CellPress

Cell Stem Cell

Letter

A subset of macrophages and monocytes in the mouse bone marrow express atypical chemokine receptor 1

Yoo-Wook Kwon,[1,2,5,*] Cheong-Whan Chae,[1,5] Hwan Lee,[1] Dongsub Shin,[1] Hyerin Yoo,[1] Choon-Soo Lee,[1] Younghyun Lee,[3] Ki Moon Seong,[3] and Hyo-Soo Kim[1,4,*]

[1]Strategic Center of Cell and Bio Therapy for Heart, Diabetes & Cancer, Biomedical Research Institute, Seoul National University Hospital, Seoul 03080, Republic of Korea

[2]Department of Medicine, Seoul National University College of Medicine, Seoul National University, Seoul 03080, Republic of Korea

[3]Lab of Biological Dosimetry, National Radiation Emergency Medical Center, Korea Institute of Radiological & Medical Sciences, Seoul 01812, Republic of Korea

[4]Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul 03080, Republic of Korea

[5]These authors contributed equally

*Correspondence: ywkwon@snu.ac.kr (Y.-W.K.), hyosoo@snu.ac.kr (H.-S.K.)

https://doi.org/10.1016/j.stem.2022.06.011

Duffy antigen receptor for chemokines (DARC)/CD234, also known as atypical chemokine receptor 1 (ACKR1), is a seven-transmembrane domain protein expressed on erythrocytes, vascular endothelium, and a subset of epithelial cells (Peiper et al., 1995). Previously, we reported that ACKR1 was expressed in bone marrow macrophages. ACKR1 interacts with CD82 on long-term repopulating hematopoietic stem cells (LT-HSCs) to maintain the dormancy of LT-HSCs during homeostasis (Hur et al., 2016). We also demonstrated that ACKR1 interacts with CD82 in HSCs from human umbilical cord blood (hUCB). These findings demonstrated that CD82 is a functional surface marker of LT-HSCs and this molecule maintains LT-HSC quiescence by interactions with ACKR1-expressing macrophages in mice and humans.

Not only our study but other groups also described that ACKR1 is expressed in various cell types such as macrophages (Kim et al., 2019), lymphocytes (Davis et al., 2015), mesenchymal stem cell-derived adipocyte (Schafer et al., 2011), and cancer cells (Jenkins et al., 2019). According to immgen microarray database (immgen.org), it has been shown that ACKR1 mRNA is expressed in several subsets of macrophages. For example, ACKR1 is expressed in splenic CD4+ T cells (CD45+MHC II+CD11c+CD4+) and splenic CD8+ dendritic cells (CD45+MHC II+CD11c+CD8+). But ACKR1 is not expressed in peritoneal macrophage (F4/80+ICAM2+). To validate these microarray data, we performed quantitative real-time PCR and we confirmed that ACKR1 mRNA was expressed in T cells, dendritic cells, monocytes, and macrophages (Figure S1A). In this study, to exclude the contamination of nucleated erythroid cells (NECs), we sorted NECs (CD71+TER119+) out first and prepared each cell with FACS sorting. To confirm the absence of contamination by erythroblasts, we performed quantitative real-time PCR with erythroblast-specific genes, such as hemoglobin subunit alpha (HBA) and beta (HBB). They were not detected (Figure S1B) in non-erythroid nucleated cells.

Rot et al. (2021) have raised questions about the cell type specificity of ACKR1 expression, which we address here. More specifically, they demonstrated that, in a comprehensive murine bone marrow single-cell RNA-sequencing (scRNA-seq) database (gexc.riken.jp), ACKR1 mRNA accumulates in NECs, whereas it is not expressed in all non-erythroid nucleated cells. However, it is suggested that an overall note of caution is required when interpreting scRNA-seq data for low-abundance transcripts (Ziegler et al., 2020), because detection inefficiencies might result in an underestimation of the actual frequencies of ACKR1+ cells in a tissue. Moreover, the protein amounts of each cell might differ from their mRNA abundance (Shalek et al., 2013).

