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Research Paper

Tumor derived EDIL3 modulates the expansion and osteoclastogenesis of myeloid derived suppressor cells in murine breast cancer model

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ABSTRACT

Epidermal growth factor-like repeats and discoidin I like domain 3 (EDIL3) is an integrin ligand which is implicated in bone metabolism and bone marrow myelopoiesis. Recently, myeloid derived suppressor cells (MDSCs) as osteoclast progenitor have been demonstrated in several kinds of cancers including breast cancer. In this paper we explored the association between tumor derived EDIL3 and MDSCs in a murine breast cancer model. Knockdown of EDIL3 in MDA-MB-231 breast cancer cells inhibited the expansion of tumor induced MDSCs in bone marrow. However, generation of bone marrow derived MDSCs in vitro was not affected by recombinant EDIL3. Osteoclastogenesis of MDSCs was dose-dependently inhibited by recombinant EDIL3 in vitro via binding to Mac-1 but not LFA-1. Moreover, in accordance with previous studies, our data showed that tumor derived EDIL3 was involved in tumor associated bone loss. The convoluted effects of EDIL3 on MDSCs compose a potential mechanism hired by tumor cells for perpetration approximately.

1. Introduction

In addition to various soluble factors, varied immune cells are also influential constituents of tumor microenvironment (TME) [1–3]. Among these immune cells, myeloid derived suppressor cells (MDSCs) are consider as the main components which are beneficial for tumor development and metastasis [4–6]. MDSCs attract huge attention because of their immunosuppressive trait as a heterogenetic population of immature myeloid cells [7,8]. Characterized by expression of both CD11b and Gr1 in tumor-bearing mice, MDSCs were reported to expand in spleen, blood, and bone marrow (BM) [9,10]. Recently, more and more evidences suggest that immunosuppressive function is not the only existed mechanism underlying the tumor promoting capacity of MDSCs [11]. Studies in immunodeficient mice have shown that MDSCs can assist tumor metastasis independent of their immunosuppressive function by promoting angiogenesis or modulating bone microenvironment [9,12–14]. In TME, MDSCs differentiation into normal myeloid cells is hampered [15–17], while an alternative that MDSCs can act as osteoclast progenitors is available [12,14,18]. However, the molecular mechanisms underlying MDSCs differentiation into osteoclasts remain unclear. Tumor cells and stromal cells are the major source of factors that determine the fate and function of MDSCs [6].

While the mortality of breast cancer has been brought down by development of early diagnosis and emergence of new therapy strategies [19–22], treating complications and improving life quality of patients become the most urgent need, especially for advanced breast cancer patients with metastatic bone diseases [23]. The interaction between tumor cells and osteoclasts plays a critical role in the process of bone metastasis, often resulting in bone loss and bone destruction [2]. After arriving at metastasis sites, tumor cells secrete various mediators inducing activation and differentiation of osteoclasts which contribute to bone resorption, and bone destruction releases cytokines and growth factors further promoting tumor cells growth [24–26]. As new osteoclast progenitors identified recently, MDSCs are also included in this notorious “vicious cycle” which has been introduced in numerous studies [14,18].

Epidermal growth factor (EGF)-like repeats and discoidin I-like domains 3 (EDIL3), also known as developmental endothelial locus-1 (Del-1), is composed of two discoidin I–like domains and three EGF-like repeats, the second of which contains an Arg-Gly-Asp (RGD) motif binding to integrins [27,28]. Targeting lymphocyte function-associated antigen (LFA-1; CD11a/CD18), a member of integrins family, EDIL3

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inhibits LFA-1-dependent recruitment of neutrophils linking to inflammatory bone loss [29]. Besides that, the interaction between EDIL3 and Macrophage-1 antigen (Mac-1; CD11b/CD18), also named as integrin $\alpha_M\beta_2$, impairs complement-dependent phagocytosis and modulates osteoclastogenesis, in inflammatory disease [30,31]. In breast cancer, EDIL3 is associated with the diagnosis and outcome of patients. High expression of EDIL3 is related to early breast cancer with worse survival trend, especially in triple-negative breast cancer(TNBC) [32–34]. Moreover, EDIL3 secreted by cellular ingredients of hematopoietic stem cells (HSCs) niche is indispensable to myelopoiesis in both physiologic and pathologic conditions [35].

Given that MDSCs were identified as novel osteoclast progenitors, we hypothesized that tumor cells derived EDIL3 regulated MDSCs expansion and affected MDSCs differentiation into osteoclast. In this work, we knocked down the expression of EDIL3 in MDA-MB-231, and then a reduction of MDSCs expansion in bone marrow was observed in a breast cancer metastasis model. But we failed to promote generation of MDSCs in vitro with recombinant EDIL3. We also found that EDIL3 reduced osteocalstogenesis of BM derived MDSCs under osteoclastogenic conditions via interaction with Mac-1 but not LFA-1. Furthermore, knockdown of EDIL3 in tumor cells promoted osteoclast differentiation and increased bone mass loss. Hence, our data suggest that EDIL3 may be an important factor employed by tumor cells to encourage MDSCs accumulation in tumor bearing host.

2. Material and methods

2.1. Cell cultures

A bone-trophic MDA-MB-231 (fLuc) cell line is a kind gift from Dr. Guohong Hu (Chinese Academy of Sciences) that has been frequently used by us and many other groups [36,37]. Cells was cultivated in DMEM (Hyclone, Logan, Utah, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, Utah, USA) and 1% penicillin/streptomycin (Mediatech, Manassas, VA, USA).

2.2. Animals

All procedures were approved by the institutional Ethics Committee on Ethics of Biomedicine, Second Military Medical University. Tumor cells were resuspended in PBS at a final concentration of 1×10^6 cells/ml. Nude mice were anesthetized with isoflurane. One $\times 10^5$ tumour cells were injected into mice via the intracardiac route as described previously [18,37]. Bone metastasis were observed by noninvasive luciferase imaging, after 12–14 days.

