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JAM-C/*Jam-C* Expression Is Primarily Expressed in Mouse Hematopoietic Stem Cells

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Hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs) are at the top of hematopoiesis hierarchy and are responsible for sustaining blood cell production all along life. HSC are known to be multipotent with a high self-renewal potential whereas MPP exhibit, if any, weak self-renewal capacities. The identification by flow cytometry of the most enriched HSC population is of importance to precisely decipher mechanisms regulating HSC biology such as self-renewal potential, HSC division/activation and microenvironment involvement. This could help to identify novel therapeutic targets for ex vivo expansion of HSC or inhibition of leukemic cells that share HSC properties.[1] In mouse, HSC and MPP are enriched in the cell population characterized by the Lin^{neg} $SSCA\text{-}1^{hi}$ $c\text{-}KIT^{hi}$ (LSK) phenotype, which can be further divided using the signaling lymphocyte activation molecule signaling lymphocyte activation molecule (SLAM) markers CD150 and CD48.[2,3] LSK $CD150^{+}$ $CD48^{-}$ population is largely enriched in HSC (here named SLAM-HSC) while LSK $CD150^{+}$ $CD48^{+}$ (MPP2) and $CD150^{-}$ $CD48^{+}$ (MPP3/4) populations contain subsets of MPP cells.[2,4]

Several members of the junctional adhesion molecule (JAM) family[5] such as JAM-A, JAM4 and endothelial-cell selective adhesion molecule (ESAM) are expressed in mouse HSC[6–9] and have been involved in the retention and engraftment of HSC within their niches.[10] The expression of the JAM-B and JAM-C proteins on HSC surface was more challenging to assess: several groups reported JAM-C expression on HSC,[4,6,11–13] however, 1 group failed to detect it but observed the expression of JAM-B.[14] Moreover, several studies have shown that the preferential ligand of JAM-B is JAM-C.[15] JAM-C function in hematopoiesis was reported in several studies.[11–13,16] Indeed, JAM-C via its interaction with JAM-B was involved in HSC maintenance, homing engraftment and retention into the bone marrow (BM).[11,16] Disrupting JAM-C/JAM-B interaction induced HSC mobilization and enhanced the effect of plerixafor.[16] Furthermore, the maintenance of HSC stemness potential ex vivo was reported to be preserved by the interaction of HSC ($JAM\text{-}C^{+}$ and $ESAM^{+}$) with JAM-C and ESAM expressed by an artificial in vitro niche.[13]

Therefore, clarifying JAM-C expression pattern in hematopoietic stem and progenitor cells is of major importance. In this study, *Jam-C*/JAM-C and *Jam-B*/JAM-B will be used to refer to the gene and protein. We definitively assessed *Jam-C*/JAM-C expression on mouse HSC using transcriptomic data together with experimental quantification by reverse transcription quantitative PCR and flow cytometry using anti-JAM-C antibodies and a JAM-B recombinant soluble protein that can bind JAM-C.

We first analyzed *Jam-C* expression on publicly available transcriptomic data. We took advantage of transcriptomic data on purified HSC and MPP cells produced by the Trumpp' lab (GSE52709).[2] The analysis showed that *Jam-C* was preferentially expressed in HSC and its expression decreased with hematopoietic differentiation along with *Jam-B* and *Esam* gene expression (Figure 1A). *Jam-C* expression in mouse HSC was also found in other microarray and RNA-Sequencing analyses.[6,17,18] Importantly, single cell transcriptomes generated by the Gottgens' lab (http://blood.stemcells.cam.ac.uk/single_cell_atlas.html),[19] confirmed that *Jam-C* was expressed mainly by long-term HSC (LT-HSC) (blue, Figure 1B). *Jam-B* could be also detected in LT-HSC but at lesser extent (Figure 1B). To experimentally confirm expression pattern of *Jam-C* in hematopoietic progenitors, LSK, SLAM-HSC (LSK $CD48^{-}CD150^{+}$), MPP2 (LSK $CD48^{+}CD150^{+}$) and MPP3/4 (LSK $CD48^{+}CD150^{-}$) cell populations from the bone marrow were purified by cell sorting and *Jam-C* expression was analyzed by quantitative RT-PCR (detailed method procedure is available in Supplemental Document 1, http://links.lww.com/HS/A159). As shown in Figure 1C, *Jam-C* was preferentially expressed in SLAM-HSC and its expression decreased with hematopoietic differentiation. Indeed, the expression of *Jam-C* was very weak in MPP2 and MPP3/4 cells. *Jam-B* expression could also be detected in SLAM-HSC (Figure 1D).