To investigate this discrepancy, we performed immunofluorescence of mouse bone marrow sections using macrophage markers including F4/80 (Ebioscience, 13-4801-82), CD11b (Abcam, ABC-AB184308), and ACKR1 antibody (Santa Cruz, sc-27817). We observed a small proportion (about 2%) of bone marrow macrophages expressing ACKR1 (Figure S1C). To confirm these findings using the same validated antibody and technique employed by Rot and colleagues, we analyzed by "ImageStream" 2,320 CD11b+Ly6G−F4/80+ BM macrophages and found that 2.7% of them were ACKR1+. While this frequency is 15-fold lower compared with our original report using less-specific antibodies, the results confirm the presence of a rare population of ACKR1+ macrophages in the BM (Figure S1E).

Ludin et al. reported in 2012 that LT-HSCs are maintained in an undifferentiated state by a rare population of bone marrow mono-macrophages expressing α-smooth muscle actin (α-SMA) (Ludin et al., 2012). Among those monocytes and macrophages, a minority fraction expressed COX-2 (cyclooxygenase-2) and preserved the primitive HSPC (hematopoietic stem and progenitor cell) population in a COX-2-dependent manner. In detail, COX-2-induced production and secretion of PGE2 from a rare α-SMA+ BM macrophage population prevents ROS production in LT-HSCs, thereby maintaining the undifferentiated status of LT-HSCs. In our re-analysis we found that 95% of ACKR1+ macrophages (75.3% low and 20% high) expressed α-SMA and COX-2. These results suggest that α-SMA and ACKR1 double-positive macrophages protect the HSPC pool by inducing a quiescent status. Given that after macrophages lose COX-2 activity,

they no longer maintain LT-HSC quiescence, there is a possibility that crosstalk exists between the ACKR1/CD82 axis and COX-2-mediated PGE2 generation. As shown in Figures S1F and S1G, not only the ACKR1+ macrophages (about 2%) but also α-SMA/COX-2 and ACKR1 triple-positive macrophages (about 2%) exist in the bone marrow. These data reconfirmed the existence of the unique subset of macrophages to preserve hematopoietic stem cells. Thus, we propose that ACKR1-positive macrophages are a unique subset of the macrophage population having a specific role for HSCs and that they do not represent the whole mono-macrophage lineage. Such a small but unique subset of mono-macrophages expressing ACKR1 plays an important role to maintain dormancy of HSCs.

To investigate the *in vivo* function of ACKR1+ macrophages, we generated a tamoxifen-inducible macrophage-specific ACKR1 knockout mouse (Figure S1H). This mouse was made by crossing a CSF1R (colony stimulating factor 1 receptor) promoter driven cre (Jackson Laboratory) and ACKR1 flox mouse (Macrogen Co). CSF1R is a well-known gene that is only expressed in monocytes or macrophages. When we treated this mouse with tamoxifen for 3 days, the population of ACKR1+ monocytes (CD11b+Ly6G−CD115+F4/80−) was dramatically reduced (Figure S1I). ACKR1+ cells were reduced not only in monocytes but also in macrophages (CD11b+Ly6G−F4/80+) (Figure S1J). However, the number of ACKR1 and Ter119/CD71 triple-positive erythrocytes did not change after treatment with tamoxifen (Figure S1K). These results confirmed that macrophage and monocyte expressed ACKR1. Concurrent with the reduction of ACKR1 in macrophages, the percentage of LT-HSCs in G_0 significantly decreased (Figure S1L) and we found that the number of WBCs and neutrophils increased (Figure S1M), suggesting that the activated LT-HSC may proliferate and differentiate. Therefore, this mouse should be useful to investigate the role of ACKR1+ macrophages *in vivo*; we will further investigate the function of ACKR1+ macrophages in the near future.

The study by Rot et al. importantly noted the insufficient specificity of some antibodies used in our previous study and prompted us to re-analyze ACKR1 expression using validated antibodies and higher-resolution techniques. The new results suggest that the vast majority of BM macrophages do not express ACKR1, which is partly in agreement with the interpretation by Rot el at. However, the new results also confirm the presence of an ACKR1+ subpopulation of BM macrophages (albeit rarer than we previously reported) that contributes to maintaining quiescence of HSCs.

