38MAPK, AKT, and NF-κB were analyzed by qPCR and/or Western blot. UHMWPE particles and DC2.4 cells cooperatively induced RAW264.7 macrophages to differentiate into osteoclasts, activated p38MAPK, AKT, and NF-κB pathways, and upregulated Nfatc1 and osteoclast markers, which was abolished by silencing Rank or inhibited by inhibitors of p38MAPK, AKT, and NF-κB in RAW264.7 cells. These data demonstrated the complexity of signaling network and cell-cell interactions involved in UHMWPE particles-induced osteolysis.

Keywords: UHMWPE wear particle, dendritic cells, osteoclastogenesis, RNAKL/RANK

Introduction

Ultra-high molecular weight polyethylene (UHMWPE) is the most used material for the load bearing of large joint replacements (e.g. the tibial plateau in total knee replacement and the acetabular cup in total hip prostheses) as it has excellent wear resistance and low coefficient of friction [1]. However, along with oxidative damage, wear is one of the main material-related causes of UHMWPE failure. Oxidative damage and wear are closely related as oxidative degradation leads to the decrease of wear resistance among other mechanical properties [1, 2].

Inflammation [3-6] and osteolysis [5-9] are the two major biological responses elicited by UHMWPE wear particles. Inflammation and osteolysis are two closely connected processes as UHMWPE-induced macrophage trafficking promotes inflammation and osteolysis [7, 10]. Wear particles initially promote the production and local release of pro-inflammatory cytokines and chemokines including MCP-1, TNF-α, different interleukins (IL) (IL1β, IL-6, IL-8, IL-10, IL-12p40, IL-11), and GM-CSF into the synovial fluid and interfacial tissues [6, 11]. These cytokines and chemokines can further amplify the polarization of macrophages and osteoclast precursor cells in periimplant tissues, leading to increased local osteoclastogenesis and inhibited osteoblast differentiation and function [12, 13] which results in the prosthesis loosening and eventually the UHMWPE replacement failure.

Osteoclasts are differentiated from the monocyte/macrophage hematopoietic lineage [14, 15]. The necessary and required cytokines for osteoclast differentiation and activation are macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) [15]. RANKL binds to its cognate receptor RANK on osteoclast progenitors and activates downstream signaling cascades

and transcription factors including NF-κB, AP-1 (c-Fos/c-Jun), NFATc1, and MITF, which in turn activate the expression of osteoclastogenic genes and osteoclast markers [15, 16]. Among them, NFATc1 is the master regulator of osteoclast differentiation that regulates a host of osteoclast specific genes such as calcitonin receptor, integrin β3, osteoclast-associated receptor (OSCAR), tartrate resistant acid phosphatase (TRAP), and cathepsin K [17].

UHMWPE wear particles were shown to induce murine monocytic RAW264.7 cells to spontaneously differentiate into osteoclasts [18]. Moreover, our previous study showed that dendritic cells DC2.4 cells co-culture enhanced UHMWPE wear particles-induced osteoclatogenesis in RAW264.7 cells [19]. This study aimed to elucidate the signaling pathways mediating the induction of RAW264.7 macrophages differentiation into osteoclasts by UHMWPE and DC2.4 dendritic cells.

Materials and methods

Cell culture

RAW264.7 (ATCC, Manassas, VA) and DC 2.4 (Huiying Bio. Tech, Shanghai, China) cells were cultured in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS) (ThermoFisher, Shanghai, China), 2 mM glutamine, 100 IU/ml penicillin and 100 mg/ml streptomycin (Beyotime, Shanghai, China) at 37°C in humidified air with 5% CO_2. The cells were split when necessary. The stable cell lines RAW-264.7-shControl and RAW264.7-shRank cells were selected with the G418 (ThermoFisher).

Materials

PF184 was purchased from R&D Systems (Minneapolis, MN) and SB203580 and Perifosine were purchased from Selleck Chemicals, (Houston, USA).

RANK silencing by shRNA

Three pairs of shRNA (CACCGGAGAAGATGGTTCCAGAAGATTCAAGAGATCTTCTGGAACCATCTTCTCC and AAAAGGAGAAGATGGTTCCAGAAGATCTCTTGAATCTTCTGGAACCATCTTCTCC targeting 902-922; CACCGCAGAAGTCAGAGATTCTAGGTTCAAGAGACCTAGAATCTCTGACTTCTGC and AAAAGCAGAAGTCAGAGATTCTAGGTCTCTTGAACCTAGAATCTCTGACTTCTGC targeting 972-992; and CACCGGCAGGTGATGAACTTCAAGGTTCAAGAGACCTTGAAGTTCATCACCTGCC and AAAAGGCAGGTGATGAACTTCAAGGTCTCTTGAACCTTGAAGTTCATCACCTGCC targeting 1603-1624) targeting mouse Rank gene (NM_009399) were designed using BLOCK-iT™ RNAi Designer (Invitrogen, Carlsbad, CA) and cloned into pENTR™/U6 vector (Invitrogen) according to manufacturer's protocol. The resultant vectors were transfected into RAW-264.7 cells to test their silencing efficiency by qPCR. The shRNA targeting mouse Rank gene from 972 to 992 was the most potent one in silencing Rank expression and sent for making lentiviral vector and packaging lentivirus (FulenGen, Guangzhou, China) for following silencing experiments.