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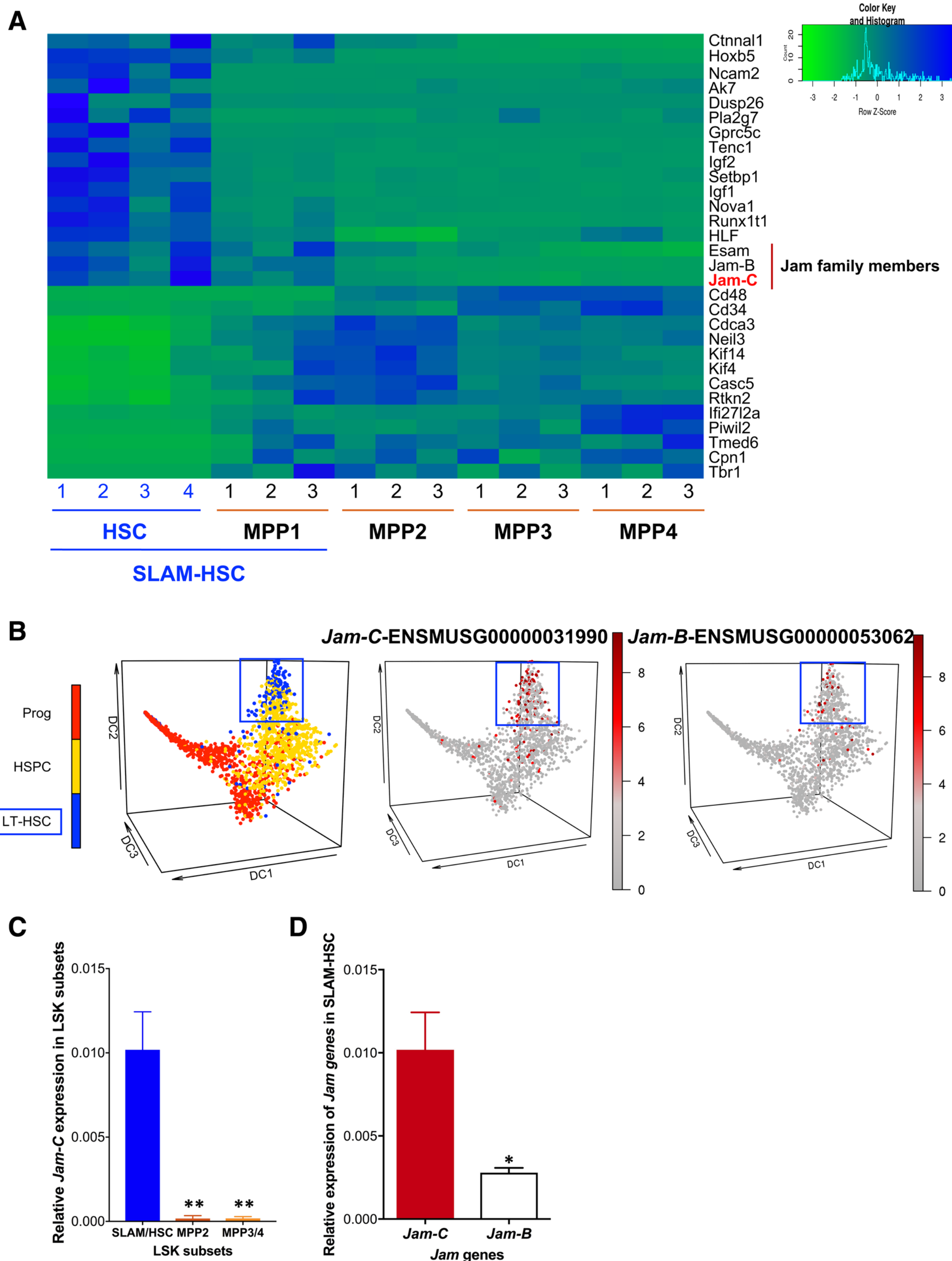