In summary, we showed evidence that ACKR1 mRNA expression varies in different subsets of macrophages and that the ACKR1 protein was expressed at the single-cell level without forming a complex with any other cells. Furthermore, we provided evidence of ACKR1 expression in monocytes and macrophages at the single-cell level with the exact same ACKR1 antibody (6B7) as was used in Rot and colleagues' experiments. Finally, we confirmed the function of ACKR1+ macrophages for LT-HSC quiescence *in vivo*. For these experiments, we used tamoxifen-inducible monocyte/macrophage-specific ACKR1 knockout mouse. In this study, we confirmed that ACKR1 was expressed not only in nucleated erythrocytes but also in a unique subset of monocytes and macrophages.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.stem.2022.06.011.

ACKNOWLEDGMENTS

This work was supported by Technology R&D Project "Strategic Center of Cell and Bio Therapy for Heart, Diabetes & Cancer (HI17C2085)" and "Korea Research-Driven Hospital (HI14C1277)" through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Davis, M.B., Walens, A., Hire, R., Mumin, K., Brown, A.M., Ford, D., Howerth, E.W., and Monteil, M. (2015). Distinct transcript Isoforms of the atypical chemokine receptor 1 (ACKR1)/Duffy antigen receptor for chemokines (DARC) gene are expressed in Lymphoblasts and Altered Isoform levels are Associated with Genetic Ancestry and the Duffy-Null Allele. PLoS One *10*, 0140098. https://doi.org/10.1371/journal.pone.0140098.

Hur, J., Choi, J.I., Lee, H., Nham, P., Kim, T.W., Chae, C.W., Yun, J.Y., Kang, J.A., Kang, J., Lee, S.E., et al. (2016). CD82/KAI1 maintains the dormancy of long-term hematopoietic stem cells through interaction with DARC-expressing macrophages. Cell Stem Cell *18*, 508–521. https://doi.org/10.1016/j.stem.2016.01.013.

Jenkins, B.D., Martini, R.N., Hire, R., Brown, A., Bennett, B., Brown, I., Elizabeth, W., Howerth, E.W., Egan, M., Yates, C., et al. (2019). Atypical chemokine receptor 1 (*DARC/ACKR1*) in Breast Tumors is Associated with Survival, Circulating chemokines, Tumor-Infiltrating immune cells, and African Ancestry. Cancer Epidemiol. Biomarkers Prev. *28*, 690–700. https://doi.org/10.1158/1055-9965.epi-18-0955.

Kim, E.Y., Noh, H.M.,3, Choi, B., Park, J.E., Kim, J.E., Jang, Y., Lee, H.K., and Chang, E.J. (2019). Interleukin-22 Induces the Infiltration of Visceral Fat tissue by a Discrete subset of Duffy antigen receptor for chemokine-positive M2-Like macrophages in Response to a high Fat Diet. Cells *8*, 1587. https://doi.org/10.3390/cells8121587.

Ludin, A., Itkin, T., Gur-Cohen, S., Mildner, A., Shezen, E., Golan, K., Kollet, O., Kalinkovich, A., Porat, Z., D'Uva, G., et al. (2012). Monocytes-macrophages that express α-smooth muscle actin preserve primitive hematopoietic cells in the bone marrow. Nat. Immunol. *13*, 1072–1082. https://doi.org/10.1038/ni.2408.

Peiper, S.C., Wang, Z.X., Neote, K., Martin, A.W., Showell, H.J., Conklyn, M.J., Ogborne, K., Hadley, T.J., Lu, Z.H., Hesselgesser, J., and Horuk, R. (1995). The Duffy antigen/receptor for chemokines (DARC) is expressed in endothelial cells of Duffy negative individuals who lack the erythrocyte receptor. J. Exp. Med. *181*, 1311–1317. https://doi.org/10.1084/jem.181.4.1311.

Rot, A., Gutjahr, J.C., Biswas, A., Aslani, M., Hub, E., Thiriot, A., von Andrian, U.H., Megens, R.T.A., Weber, C., and Duchene, J. (2021). Murine bone marrow macrophages and human monocytes do not express atypical chemokine receptor 1. Cell Stem Cell *29*, 1013–1015.

Schafer, R., Schnaidt, M., Klaffschenkel, R.A., Siegel, G., Schüle, M., Rädlein, M.A., Hermanutz-Klein, U., Ayturan, M., Buadze, M., Gassner, C., et al. (2011). Expression of blood group genes by mesenchymal stem cells. Br. J. Haematol. *153*, 520–528. https://doi.org/10.1111/j.1365-2141.2011.08652.x.