2.3. Flow cytometric analysis

BM cells were flushed from femurs with PBS containing 2% FBS (Hyclone, Logan, UT, USA). Single cell suspensions were prepared by using a 70 μm filter and then red blood cells were deleted by incubating with red blood cells lysis buffer (Biolegend, San Diego, CA). Cells were incubated with Gr1-PE and CD11b-FITC fluorescence conjugated antibodies (Biolegend, San Diego, CA) for 20 min at room temper. Analysis was performed on a flow cytometer BD FACSCalibur (Becton Dickinson).Data was analysed by using BD CellQuest Pro software. For BM derived MDSCs sorting, BM cells were labelled with anti-Gr1-PE antibody and followed by incubating with anti-PE magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and then MDSCs were isolated by running the cell samples on AutoMACS (Miltenyi Biotec).

2.4. ShRNA transfection

For knockdown of EDIL3 in MDA-MB-231 cell line, lentiviral vectors encoding shEDIL3 and control shRNA were used to target EDIL3 mRNA. A pool of three lentiviral shRNAs targeting human EDIL3 were transfected into tumor cells via using Xfect transfection reagent (CloneTech, Mountain View, CA) following the manufacturer's instructions and selected by puromycin treatment.

2.5. In vitro osteoclast differentiation

One $\times 10^5$ BM derived MDSCs were seed into 96-well plates and cultured in α-minimum essential medium (αMEM) supplemented with 10% FBS, 25 ng/mL recombinant murine macrophage-colony stimulating factor (M-CSF) (R&D Systems, UK) and 50 ng/mL receptor activator of NF-κB ligand (RANKL) (R&D Systems, Minneapolis, MN, USA), in the presence of increasing concentration of recombinant EDIL3 (R&D systems, Abingdon, UK). Media was replaced every 3 days and cells were maintained for up to 15 days for differentiation. Tartrate-resistant acid phosphatase (TRAP) staining was performed using TRAP kit (Sigma-Aldrich, St Louis, Missouri, USA) followed the manufacturer's procedure. TRAP positive cells were counted in each well (cells containing more than 3 nuclei were considered as TRAP positive cells). And Nine sections were counted in each separate experiment. Slides were viewed using a Nikon Eclipse Ni-E microscope. For some assays, MDSCs were pre-treated with antibody against CD11b or CD11a (Biolegend, San Diego, CA) or isotype control (Biolegend, San Diego, CA) (10 μg/ml).

2.6. BM derived MDSCs inducing in vitro

To acquire MDSCs in vitro, BM cells were cultured in the presence of GM-CSF and IL-6 as described previously [38,39]. Briefly, BM cells were flushed from long bones of mice. Red blood cells were lysed using red blood cell lysing buffer and then 2.5×10^6 cells were seeded into 100 mm diameter dishes (Corning) in α-MEM supplemented with combinations of GM-CSF (40 ng/ml) and IL-6 (40 ng/ml) cytokines (R&D, Minneapolis, MN). For some groups, BM cells were exposed to increasing concentrations of recombinant EDIL3 (rEDIL3). Cells were maintained at 37 °C in 5% CO2-humidified atmosphere for 4 days and analysed by flow cytometer as described above.

2.7. Quantitative RT-PCR

Total RNA was extracted from cells using TRIzol reagent (Takara, Japan). RNA was reversetranscribed into cDNA with PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, Japan). Real-time PCR was performed on a 7300 Real-Time PCR System (Applied Biosystems) using a Power SYBR Green PCR Master Mix (Applied Biosystems; Foster City, CA, USA). Data were analyzed using the $2^{-\Delta\Delta CT}$ method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control to normalized the expression of target genes. The primer sequences we used here were present in Table S1.

2.8. Bone TRAP staining

Long bones were isolated from mouse bodies after they were killed and fixed in 10% formalin for 48 h at room temperature. Then bones were decalcified in 10% EDTA for 2 weeks at 4 °C and embedded in paraffin. Five μm-thick sections were stained by TRAP kit (Sigma-Aldrich) to evaluate osteoclast differentiation in accordance to the manufacturer's procedure. Stained bone sections were examined under a microscope and osteoclasts number was quantified using Metamorph software (Molecular Devices).

2.9. Western blot analysis

The tumor cells transfected or not with shRNA were lysed using RIPA Lysis Buffer supplemented with protein inhibitor on ice. Protein concentration was determined by the BCA Protein Assay Kit (Thermo Scientific). Protein was separated by SDS-PAGE and transferred on to

PVDF membranes (Millipore). after being blocking by 5% non-fat dry milk with Tris-buffered saline (TBST) for 1 h, the membranes were reacted with primary antibody, and then incubated with DyLight 800-conjugated secondary antibody and scanned with LI-COR Infrared Imaged Odyssey (Gene Company Ltd.). The primary antibodies used here are EDIL3 (Ag3274, 1:3000; Proteintech Group, Inc., Chicago, IL, USA) and GAPDH (2118, 1:1000; Cell Signalling Technology, Beverly, MA, USA).

2.10. Micro-CT analysis

To conduct Micro-CT analysis, the bone was scanned by X-ray microtomography (Skyscan 1076, Bruker microCT) at a 15-μm resolution. The results were analysed in accordance to the manufacturer's instructions. The tibial trabeculae of the proximal metaphysis below the growth plate (0.228–1.85 mm) was considered as the region of interest (ROI).

2.11. Statistics

Data were analyzed by ANOVA and the Dunnett's multiple-comparison test using GraphPad Prism 6 (GraphPad Software Inc.). Comparisons of two groups were performed by unpaired *t*-test. All data were presented as means ± SEM. $P < 0.05$ were considered to be statistically significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$.