TRAP staining

TRAP staining was performed according to method described previously [19]. Twenty thousand cells/well were plated in 4-well Lab-Tek TM chamber slides (Thermo Fisher, Waltham, MA) in the presence of specified treatment for 4 days. Cells were washed twice with PBS, fixed with 10% buffered formalin for 5 min and subjected to tartrate resistant acid phosphatase (TRAP) staining (Sigma, St Louis, MO) following the manufacturer's instructions. Slides were mounted with Aqua-Mount and images were obtained using an upright microscope (Olympus IX71).

Quantitative real-time polymerase chain reaction (RT-qPCR)

RNA extraction, reverse transcription, and quantitative real-time PCR were performed as we described previously [19]. Briefly, total RNA (0.5 μg) was reverse-transcribed into cDNA using an M-MLV Reverse Transcriptase kit (Invitrogen, Carlsbad, CA). Real-time RT-PCR amplification was carried on an ABI 7500 Fast (Applied Biosystems, Foster City, CA) using SYBR Premix Ex Taq™ kit (Takara, Dalian, China). The PCR primers were CTCCGTGTCCTGTAAATCTGC and TCTGACCTGAACCATAACGCA for matrix metallopeptidase 9 (MMP9), CCTGTTCACGAGAAGGAACC and GGGAGCAGGGCTACTACACA for calcitonin receptor (Calcr), CACCCTTAGTCTTCCGCTCAC and CCCACATCCTGCTGTTGAGAA for cathepsin K (Cstk), TCCCCTGAAAAGCACCTGAC and CTGCCTGTGTAGCCATCTGT for TNF receptor superfamily member