Shalek, A.K., Satija, R., Adiconis, X., Gertner, R.S., Gaublomme, J.T., Raychowdhury, R., Schwartz, S., Yosef, N., Malboeuf, C., Lu, D., et al. (2013). Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature *498*, 236–240. https://doi.org/10.1038/nature12172.

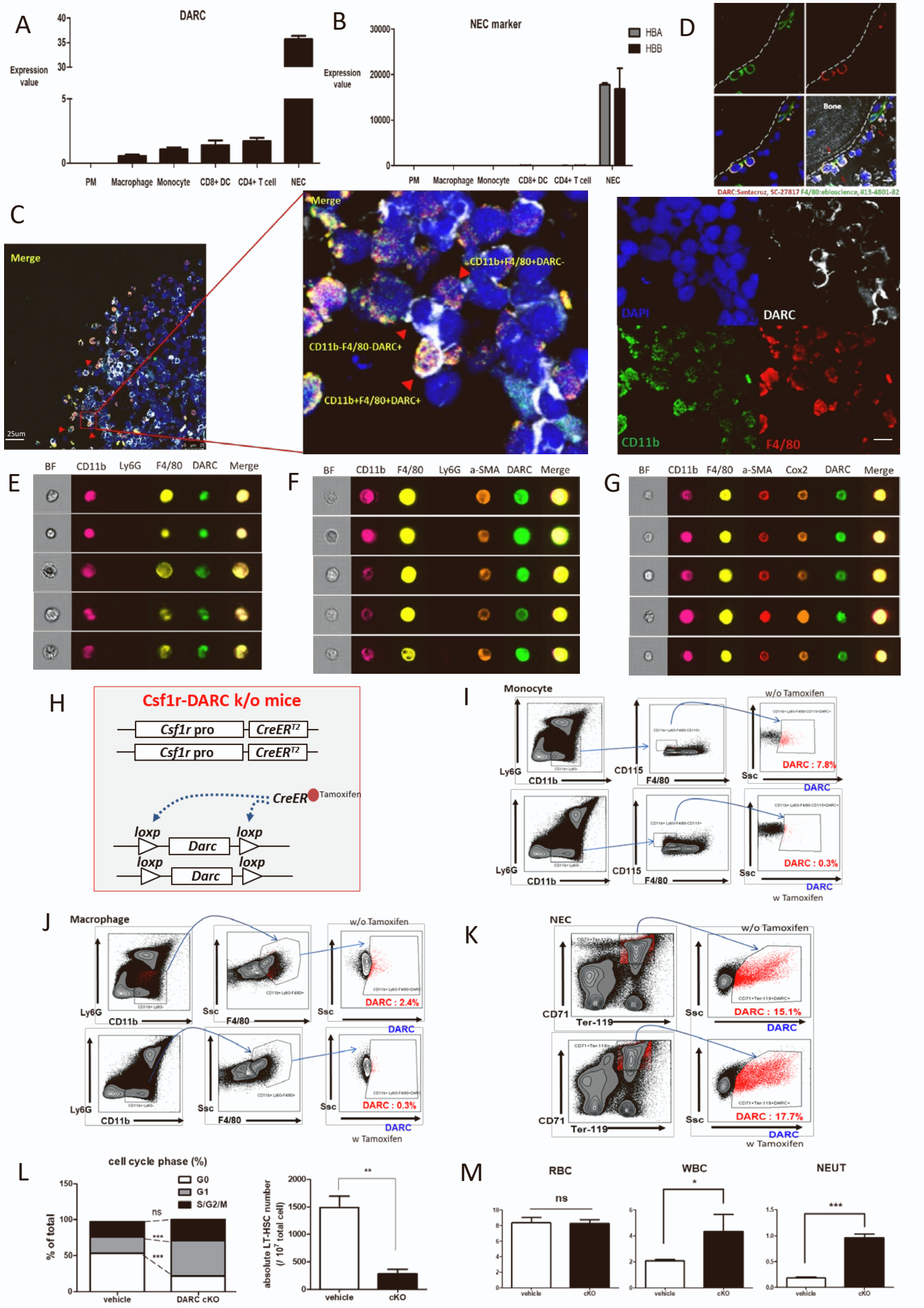
Ziegler, C.G.K., Allon, S.J., Nyquist, S.K., Mbano, I.M., Miao, V.N., Tzouanas, C.N., Cao, Y., Yousif, A.S., Bals, J., Hauser, B.M., et al.; HCA Lung Biological Network. Electronic address: lung-network@humancellatlas.org; HCA Lung Biological Network (2020). SARS-CoV-2 receptor ACE2 is an Interferon-Stimulated gene in human Airway epithelial cells and is detected in specific cell subsets across tissues. Cell *181*, 1016–1035.e19. https://doi.org/10.1016/j.cell.2020.04.035.