3. Result

3.1. Tumor derived EDIL3 inhibits MDSCs expansion in vivo

High expression of EDIL3 is closely related to breast cancer and predicts worse outcome in especially triple-negative breast cancer (TNBC) [32]. To investigate the correlation between EDIL3 and MDSCs in breast cancer, EDIL3 knockdown was performed in MDA-MB-231 tumor cells and confirmed by western blotting (FIG S1). Then we inoculated immunodeficient nude mice with tumor cells via intracardiac route. Approximate two weeks after inoculation, mice were sacrificed and BM cells were flushed from long bones and incubated with CD11b-FITC and Gr1-PE fluorescence conjugated antibodies. $CD11b^+/Gr1^+$ cells expansion was analysed by FACS. MDSCs expansion was observed in tumor bearing mice as reported previously, that MDSCs reach more than 60% of total cells, while the ratio of MDSCs account for approximate 40% of total cells in normal mice. However, this ratio decreased significantly in mice inoculated with MDA-MB-231 cells, in which EDIL3 was knocked down (Fig. 1A and B). These data indicate that EDIL3 is beneficial for MDSCs expansion in this murine breast cancer model.

3.2. EDIL3 fails to promote MDSCs differentiation in vitro

It has been reported previously that MDSCs can be generated in vitro from BM cells in the presence of GM-CSF + IL-6 combination [38,39]. Based on the results obtained in vivo, we further investigated whether EDIL3 could promote MDSCs differentiation in vitro. Increasing concentrations of recombinant EDIL3 (rEDIL3) were added to BM cells cultures in the presence of GM-CSF + IL-6 combination to evaluate the effect of rEDIL3 on MDSCs generation in vitro. Beyond our expectation, no difference in the ratio of $CD11b^+/Gr1^+$ cells to total cultured BM cells was observed between non-rEDIL3 group and rEDIL3 affected groups (Fig. 2A and B). Our results suggested that EDIL3 failed to enhance the expression of both CD11b and Gr1 markers in BM cells cultured with GM-CSF + IL-6 combination.

3.3. EDIL3 decreases tumor induced MDSCs differentiation into osteoclasts in vitro

EDIL3 is a crucial factor involved in osteoclast differentiation in inflammatory disease [28,29,31]. Here, we focused on the effect of EDIL3 on tumor induced MDSCs as osteoclast progenitor cells. Using MACS sorting, we isolated BM derived MDSCs from mice bearing MDA-MB-231 tumor cells. The purity of $CD11b^+/Gr1^+$ cells met the requirements of following assays (FIG S2). MDSCs were stimulated by increasing concentrations of rEDIL3 under osteoclastogenic condition and TRAP straining was performed to evaluate osteoclastogenesis. We found that MDSCs differentiation into osteoclasts was dose-dependently inhibited by rEDIL3 (Fig. 3A and B). We further examined the expression of nuclear factor of activated T cells c1 (NFATc1), calcitonin receptor (CTR), cathepsin K, and TRAP which are osteoclast differentiation and functional markers [30], and found that the expression of these markers was also inhibited (Fig. 3C), consistent with results of TRAP straining. Therefore, EDIL3 negatively regulates MDSCs differentiation into osteoclasts in vitro.

3.4. EDIL3 inhibits MDSCs differentiation toward osteoclast via binding to Mac-1 (CD11b/CD18) but not LFA-1 (CD11a/CD18)

Previous study has reported that EDIL3 suppress NFATc1 by binding to Mac-1 but not LFA-1 in murine RAW264.7 macrophages [30]. NFATc1 is considered as a key transcriptional factor required for osteoclastogenesis and modulates expression of many genes involved in osteoclast differentiation and function [40]. So we hypothesized that EDIL3 inhibited MDSCs differentiation toward osteoclast follow the same mechanism. Indeed, the effect of EDIL3 on NFATc1 expression was significantly inhibited by blocking CD11b but not CD11a (Fig. 4A). Because B cell lymphoma 6 (Bcl6) is a transcriptional repressor of NFATc1, we also examined Bcl6 expression and found Bcl6 expression was CD11b-dependently upregulated (Fig. 4B).

3.5. Diminished osteoclast differentiation is observed after down-regulating EDIL3 in tumor cells

EDIL3 was knocked down in MDA-MB-231 cells which were injected into nude mice via intracardiac route. Two weeks later, long bones were obtained from tumor bearing mice and the TRAP straining of tibia sections indicated that significantly increasing of osteoclast number occurred in mice inoculated with EDIL3-knockdown tumor cells compared with normal tumor cells (Fig. 5A and B). Moreover, micro-CT analysis were performed and we found that the trabecular bone volume

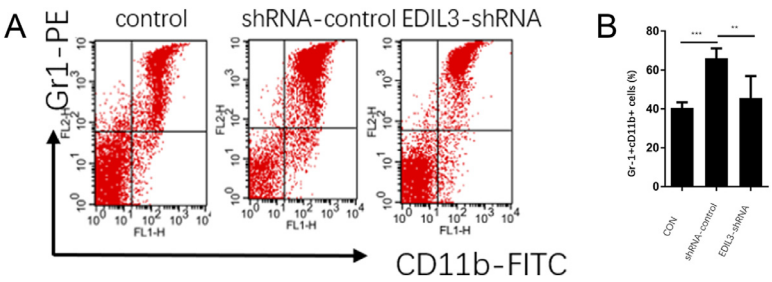

Fig. 1. Tumor derived EDIL3 inhibits MDSCs expansion in vivo. Nude mice were injected with MDA-MB-231 shEDIL3 cells or MDA-MB-231 shRNA control cells via intracardiac route. Normal MDA-MB-231 cells were set to be control. (A) BM cells were isolated and $CD11b^+/Gr1^+$ cells were analysed by flow cytometry. (B) Quantitative analysis of the expansion of $CD11b^+/Gr1^+$ cells in bone marrow of tumor bearing mice one month after tumor cells inoculation. All data are means ± SD (5 mice per group).