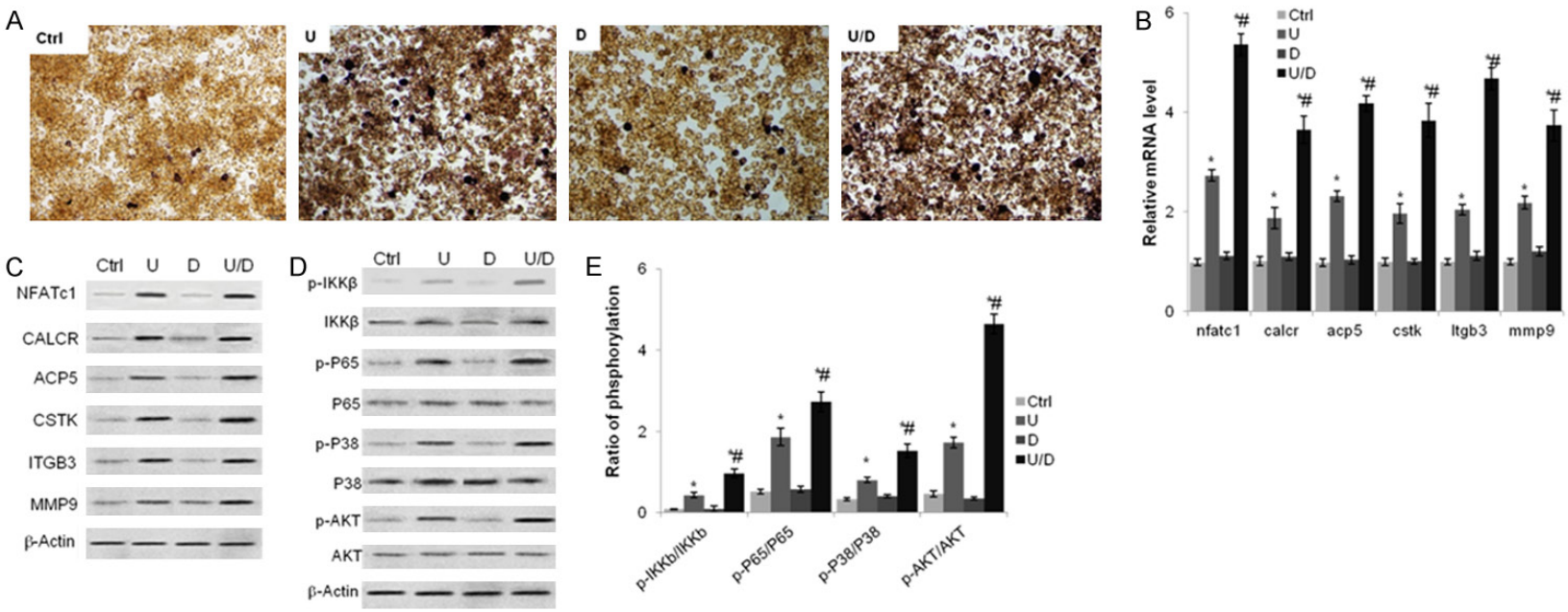

Figure 1. DC 2.4 cells enhanced UHMWPE particles induced osteoclastogenic responses of RAW264.7 cells. (A) TRAP staining of RAW264.7 cells treated with or with UHMWPE particles in the presence or absence of DC 2.4 cells (200×). The mRNA (B) and protein (C) levels of Nfatc1, Calcr, Acp5, Cstk, Itgb3, and Mmp9 were assessed by qPCR and western blot. (D) The phosphorylated and total IKKβ, NF-κB p65, p38MAPK, and AKT were assessed by western blot. (E) Quantitative analyses of the ratio between phosphorylated and total IKKβ, NF-κB p65, p38MAPK, and AKT proteins. Ctrl, vehicle control; U, UHMWPE particles (cell/particle ratio at 1:300); D, DC2.4 cells (1/10 of RAW264.7). Data expressed as mean ± standard deviation from three independent experiments. *, $P < 0.05$ compared to Ctrl; #, $P < 0.05$ compared to U.

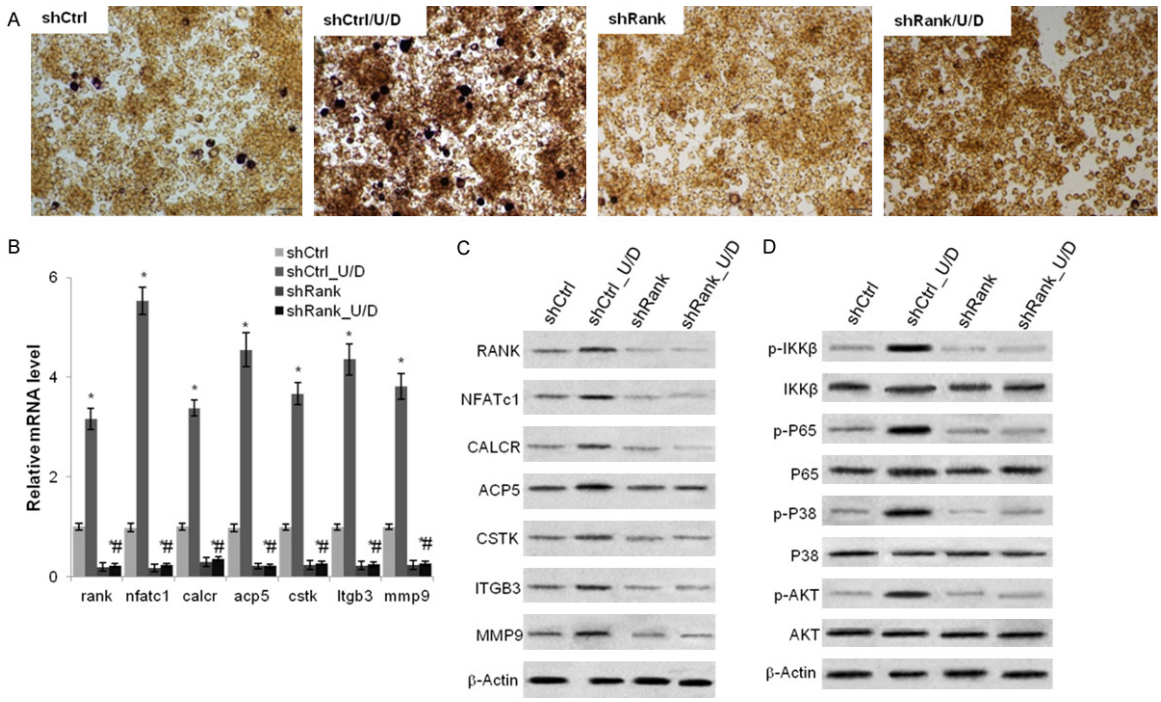

Figure 2. Silencing Rank expression abolished UHMWPE particles and DC 2.4 cells induced osteoclastogenic responses of RAW264.7 macrophages. (A) TRAP staining showed that RAW264.7-shRank almost did not have osteoclast after UHMWPE particles and DC 2.4 cells treatment whereas RAW264.7-shCtrl had large amount of osteoclasts upon induction. The upregulation of mRNA (B) and protein (C) levels of Nfatc1, Calcr, Acp5, Cstk, Itgb3, and Mmp9 upon UHMWPE particles and DC 2.4 cells treatment was lost in RAW264.7-shRank cells. (D) Western blot showed that silencing Rank expression blocked UHMWPE particles and DC 2.4 cells induced activation of IKKβ, NF-κB p65, p38MAPK, and AKT in RAW264.7 macrophages. U, UHMWPE particles (cell/particle ratio at 1:300); D, DC2.4 cells (1/10 of RAW264.7). Data expressed as mean ± standard deviation from three independent experiments. *, $P < 0.05$ compared to Ctrl; #, $P < 0.05$ compared to U.