Figure 1. Jam-C and Jam-B gene expression pattern in hematopoietic stem and progenitor cells. (A), Analysis of *Jam-C* expression on RNA-Sequencing data generated by the Trumpp's lab[2] and obtained in GEO under accession number GSE52709. The heatmap was generated using http://shiny-heatmap.com. (B), Dotplots representing, at single cell level, the different clusters corresponding to the different LSK subsets (left) and the expression of *Jam-C* and *Jam-B* in these different clusters[19] and http://blood.stemcells.cam.ac.uk/single_cell_atlas.html. (C) and (D), *Jam-C* and *Jam-B* expressions were determined by quantitative RT-PCR on LSK subsets. In this purpose, LSK, LSK CD48$^+$CD150$^-$(MPP3/4), LSK CD48$^+$CD150$^+$(MPP2) and LSK CD48$^-$CD150$^+$ (SLAM-HSC) cell populations were purified by cell sorting (C) *Jam-C* gene expression in different LSK cell subsets (4 independent experiments, primer sequences for *Jam-C*: forward GAACTCGGAGACAGGCACTC, reverse CAACAAGGACTCCCCCAATA). (D), Comparison of *Jam-C* and *Jam-B* gene expression in SLAM-HSC cells (n = 4 independent experiments, primer sequences for *Jam-B*: forward TAGTGGCTCCTGCTGTTCCT, reverse GGAGCTGGGTTTCCTTCTTT). Statistical analysis was performed with a 1-way ANOVA test, *$P < 0.05$, **$P < 0.01$. GEO = Gene Expression Omnibus; HSC = hematopoietic stem cell; HSPC = hematopoietic stem/progenitor cells; JAM = junctional adhesion molecule; LSK = Lin^{neg} SCA-1^{hi} c-KIT^{hi}; LT-HSC = long-term HSC; MPP = multipotent progenitor; Prog = progenitor cells; RT-PCR = reverse transcription PCR; SLAM = signaling lymphocyte activation molecule.