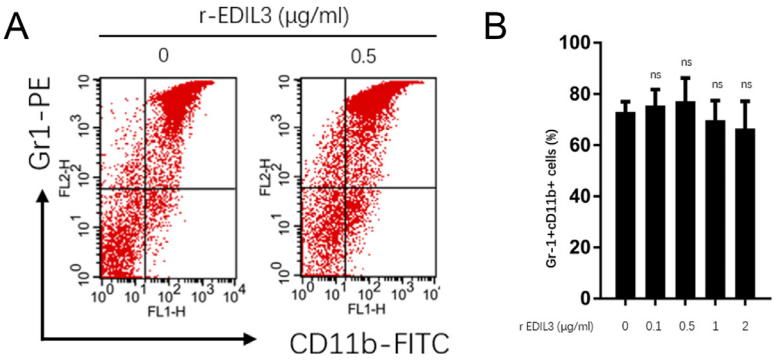

Fig. 2. The effect of EDIL3 on MDSCs generation from BM cells in vitro. (A) BM cells were cultured in medium supplemented with GM-CSF + IL-6 in the presence of recombinant EDIL3 or not for 4 days and then were analysed by flow cytometry. Only image of r-EDIL3 = 0.5 μg/ml was shown here for compare. (B) Quantitative analysis of $CD11b^+/Gr1^+$ cells. All data are means ± SD (n = 6).

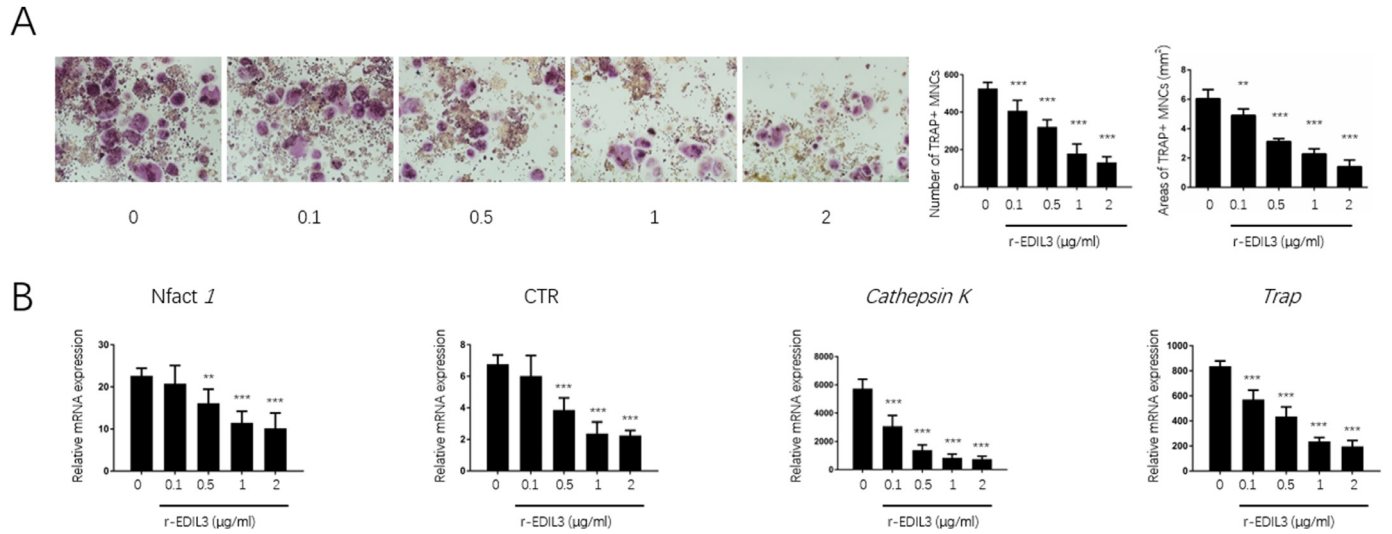

Fig. 3. EDIL3 dose-dependently inhibits osteoclastogenesis of MDSCs. (A) TRAP staining were performed. TRAP-positive cells as well as area of Trap-positive osteoclasts per field were counted. (B) Osteoclasts were harvested for mRNA expression of the osteoclast-specific genes by quantitative PCR. Results were normalized to those of β-actin mRNA and are presented relative to those of undifferentiated control. All data are means ± SD (n = 3).

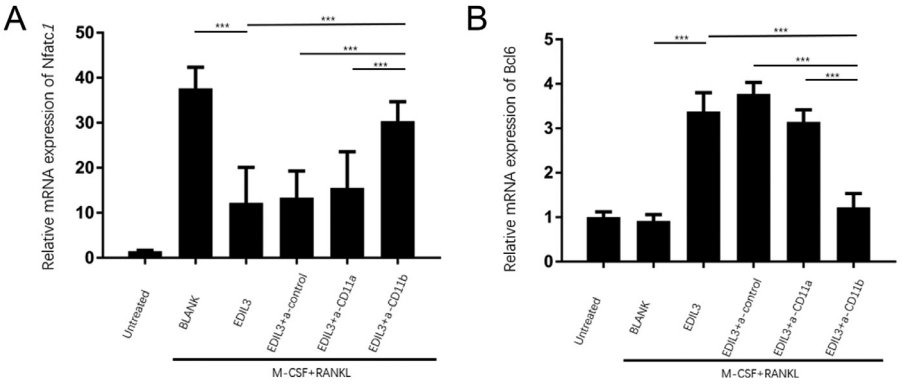

Fig. 4. EDIL3 regulates expression of Nfatc1 and Bcl6 via binding to Mac-1. Effect of r-EDIL3 (2 μg/ml) on Nfatc1 (A) and Bcl6 (B) mRNA expression during osteoclastogenesis of MDSCs which were pre-treated with antibody against CD11b or CD11a. Bcl6 and Nfatc1 expression was determined at 24 and 12 h, respectively. Data were normalized to β-actin mRNA and are relative to undifferentiated controls. All data are means ± SD (n = 5).

and trabecular thickness significantly decreased in EDIL3-knockdown tumor cells bearing mice Fig. 6, consistent with what we observed in TRAP straining of bone sections. In sum, osteoclast differentiation is suppressed by tumor derived EDIL3 in this breast cancer model.