11a (Rank), ACTTCGATTTCCTCTTCGAGTTC and GGTGACACTAGGGGACACATAA for Nuclear factor of active T cells1 (NFATc1), AAGTGGAACATTGAGGACAAAGG and CACAAATCTCAGGGTGGGAGTG for acid phosphatase 5, tartrate resistant (Acp5), CTTGGCTTAGGTGGTGGGAG and CCCCACTCTACTCTCACGGA for integrin β3 (Itgb3), and CCGAGAATGGGAAGCTTGTC and AAGCACCAACGAGAGGAGAA for glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The relative gene expression level was calculated with $2^{-\Delta\Delta Ct}$ method using GAPDH as internal control.

Western blot

For gene expression, RAW264.7 macrophages were culture in plates coated with or without UHMWPE particles [19] combined with specified treatments for 24 hr before collected for protein extraction whereas RAW264.7 cells were treated for 45 min for protein phosphorylation analyses. Western blot was performed as we described earlier [19]. The primary antibodies against MMP-9 (ab194314), TRAP (ab133238), cathepsin K (ab19027), calcitonin receptor (ab11042), Integrin β3 (ab38460), NFATc1 (ab3447), RANK (ab13918), and β-Actin (ab8226) were purchased from Abcam (Shanghai, China). Antibodies against NF-κB p65 (#6956), phospho-NF-κB p65 (#3036), p-IKKβ (#2694), IKKβ (#2684), p-p38MAPK (#9216), p38 (#9212), AKT (#4685), and p-AKT (#9271) were purchased from Cell Signaling (Danvers, MA).

Statistical analysis

Every experiment was independently carried out at least three times with more than three repeats each time. The data was expressed as mean ± standard deviation. Statistical analyses were done using Graphpad 6 and the differences between treated groups and control were assessed using student t-test. It was con-