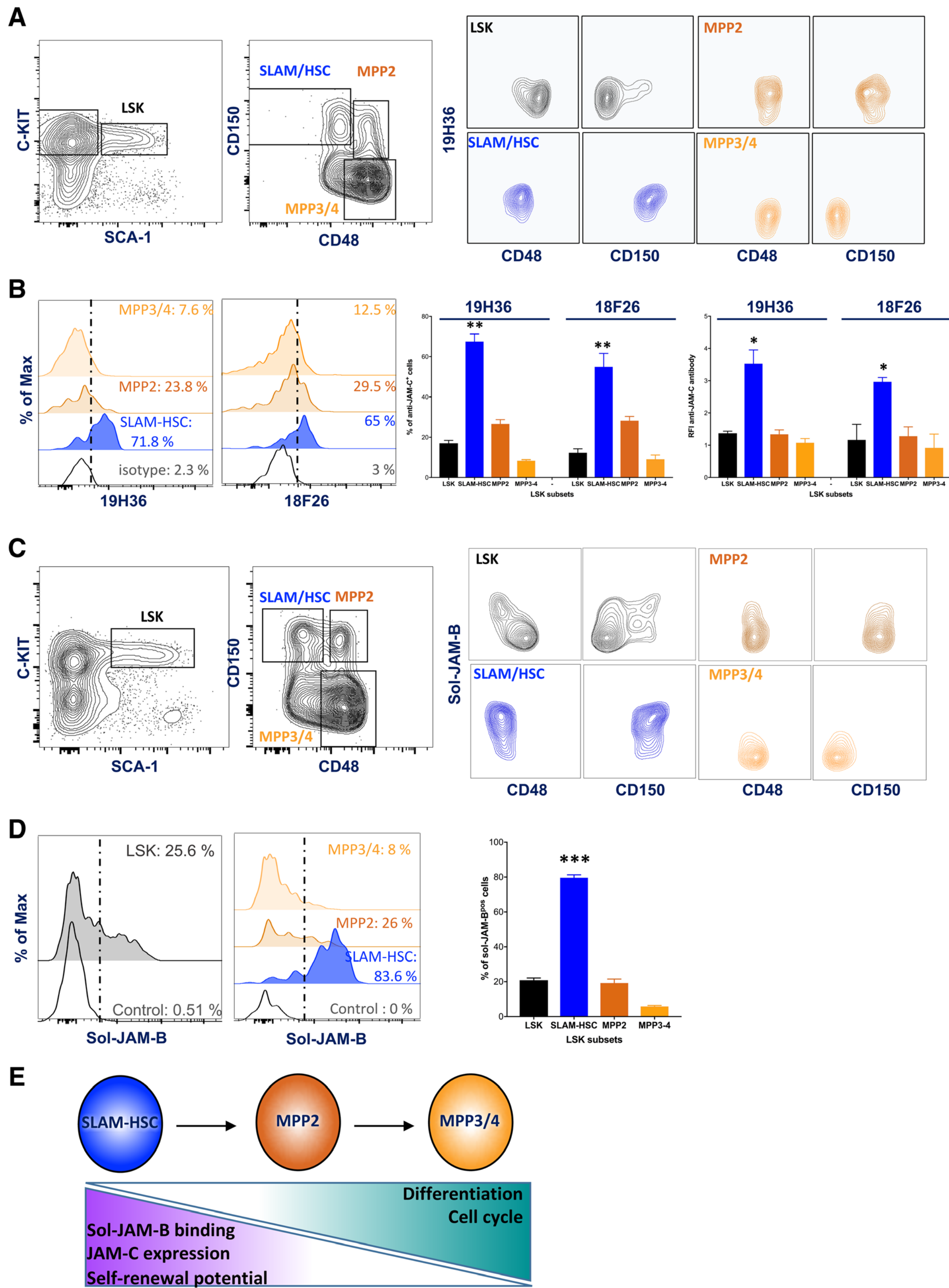

Figure 2. JAM-C is preferentially expressed on SLAM-HSC within the LSK cell compartment. JAM-C surface expression was examined on total LSK cells (black) and on different LSK cell subsets. SLAM-HSC (blue) are LSK CD150$^+$ CD48$^-$, MPP2 cells (dark orange) are LSK CD150$^+$ CD48$^+$ and MPP3/4 cells (light orange) cells are LSK CD150$^-$ CD48$^+$.[3] (A), Representative dot plots of LSK and CD150/CD48 subsets, left. Back-gating analysis shows expression of 19H36 in function of CD48 or CD150 in each 4 populations, right. (B), Left, representative flow cytometry analysis showing JAM-C expression using 19H36 or 18F26 antibodies on different LSK cell subsets. Dark line represent signals obtained with isotype control. Center, percentages of JAM-C-expressing cells in indicated cell populations, using either 19H36 antibody (n = 3 mice), or 18F26 antibody (n = 3 mice). Right, RFIs are calculated by the MFI of anti-JAM-C antibodies divided by the MFI of the control isotype (n = 3). (C), Representative dot plots of LSK and CD150/CD48 subsets, left. Back-gating analysis shows the binding of sol-JAM-B in function of CD48 or CD150 in each 4 populations, right. (D), Representative flow cytometry histograms showing the binding of recombinant soluble JAM-B compared to irrelevant soluble protein (dark line) on LSK cells (left) or on the different LSK cell subsets (center). Right, recapitulation for 6 independent mice showing soluble JAM-B binding on LSK cells and LSK cell populations. (E), Schema representing JAM-C expression on the different hematopoietic progenitor populations associated with different self-renewal potential and cell cycle and differentiation states. Statistical analysis was performed with a 1-way ANOVA test, *P < 0.05, **P < 0.01, ***P < 0.001. HSC = hematopoietic stem cell; JAM = junctional adhesion molecule; LSK = Lin^{neg} SCA-1^{hi} c-KIT^{hi}; MFI = mean fluorescence intensity; MPP = multipotent progenitor; RFI = ratio of fluorescence intensity; SLAM = signaling lymphocyte activation molecule; sol-JAM-B = soluble form of junctional adhesion molecule B.