4. Discussion

In this study, we demonstrated that tumor derived EDIL3 promoted MDSCs expansion in bone marrow. Consistent with previous study results [14,18], we observed that tumor induced MDSCs isolated from bone marrow could differentiate into osteoclasts under osteoclastogenic condition. Moreover, EDIL3 dose-dependently inhibited MDSCs differentiation into osteoclasts in vitro via binding to Mac-1 but not LFA-1. However, EDIL3 had no effect on generation of MDSCs in vitro from bone marrow cells. Our work aimed to explore the association between EDIL3 and BM-derived MDSCs in TME.

To our knowledge, most studies related to MDSCs in cancer prefer their immunosuppressive role [4,9]. Recently, MDSCs were introduced as osteoclast precursors in some studies which revealed another face of this heterogeneous population of myeloid cells [6,14]. In breast cancer, prior studies have demonstrated that bone marrow MDSCs were characterized by the capacity of differentiation into osteoclasts in a TME dependent manner [18]. These findings raise a question about the mechanism for manipulating osteoclastogenesis of MDSCs in TME.

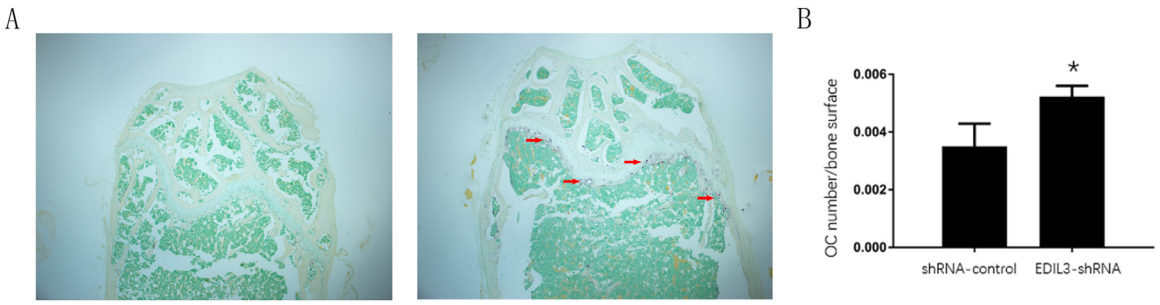

Fig. 5. Tumor derived EDIL3 decreases the osteoclast number in tumor bearing mice. (A) Representative images of TRAP staining on bone sections from mice inoculated with shEDIL3 MDA-MB-231 cells or control 4 weeks after sacrifice. Osteoclasts were marked by red arrow. (B) Quantitative analysis of the ratio bone surface/osteoclast number. (n = 8, two images each mice).

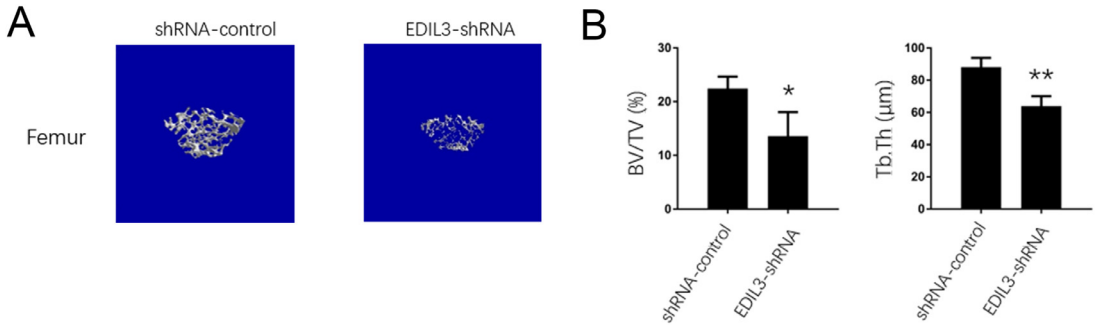

Fig. 6. Tumor derived EDIL3 increases bone mass. (A) Representative micro-CT images. (B) Bone mass (bone volume/total volume and trabecular thickness) was assessed by micro-CT analysis of left tibias from both groups. Data are presented as the mean ± SD. (n = 8, one image each mice).

Hence we supposed that some factors derived from tumor cells must be involved in this process. Indeed, our data indicate that EDIL3 plays a suppressive role in osteoclastogenesis of MDSCs in vitro. However, it has been demonstrated that EDIL3 is highly expressed in MDA-MB-231 tumor cells [32,33], and tumor cells induced osteoclastogenesis of MDSCs deteriorates bone loss [12]. Our observations that tumor derived EDIL3 inhibits osteoclastogenesis of MDSCs seem to be paradoxical with previous studies. The possible explanation is that endogenous EDIL3 is a homeostatic anti-inflammatory factor [27]. Moreover, osteoclastogenesis is indirectly regulated by tumor secreted hormones and directly regulated by osteoblasts derived RANKL in tumor-bearing host [24,25]. Interestingly, as an important component of HSCs niche, EDIL3 promotes osteoblasts differentiation [41], and the interaction between osteoblasts and MDSCs is needed to be illuminated further. One limitation of our study is that the validity of these results is based on in vitro condition, so it is not known whether our results are applicable to in vivo experiments.