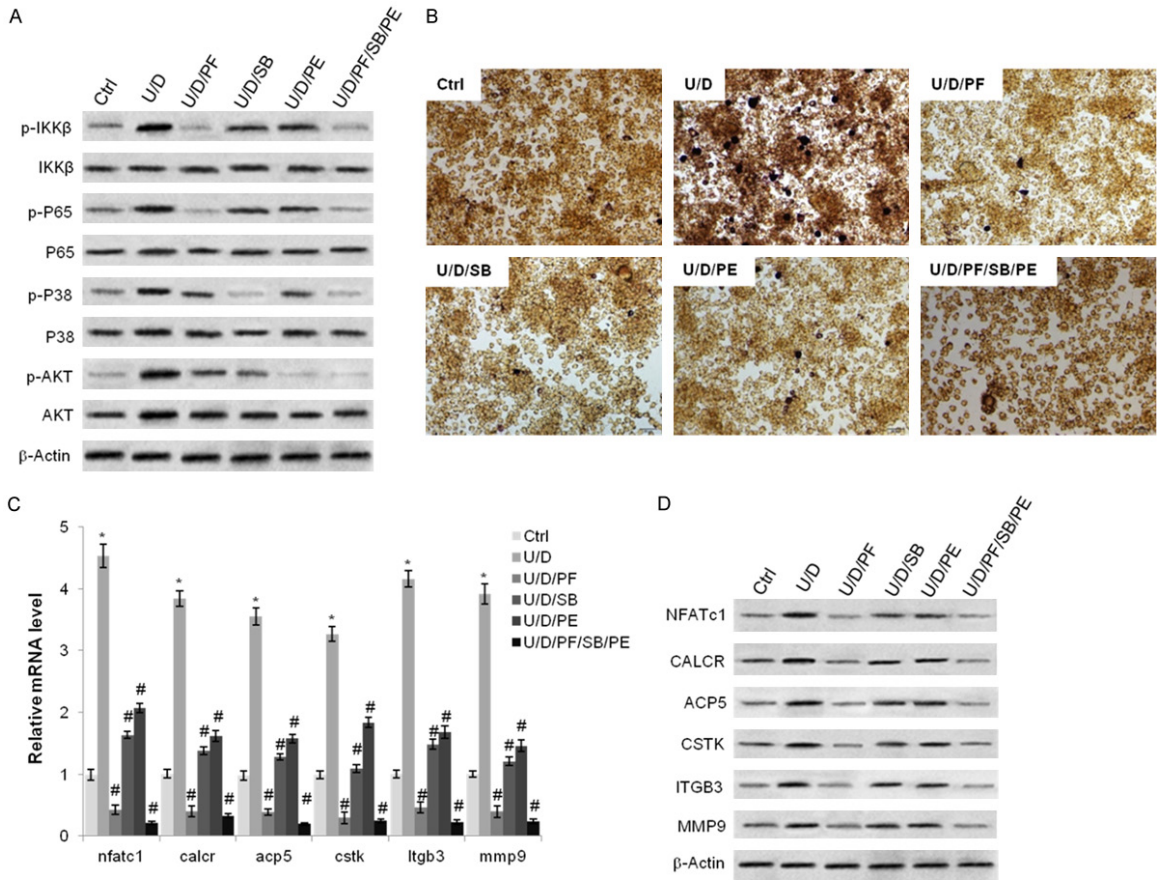

Figure 3. Inhibition of NF-κB, p38MAPK, and AKT activation blocked or weakened UHMWPE particles and DC 2.4 cells induced osteoclastogenic responses in RAW264.7 macrophages. (A) Western blot showed the inhibition of UHMWPE particles and DC 2.4 cells induced activation of IKKβ/NF-κB p65, p38MAPK, and AKT in RAW264.7 macrophages by PF184, SB203580, and Perifosine, respectively. (B) TRAP staining showed that PF184, SB203580, and Perifosine significantly reduced the number of RAW264.7-differentiated osteoclasts after UHMWPE particles and DC 2.4 cells treatments. The upregulation of mRNA (C) and protein (D) levels of Nfatc1, Calcr, Acp5, Cstk, Itgb3, and Mmp9 in RAW264.7 macrophages upon UHMWPE particles and DC 2.4 cells treatment was inhibited by PF184, SB203580, and Perifosine. U, UHMWPE particles (cell/particle ratio at 1:300); D, DC2.4 cells (1/10 of RAW264.7); PF, PF184; SB, SB203580; PE, Perifosine. Data expressed as mean ± standard deviation from three independent experiments. *, $P < 0.05$ compared to Ctrl; #, $P < 0.05$ compared to U.

sidered statistically significant when a *p* value was less than 0.05.

Results

UHMWPE wear particles and dendritic cells cooperatively induced osteoclastogenesis in RAW264.7 macrophages by activating MAPK and AKT pathways

TRAP staining showed that UHMWPE wear particles induced significant osteoclast differentiation from RAW264.7 macrophages, which was strongly enhanced by the coculturing of dendritic cells DC2.4 even though DC2.4 coculturing did not induce obvious osteoclast differentiation by itself (**Figure 1A**). Consistently, the mRNA (**Figure 1B**) and protein (**Figure 1C**) levels of master regulator Nfatc1 and osteoclast marker genes Calcr, Acp5, Cstk, Itgb3, and Mmp9 were substantially increased by UHMWPE particles and further elevated by dendritic cell coculturing in the presence of UHMWPE particles.

UHMWPE wear particles promoted the activation of p38MAPK, AKT, and IKKβ/NF-κB pathways in RAW264.7 cells (**Figure 1D** and **1E**). After 45 min treatment with UHMWPE particle alone or with DC2.4 co-culturing, the phosphorylation of IKKβ, NF-κB p65, p38MAPK, and AKT were markedly increased compared to

RAW264.7 treated with vehicle or co-cultured with DC2.4 alone (**Figure 1D** and **1E**).

RANK/RANKL pathway was required for the osteoclastogenesis induced by UHMWPE alone and cooperatively with DC2.4

Next we tested whether RANK/RANKL signaling pathway was required for UHMEPE wear particles alone or cooperatively with dendritic cells induced osteoclastogenesis of RAW264.7 macrophages. Rank expression was silenced by shRNA targeting nucleotides 972 to 992 of mouse rank gene (NM_009399) compared to scrambled shRNA (shCtrl) (**Figure 2B** and **2C**). Consequently, the expression levels of Nfatc1, Calcr, Acp5, Cstk, Itgb3, and Mmp9 were drastically reduced in RAW264.7-shRank cells compared to RAW264.7-shCtrl cells (**Figure 2B** and **2C**). Moreover, RAW264.7-shRank macrophages greatly lost osteoclastogenic responses when treated with UHMWPE particles in conjunction with DC2.4 coculturing (**Figure 2A**). UHMWPE wear particles and DC2.4 induced phsophorylation of p38MAPK, AKT, IKKβ, and NF-κB was blocked in Rank-silenced RAW264.7 cells (**Figure 2D**).