Cell Stem Cell, Volume *29*

Supplemental Information

A subset of macrophages and monocytes in the mouse bone marrow express atypical chemokine receptor 1

Yoo-Wook Kwon, Cheong-Whan Chae, Hwan Lee, Dongsub Shin, Hyerin Yoo, Choon-Soo Lee, Younghyun Lee, Ki Moon Seong, and Hyo-Soo Kim


A
DARC
Expression value
PM
Macrophage
Monocyte
CD8+ DC
CD4+ T cell
NEC
B
NEC marker
HBA
HBB
C
Merge
CD11b+F4/80+DARC-
CD11b-F4/80-DARC+
CD11b+F4/80+DARC+
25um
D
Bone
DARC:Santacruz, SC-27817 F4/80:ebioscience, #13-4801-82
DAPI
DARC
CD11b
F4/80
E
BF
CD11b
Ly6G
F4/80
DARC
Merge
F
a-SMA
G
Cox2
H
Csf1r-DARC k/o mice
Csf1r pro
CreER^T2
CreER
Tamoxifen
loxp
Darc
I
Monocyte
w/o Tamoxifen
Ly6G
CD11b
CD115
F4/80
Ssc
DARC : 7.8%
DARC : 0.3%
w Tamoxifen
J
Macrophage
DARC : 2.4%
DARC : 0.3%
K
NEC
CD71
Ter-119
DARC : 15.1%
DARC : 17.7%
L
cell cycle phase (%)
G0
G1
S/G2/M
ns
% of total
vehicle
DARC cKO
absolute LT-HSC number (/ 10^7 total cell)
cKO
M
RBC
WBC
NEUT

Supplemental Figure 1. Murine bone marrow macrophages and human monocytes express ACKR1 that interacts with CD82 leading to dormancy of stem cells.

(A) ACKR1 mRNA expression in mouse non-nucleated erythrocyte cells: Peritoneal macrophages (PM), Bone marrow derived macrophages and monocytes, Splenic CD8+ DC (Dendritic cells), Splenic CD4+ T cells and nucleated erythrocytes (NEC) (B) mRNA expression of NEC specific genes, Hemoglobin subunit alpha (HBA) and beta (HBB) in non-NECs and NECs. (C, D) Representative images of Immuno-fluorescent staining of macrophage marker F4/80 (red), CD11b (green) and ACKR1 (white) in bone marrow endosteal niche. Scale bar means 5 um. (E-G) Representative visualized FACS single cell images of (E) ACKR1+ macrophages (CD11b+Ly6G-F4/80+) (F) ACKR1 and □ -SMA double positive macrophages (G) ACKR1, □ -SMA and COX2 triple-positive macrophages. (H) Schematic figure of tamoxifen-inducible Csf1r-specific conditional knockout mouse. (I-K) Fluorescence-activated cell sorting (FACS) analysis of (I) Monocyte (CD11b+CD115+F4/80-Ly6G-), (J) Macrophage (CD11b+Ly6G-F4/80+) and (K) NEC (Ter119+CD71+) with or without tamoxifen, stained with validated ACKR1 antibody. (L) (Left) Quantification of the plot for Cell-cycle status of the primary LT-HSC with or without tamoxifen (***$p<0.01$, n=3) (Right) The number of LT-HSC cells with or without tamoxifen (**$p<0.05$, n=3). (M) The test of Complete Blood Counting (CBC) in the mouse with or without tamoxifen (*$p<0.1$, ***$p<0.001$, n=3)