Bone marrow is considered to be the main site of MDSCs accumulation in divers carcinomas including breast cancer [9]. In line with previous studies [42], we observed that MDSCs accounted for approximately 70% of total BM cells in a murine breast cancer model. We further found that knockdown of EDIL3 in tumor cells brought down the expansion of MDSCs in bone marrow. This result ties well with a previous study that EDIL3 promoted myelopoiesis of hematopoietic stem cells [35]. To confirm the role of EDIL3 in MDSCs generation from BM cells, we cultured BM cells in the medium supplemented with GM-CSF + IL-6 cytokines combination which was reported previously to foster MDSCs generation in vitro [38]. However, the results in vitro were not in accordance with our observation in vivo (Fig 2A and B). We speculate that this might be due to the imperfect simulation of HSCs niche in vitro with combination of GM-CSF + IL-6 cytokines. Actually, myelopoiesis under TME is complicated and varied factors implicate in tumor induced MDSCs differentiation, such as prostaglandin E2 (PGE2), interleukin 10 (IL10), IL-1β, stem cell factor (SCF), transforming growth factor (TGF)-β and vascular endothelial growth factor (VEGF) [4,6,9]. Therefore, possibly, tumor secreted EDIL3 promotes MDSCs expansion indirectly.

Inhibition of leukocyte-endothelial adhesion via binding to LFA-1 is one of the main functions of EDIL3 in inflammation-associated diseases [27,29]. Recently, it has been reported in RAW264.7 cells that EDIL3 suppress expression of NFATc1, which induce many osteoclast-specific genes expression, and then inhibited osteoclastogenesis via binding to Mac-1 but not LFA-1 [30]. In this study, our results (Fig. 4A) were consistent with those reported previously. Therefore, our data support the previous contention that MDSCs are a population of osteoclast precursors.

MDSCs direct differentiation into osteoclasts has been demonstrated previously in murine breast cancer model [18]. In addition, inflammatory disorders associated bone loss can be blocked by EDIL3 [29,30]. So we hypothesized that tumor derived EDIL3 possibly inhibited osteoclastogenesis and further impeded bone loss. Indeed, our results from micro CT and TRAP straining of bone sections support this speculation, despite we are not certain whether this effect is related to ablated osteoclastogenesis of MDSCs by EDIL3.

Previously, it was reported that high EDIL3 expression was associated with high invasive activity and metastasis of tumor cells as well as worse outcome [32]. As is known, MDSCs accelerate tumor development and metastasis by immune and non-immune mechanisms [4,11]. In summary, the present study showed that EDIL3 promoted MDSCs expansion but hampered their osteoclastogenesis. Moreover, the limitations of the present study are apparent, including the nude mouse model and single breast cancer cell line used here. It's urgent to investigate if the same result could be acquired in immune-competent mouse and other tumor cell lines in the future.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jbo.2019.100238.

References

[1] M. Binnewies, E.W. Roberts, K. Kersten, V. Chan, D.F. Fearon, M. Merad, L.M. Coussens, D.I. Gabrilovich, S. Ostrand-Rosenberg, C.C. Hedrick, R.H. Vonderheide, M.J. Pittet, R.K. Jain, W. Zou, T.K. Howcroft, E.C. Woodhouse, R.A. Weinberg, M.F. Krummel, Understanding the tumor immune microenvironment (TIME) for effective therapy, Nat. Med. 24 (5) (2018) 541–550.

[2] M. Futakuchi, K. Fukamachi, M. Suzui, Heterogeneity of tumor cells in the bone microenvironment: mechanisms and therapeutic targets for bone metastasis of prostate or breast cancer, Adv. Drug Deliv. Rev. 99 (Pt B) (2016) 206–211.

[3] J.M. Taube, J. Galon, L.M. Sholl, S.J. Rodig, T.R. Cottrell, N.A. Giraldo, A.S. Baras, S.S. Patel, R.A. Anders, D.L. Rimm, A. Cimino-Mathews, Implications of the tumor immune microenvironment for staging and therapeutics, Mod. Pathol. 31 (2) (2018) 214–234.

[4] V. Bronte, S. Brandau, S.H. Chen, M.P. Colombo, A.B. Frey, T.F. Greten, S. Mandruzzato, P.J. Murray, A. Ochoa, S. Ostrand-Rosenberg, P.C. Rodriguez, A. Sica, V. Umansky, R.H. Vonderheide, D.I. Gabrilovich, Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards, Nat. Commun. 7 (2016) 12150.

[5] C. Groth, X. Hu, R. Weber, V. Fleming, P. Altevogt, J. Utikal, V. Umansky, Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression, Br. J. Cancer 120 (1) (2019) 16–25.

[6] F. Veglia, M. Perego, D. Gabrilovich, Myeloid-derived suppressor cells coming of age, Nat. Immunol. 19 (2) (2018) 108–119.

[7] A. Pastaki Khoshbin, M. Eskian, M. Keshavarz-Fathi, N. Rezaei, Roles of myeloid-derived suppressor cells in cancer metastasis: immunosuppression and beyond, Arch. Immunol. Ther. Exp. (Warsz) 67 (2) (2019) 89–102.

[8] E. Safarzadeh, M. Orangi, H. Mohammadi, F. Babaie, B. Baradaran, Myeloid-derived suppressor cells: Important contributors to tumor progression and metastasis, J. Cell Physiol. 233 (4) (2018) 3024–3036.

[9] J.E. Talmadge, D.I. Gabrilovich, History of myeloid-derived suppressor cells, Nat. Rev. Cancer 13 (10) (2013) 739–752.