Inhibition of NF-κB, p38MAPK, and AKT activation attenuated UHMWPE and DC2.4 induced osteoclastogenesis of RAW264.7 macrophages

As p38MAPK, AKT, and NF-κB were activated in RAW264.7 macrophages by UHMWPE particles and DC2.4 coculturing, we next examined the effects of the blockade of these signaling pathways on the osteoclastogenesis. The activation of IKKκ/NF-κB, p38MAPK, and AKT in RAW264.7 by UHMWPE particles and DC2.4 was inhibited by PF184, SB203580, and Perifosine, respectively (**Figure 3A**), which in turn led to drastic reduction of osteoclasts differentiated from RAW264.7 cells induced by UHMWPE wear particles and DC2.4 coculturing (**Figure 3B**). The combination of PF184, SB203580, and Perifosine almost completely blocked UHMWPE wear particles and DC2.4 coculturing induced osteoblast differentiation of RAW264.7 macrophages (**Figure 3B**). The expression levels of Nfatc1, Calcr, Acp5, Cstk, Itgb3, and Mmp9 were also significantly inhibited by blockade of p38MAPK, AKT and NF-κB activation (**Figure 3C** and **3D**).

Discussion

UHMWPE wear particles and co-cultured dendritic cells cooperatively promoted osteoclastogenesis of RAW264.7 macrophages with activation of p38MAPK, AKT and IKKβ/NF-κB signaling pathways and upregulation of osteoclastogenic genes. Using shRNA silencing Rank expression stopped osteoclastogenic responses of RAW264.7 cells upon the induction of UHMWPE particles and DC2.4 cells. Moreover, inhibitors of IKKβ/NF-κB, p38MAPK, and AKT (PF184, SB203580, and Perifosine, respectively) substantially inhibited UHMWPE particles and DC2.4 cells induced osteoclast differentiation of RAW264.7 macrophages and upregulation of osteoclastogenic genes.

We and others have demonstrated that dendritic cells augmented UHMWPE wear particles-induced inflammatory and osteoclatogenic responses [19, 20]. The activation of NF-κB pathway was at the center of wear particle-induced inflammatory cytokines and chemokines production by macrophages and periprosthetic osteolysis [19-21]. UHMWPE wear particles induced activation of NF-κB was dependent on receptors expressed by macrophages [22, 23]. RANK was required for UHMWPE-induced osteoclastogenesis as UHMWPE particles failed to induce osteoclast differentiation and bone resorption in $RANK^{-/-}$ mice despite strong inflammatory response [23], which was consistent with the current data that silencing Rank expression in RAW264.7 macrophages abolished UHMWPE particles and DC2.4 induced osteoclastogenesis. On the other hand, UHMWPE particles stimulated RANKL expression at both low and high concentration (cell/particle ratios 1:100 and 1:500) and downregulated osteoprotegerin (OPG) at high concentration in primary osteoblasts [24]. Moreover, UHMWPE particles exposure switched osteocytes from anabolic state to catabolic state and resulted in loss of osteocyte perilacunar bone [25]. These results showed that UHMWPE wear particles could impact both sides of the balance between osteogenesis and osteoclastogenesis.

UHMWPE particles caused significantly increased RANKL level and decreased OPG level [24] led to the promotion of osteoclast differen-

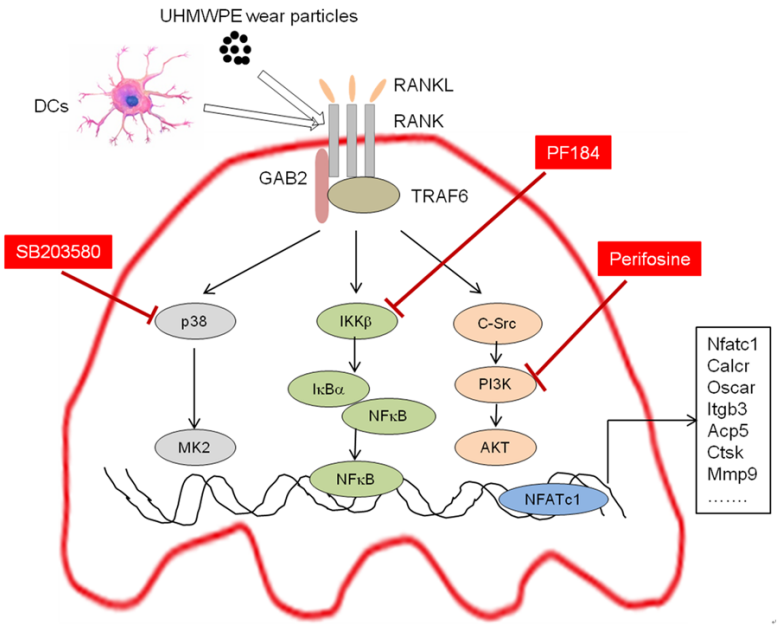

Figure 4. A proposed working model for UHMWPE particles and DC 2.4 cells-induced osteoclastogenesis. Refer to the text for details.