Then, to examine the expression of the JAM-C protein, we performed flow cytometry analyses on LSK cell subsets using 2 different commercially available antibodies (clone 19H36 and 18F26 coupled to fluorescein isothiocyanate, reference numbers MA5-28230 and MA5-28229, respectively (Thermofisher, detailed in Supplemental Document 1, http://links.lww.com/HS/A159). As shown in Figure 2A and B, JAM-C was expressed mainly in HSC (blue) and its cell surface expression decreased with HSC differentiation toward MPP cells (Figure 2B, left). The mean fluorescence intensity (MFI) compared to the one of isotype control (ratio of fluorescence intensity [RFI]) was analyzed in each LSK cell subsets (Figure 2B, right). The highest RFI was found in HSC for both used antibodies. These results further support the reports of JAM-C protein expression on HSC by 3 independent teams using the same antibodies[4,6,13] and by us and others using 2 different homemade rabbit-polyclonal antibodies.[11,12] Concerning JAM-B surface expression, we have previously shown, using homemade rabbit anti-mouse polyclonal antibody (p829) validated for its specificity to the extracellular part of JAM-B by the use of *Jam-B* knock-out mice and small interfering RNA directed against *Jam-B*, that JAM-B was expressed by BM stroma in close contact to SLAM-HSC, but not on LSK cells.[11]

Finally, JAM-C expression was monitored by the binding of a recombinant soluble form of JAM-B (sol-JAM-B) to the extracellular part of the JAM-C protein in a specific manner. This binding cannot be detected on JAM-C$^{-/-}$ LSK cells[11] (protocol detailed in Supplemental Document 1, http://links.lww.com/HS/A159). Sol-JAM-B binding was detected in >80% of HSC and the frequency of sol-JAM-B^{+} cells decreased with the hematopoietic differentiation (Figure 2C and D). To note, when gated on JAM-C^{neg} (19H36; Sup Figure 1A, http://links.lww.com/HS/A159) or sol-JAM-B^{neg} (Sup Figure 1B, http://links.lww.com/HS/A159) LSK subset, very few phenotypic SLAM-HSC can be found. This is in accordance with functional data published by Praetor et al[12] and us reporting that, in vivo, the HSC potential resides only respectively, in JAM-C^{+} and sol-JAM-B^{+}[11] LSK fraction. To conclude, the expression pattern of JAM-C at cell surface in the different hematopoietic stem and progenitor subsets correlates with the *Jam-C* gene expression pattern during hematopoiesis. Notably, both transcriptome and cell surface expression analyses indicate that *Jam-C*/JAM-C expression is down-regulated when HSC are mobilized by granulocyte colony-stimulating factor and cyclophosphamide.[16,18]

Altogether, our novel analyses and publicly available expression data[2,6,11,12,17,18] demonstrate that JAM-C is mainly expressed by HSC. JAM-C expression functionally correlates with HSC stemness and their mobilization property (Figure 2E). Very recent data have shown that JAM-C was asymmetrically distributed upon HSC division after in vivo IFNα activation, the paired daughter cells expressing JAM-C being the most immature one.[20] This demonstrates that JAM-C is a robust marker/tool to decipher mechanisms controlling HSC maintenance and activation within the BM microenvironment.

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Disclosures

The authors have no conflicts of interest to disclose.

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