[10] L. Yang, C.M. Edwards, G.R. Mundy, Gr-1 + CD11b + myeloid-derived suppressor cells: formidable partners in tumor metastasis, J. Bone Miner. Res. 25 (8) (2010) 1701–1706.

[11] A. Calcinotto, C. Spataro, E. Zagato, D. Di Mitri, V. Gil, M. Crespo, G. De Bernardis, M. Losa, M. Mirenda, E. Pasquini, A. Rinaldi, S. Sumanasuriya, M.B. Lambros, A. Neeb, R. Luciano, C.A. Bravi, D. Nava-Rodrigues, D. Dolling, T. Prayer-Galetti, A. Ferreira, A. Briganti, A. Esposito, S. Barry, W. Yuan, A. Sharp, J. de Bono, A. Alimonti, IL-23 secreted by myeloid cells drives castration-resistant prostate cancer, Nature 559 (7714) (2018) 363–369.

[12] S. Danilin, A.R. Merkel, J.R. Johnson, R.W. Johnson, J.R. Edwards, J.A. Sterling, Myeloid-derived suppressor cells expand during breast cancer progression and promote tumor-induced bone destruction, Oncoimmunology 1 (9) (2012) 1484–1494.

[13] H. Qin, B. Lerman, I. Sakamaki, G. Wei, S.C. Cha, S.S. Rao, J. Qian, Y. Hailemichael, R. Nurieva, K.C. Dwyer, J. Roth, Q. Yi, W.W. Overwijk, L.W. Kwak, Generation of a new therapeutic peptide that depletes myeloid-derived suppressor cells in tumor-bearing mice, Nat. Med. 20 (6) (2014) 676–681.

[14] J. Zhuang, J. Zhang, S.T. Lwin, J.R. Edwards, C.M. Edwards, G.R. Mundy, X. Yang, Osteoclasts in multiple myeloma are derived from Gr-1 + CD11b + myeloid-derived suppressor cells, PLoS One 7 (11) (2012) e48871.

[15] D.R. Barreda, P.C. Hanington, M. Belosevic, Regulation of myeloid development and function by colony stimulating factors, Dev. Comp. Immunol. 28 (5) (2004) 509–554.

[16] F. Geissmann, M.G. Manz, S. Jung, M.H. Sieweke, M. Merad, K. Ley, Development of monocytes, macrophages, and dendritic cells, Science 327 (5966) (2010) 656–661.

[17] D. Marvel, D.I. Gabrilovich, Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected, J. Clin. Invest. 125 (9) (2015) 3356–3364.

[18] A. Sawant, J. Deshane, J. Jules, C.M. Lee, B.A. Harris, X. Feng, S. Ponnazhagan, Myeloid-derived suppressor cells function as novel osteoclast progenitors enhancing bone loss in breast cancer, Cancer Res. 73 (2) (2013) 672–682.

[19] E. Azizi, A.J. Carr, G. Plitas, A.E. Cornish, C. Konopacki, S. Prabhakaran, J. Nainys, K. Wu, V. Kiseliovas, M. Setty, K. Choi, R.M. Fromme, P. Dao, P.T. McKenney, R.C. Wasti, K. Kadaveru, L. Mazutis, A.Y. Rudensky, D. Pe'er, Single-cell map of diverse immune phenotypes in the breast tumor microenvironment, Cell 174 (5) (2018) 1293–1308 e36.

[20] M. Kwa, A. Makris, F.J. Esteva, Clinical utility of gene-expression signatures in early stage breast cancer, Nat. Rev. Clin. Oncol. 14 (10) (2017) 595–610.

[21] N.F. Ponde, D. Zardavas, M. Piccart, Progress in adjuvant systemic therapy for breast cancer, Nat. Rev. Clin. Oncol. 16 (1) (2019) 27–44.

[22] E.P. Mamounas, T. Kuehn, E.J.T. Rutgers, G. von Minckwitz, Current approach of the axilla in patients with early-stage breast cancer, The Lancet (2017).

[23] C. Cleeland, R. von Moos, M.S. Walker, Y. Wang, J. Gao, M. Chavez-MacGregor, A. Liede, J. Arellano, A. Balakumaran, Y. Qian, Burden of symptoms associated with development of metastatic bone disease in patients with breast cancer, Support Care Cancer 24 (8) (2016) 3557–3565.

[24] S.J. Coniglio, Role of tumor-derived chemokines in osteolytic bone metastasis, Front. Endocrinol. (Lausanne) 9 (2018) 313.

[25] K.L. Owen, B.S. Parker, Beyond the vicious cycle: the role of innate osteoimmunity, automimicry and tumor-inherent changes in dictating bone metastasis, Mol. Immunol. (2017).

[26] H. Yao, D.M. Veine, D.L. Livant, Therapeutic inhibition of breast cancer bone metastasis progression and lung colonization: breaking the vicious cycle by targeting alpha5beta1 integrin, Breast Cancer Res. Treat 157 (3) (2016) 489–501.

[27] E.Y. Choi, E. Chavakis, M.A. Czabanka, H.F. Langer, L. Fraemohs, M. Economopoulou, R.K. Kundu, A. Orlandi, Y.Y. Zheng, D.A. Prieto, C.M. Ballantyne, S.L. Constant, W.C. Aird, T. Papayannopoulou, C.G. Gahmberg, M.C. Udey, P. Vajkoczy, T. Quertermous, S. Dimmeler, C. Weber, T. Chavakis, Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment, Science 322 (5904) (2008) 1101–1104.

[28] A. Klotzsche-von Ameln, S. Cremer, J. Hoffmann, P. Schuster, S. Khedr, I. Korovina, M. Troullinaki, A. Neuwirth, D. Sprott, A. Chatzigeorgiou, M. Economopoulou, A. Orlandi, A. Hain, A.M. Zeiher, A. Deussen, G. Hajishengallis, S. Dimmeler, T. Chavakis, E. Chavakis, Endogenous developmental endothelial locus-1 limits ischaemia-related angiogenesis by blocking inflammation, Thromb. Haemost. 117 (6) (2017) 1150–1163.