tiation via the RANK/RANKL/OPG system [26]. Studies have shown that the RANK/RANKL/OPG system plays an indispensable role in bone resorption induced by UHMWPE particles and activation of inflammatory responses only was not enough to promote osteoclastogenesis [23, 24]. Simultaneously activation of a host of RANKL/RANK downstream signaling pathways was a prerequisite for UHMWPE particle-induced osteoclast differentiation and osteolysis, which could be inhibited by blocking any o f the signaling pathways [5, 6, 9, 11, 22]. Using shRNA targeting RANK and using specific inhibitors we demonstrated that among others, p38MAPK, AKT, and NF-κB were activated in RAW264.7 macrophages downstream of RANKL/RANK signaling by concurrent treatment of UHMWPE particles and DC2.4 co-culture. NF-κB was the main player mediating RANKL/RANK regulation of osteoclastogenic gene expression besides its role in inflammatory responses [21] whereas PI3K/AKT [27] and p38MAPK [28] were participated in mediating RANKL/RANK signaling to promote osteoclast differentiation.

Taken together, the cooperative effects of UHMWPE particles and DC2.4 dendritic cells on osteoclastogenensis were through RANK receptor on RAW264.7 macrophages, which further activated downstream signaling network including p38MAPK, AKT, and NF-κB among other players. The osteoclastogenic effects of UHMWPE particles and DC2.4 were abolished by silencing Rank expression or inhibited by specific inhibitors against p38MAPK (SB-203580), AKT (Perifosine), and NF-κB (PF184) (**Figure 4**), indicating the complexity of UHMWPE-induced periprosthetic osteolysis.

Acknowledgements

This work was supported by National Natural Science Foundation of China (816-72184) and Six Talent Peak Projects of Jiangsu Province (2015-WSN-065).

Disclosure of conflict of interest

None.

Address correspondence to: Fengchao Zhao, Department of Orthopedics, Affiliate Hospital of Xuzhou Medical University, 99 West Huaihai Road, Xuzhou 221006, Jiangsu, China. Tel: +86-516-85802116; Fax: +86-516-85802120; E-mail: zhjianzh@sina.com

References

[1] Ansari F, Ries MD and Pruitt L. Effect of processing, sterilization and crosslinking on UHMWPE fatigue fracture and fatigue wear mechanisms in joint arthroplasty. J Mech Behav Biomed Mater 2016; 53: 329-340.

[2] Slouf M, Kotek J, Baldrian J, Kovarova J, Fencl J, Bouda T and Janigova I. Comparison of one-step and sequentially irradiated ultra-high molecular weight polyethylene for total joint replacements. J Biomed Mater Res Part B: Appl Biomater 2013; 101B: 414-422.

[3] Sieving A, Wu B, Mayton L, Nasser S and Wooley PH. Morphological characteristics of total joint arthroplasty-derived ultra-high molecular weight polyethylene (UHMWPE) wear debris that provoke inflammation in a murine model of inflammation. J Biomed Mater Res A 2003; 64: 457-464.

[4] Ingram JH, Stone M, Fisher J and Ingham E. The influence of molecular weight, crosslinking