[29] M.A. Eskan, R. Jotwani, T. Abe, J. Chmelar, J.H. Lim, S. Liang, P.A. Ciero, J.L. Krauss, F. Li, M. Rauner, L.C. Hofbauer, E.Y. Choi, K.J. Chung, A. Hashim, M.A. Curtis, T. Chavakis, G. Hajishengallis, The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss, Nat. Immunol. 13 (5) (2012) 465–473.

[30] J. Shin, T. Maekawa, T. Abe, E. Hajishengallis, K. Hosur, K. Pyaram, I. Mitroulis, T. Chavakis, G. Hajishengallis, DEL-1 restrains osteoclastogenesis and inhibits inflammatory bone loss in nonhuman primates, Sci. Transl. Med. 7 (307) (2015) 307ra155.

[31] I. Kourtzelis, K. Kotlabova, J.H. Lim, I. Mitroulis, A. Ferreira, L.S. Chen, B. Gercken, A. Steffen, E. Kemter, A. Klotzsche-von Ameln, C. Waskow, K. Hosur, A. Chatzigeorgiou, B. Ludwig, E. Wolf, G. Hajishengallis, T. Chavakis, Developmental endothelial locus-1 modulates platelet-monocyte interactions and instant blood-mediated inflammatory reaction in islet transplantation, Thromb. Haemost. 115 (4) (2016) 781–788.

[32] S.J. Lee, J. Lee, W.W. Kim, J.H. Jung, H.Y. Park, J.Y. Park, Y.S. Chae, Del-1 expression as a potential biomarker in triple-negative early breast cancer, Oncology 94 (4) (2018) 243–256.

[33] J.E. Lee, P.G. Moon, Y.E. Cho, Y.B. Kim, I.S. Kim, H. Park, M.C. Baek, Identification of EDIL3 on extracellular vesicles involved in breast cancer cell invasion, J. Proteomics 131 (2016) 17–28.

[34] P.G. Moon, J.E. Lee, Y.E. Cho, S.J. Lee, J.H. Jung, Y.S. Chae, H.I. Bae, Y.B. Kim, I.S. Kim, H.Y. Park, M.C. Baek, Identification of developmental endothelial locus-1 on circulating extracellular vesicles as a novel biomarker for early breast cancer detection, Clin Cancer Res 22 (7) (2016) 1757–1766.

[35] I. Mitroulis, L.S. Chen, R.P. Singh, I. Kourtzelis, M. Economopoulou, T. Kajikawa, M. Troullinaki, A. Ziogas, K. Ruppova, K. Hosur, T. Maekawa, B. Wang, P. Subramanian, T. Tonn, P. Verginis, M. von Bonin, M. Wobus, M. Bornhauser, T. Grinenko, M. Di Scala, A. Hidalgo, B. Wielockx, G. Hajishengallis, T. Chavakis, Secreted protein Del-1 regulates myelopoiesis in the hematopoietic stem cell niche, J. Clin. Invest. 127 (10) (2017) 3624–3639.

[36] X. Zhuang, H. Zhang, X. Li, X. Li, M. Cong, F. Peng, J. Yu, X. Zhang, Q. Yang, G. Hu, Differential effects on lung and bone metastasis of breast cancer by Wnt signalling inhibitor DKK1, Nat. Cell. Biol. 19 (10) (2017) 1274–1285.

[37] Y. Kang, P.M. Siegel, W. Shu, M. Drobnjak, S.M. Kakonen, C. Cordón-Cardo, T.A. Guise, J. Massagué, A multigenic program mediating breast cancer metastasis to bone, Cancer Cell 3 (6) (2003) 537–549.

[38] I. Marigo, E. Bosio, S. Solito, C. Mesa, A. Fernandez, L. Dolcetti, S. Ugel, N. Sonda, S. Bicciato, E. Falisi, F. Calabrese, G. Basso, P. Zanovello, E. Cozzi, S. Mandruzzato, V. Bronte, Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor, Immunity 32 (6) (2010) 790–802.

[39] A. Stiff, P. Trikha, R. Wesolowski, K. Kendra, V. Hsu, S. Uppati, E. McMichael, M. Duggan, A. Campbell, K. Keller, I. Landi, Y. Zhong, J. Dubovsky, J.H. Howard, L. Yu, B. Harrington, M. Old, S. Reiff, T. Mace, S. Tridandapani, N. Muthusamy, M.A. Caligiuri, J.C. Byrd, W.E. Carson 3rd, Myeloid-derived suppressor cells express bruton's tyrosine kinase and can be depleted in tumor-bearing hosts by ibrutinib treatment, Cancer Res. 76 (8) (2016) 2125–2136.

[40] T. Koga, Y. Matsui, M. Asagiri, T. Kodama, B. de Crombrugghe, K. Nakashima, H. Takayanagi, NFAT and Osterix cooperatively regulate bone formation, Nat. Med. 11 (8) (2005) 880–885.

[41] S.H. Oh, J.W. Kim, Y. Kim, M.N. Lee, M.S. Kook, E.Y. Choi, S.Y. Im, J.T. Koh, The extracellular matrix protein Edil3 stimulates osteoblast differentiation through the integrin alpha5beta1/ERK/Runx2 pathway, PLoS One 12 (11) (2017) e0188749.

[42] F. Yu, Y. Shi, J. Wang, J. Li, D. Fan, W. Ai, Deficiency of Kruppel-like factor KLF4 in mammary tumor cells inhibits tumor growth and pulmonary metastasis and is accompanied by compromised recruitment of myeloid-derived suppressor cells, Int. J. Cancer 133 (12) (2013) 2872–2883.