and counterface roughness on TNF-alpha production by macrophages in response to ultra high molecular weight polyethylene particles. Biomaterials 2004; 25: 3511-3522.
[5] Jiang J, Jia T, Gong W, Ning B, Wooley PH and Yang SY. Macrophage polarization in IL-10 treatment of particle-induced inflammation and osteolysis. Am J Pathol 2016; 186: 57-66.
[6] Jiang X, Sato T, Yao Z, Keeney M, Pajarinen J, Lin TH, Loi F, Egashira K, Goodman S and Yang F. Local delivery of mutant CCL2 protein-reduced orthopaedic implant wear particle-induced osteolysis and inflammation in vivo. J Orthop Res 2016; 34: 58-64.
[7] Ren PG, Irani A, Huang Z, Ma T, Biswal S and Goodman SB. Continuous infusion of UHMWPE particles induces increased bone macrophages and osteolysis. Clin Orthop Relat Res 2011; 469: 113-122.
[8] Neuerburg C, Loer T, Mittlmeier L, Polan C, Farkas Z, Holdt LM, Utzschneider S, Schwiesau J, Grupp TM, Böcker W, Aszodi A, Wedemeyer C and Kammerlander C. Impact of vitamin E-blended UHMWPE wear particles on the osseous microenvironment in polyethylene particle-induced osteolysis. Int J Mol Med 2016; 38: 1652-1660.
[9] Zaveri TD, Dolgova NV, Lewis JS, Hamaker K, Clare-Salzler MJ and Keselowsky BG. Macrophage integrins modulate response to ultra-high molecular weight polyethylene particles and direct particle-induced osteolysis. Biomaterials 2017; 115: 128-140.
[10] Ren PG, Huang Z, Ma T, Biswal S, Smith RL and Goodman SB. Surveillance of systemic trafficking of macrophages induced by UHMWPE particles in nude mice by noninvasive imaging. J Biomed Mater Res A 2010; 94: 706-711.
[11] Yao Z, Keeney M, Lin TH, Pajarinen J, Barcay K, Waters H, Egashira K, Yang F and Goodman S. Mutant monocyte chemoattractant protein 1 protein attenuates migration of and inflammatory cytokine release by macrophages exposed to orthopedic implant wear particles. J Biomed Mater Res A 2014; 102: 3291-3297.
[12] Harris WH. Wear and periprosthetic osteolysis: the problem. Clin Orthop Relat Res 2001; 393: 66-70.
[13] Shanbhag AS, Hasselman CT and Rubash HE. The John Charnley Award. Inhibition of wear debris mediated osteolysis in a canine total hip arthroplasty model. Clin Orthop Relat Res 1997; 344: 33-43.
[14] Udagawa N, Takahashi N, Akatsu T, Tanaka H, Sasaki T, Nishihara T, Koga T, Martin TJ and Suda T. Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. Proc Natl Acad Sci U S A 1990; 87: 7260-7264.
[15] Boyle WJ, Simonet WS and Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423: 337-342.
[16] Asagiri M and Takayanagi H. The molecular understanding of osteoclast differentiation. Bone 2007; 40: 251-264.
[17] Kim JH and Kim N. Regulation of NFATc1 in osteoclast differentiation. J Bone Metab 2014; 21: 233-241.
[18] Sartori M, Vincenzi F, Ravani A, Cepollaro S, Martini L, Varani K, Fini M and Tschon M. RAW264.7 co-cultured with ultra-high molecular weight polyethylene particles spontaneously differentiate into osteoclasts: an in vitro model of periprosthetic osteolysis. J Biomed Mater Res A 2017; 105: 510-520.
[19] Cang D, Guo K and Zhao F. Dendritic cells enhance UHMWPE wear particle-induced osteoclast differentiation of macrophages. J Biomed Mater Res A 2015; 103: 3349-3354.
[20] Lin TH, Kao S, Sato T, Pajarinen J, Zhang R, Loi F, Goodman SB and Yao Z. Exposure of polyethylene particles induces interferon-γ expression in a natural killer T lymphocyte and dendritic cell coculture system in vitro: a preliminary study. J Biomed Mater Res A 2015; 103: 71-75.
[21] Lin TH, Yao Z, Sato T, Keeney M, Li C, Pajarinen J, Yang F, Egashira K and Goodman SB. Suppression of wear-particle-induced pro-inflammatory cytokine and chemokine production in macrophages via NF-κB decoy oligodeoxynucleotide: a preliminary report. Acta Biomater 2014; 10: 3747-3755.
[22] Gibon E, Ma T, Ren PG, Fritton K, Biswal S, Yao Z, Smith L and Goodman SB. Selective inhibition of the MCP-1-CCR2 ligand-receptor axis decreases systemic trafficking of macrophages in the presence of UHMWPE particles. J Orthop Res 2012; 30: 547-553.
[23] Ren W, Wu B, Peng X, Hua J, Hao HN and Wooley PH. Implant wear induces inflammation, but not osteoclastic bone resorption, in RANK(-/-) mice. J Orthop Res 2006; 24: 1575-1586.
[24] Kauther MD, Xu J and Wedemeyer C. Alpha-calcitonin gene-related peptide can reverse the catabolic influence of UHMWPE particles on RANKL expression in primary human osteoblasts. Int J Biol Sci 2010; 6: 525-536.
[25] Ormsby RT, Cantley M, Kogawa M, Solomon LB, Haynes DR, Findlay DM and Atkins GJ. Evidence that osteocyte perilacunar remodelling contributes to polyethylene wear particle induced osteolysis. Acta Biomater 2016; 33: 242-251.
[26] Tyrovola JB. The "Mechanostat Theory" of frost and the OPG/RANKL/RANK system. J Cell Biochem 2015; 116: 2724-2729.

[27] Wu M, Chen W, Lu Y, Zhu G, Hao L and Li YP. Gα13 negatively controls osteoclastogenesis through inhibition of the Akt-GSK3β-NFATc1 signalling pathway. Nat Commun 2017; 8: 13700.

[28] Lee K, Chung YH, Ahn H, Kim H, Rho J and Jeong D. Selective regulation of MAPK signaling mediates RANKL-dependent osteoclast differentiation. Int J Biol Sci 2016; 12: 235-245.