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N-cadherin$^+$ HSCs in fetal liver exhibit higher long-term bone marrow reconstitution activity than N-cadherin$^-$ HSCs

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ABSTRACT

Adult hematopoietic stem cells (HSCs) are maintained in a microenvironment known as the stem cell niche. The regulation of HSCs in fetal liver (FL) and their niche, however, remains to be elucidated. In this study, we investigated the role of N-cadherin (N-cad) in the maintenance of HSCs during FL hematopoiesis. By using anti-N-cad antibodies (Abs) produced by our laboratory, we detected high N-cad expression in embryonic day 12.5 (E12.5) mouse FL HSCs, but not in E15.5 and E18.5 FL. Immunofluorescence staining revealed that N-cad$^+$c-Kit$^+$ and N-cad$^+$ endothelial protein C receptor (EPCR)$^+$ HSCs co-localized with Lyve-1$^+$ sinusoidal endothelial cells (ECs) in E12.5 FL and that some of these cells also expressed N-cad. However, N-cad$^+$ HSCs were also observed to detach from the perisinusoidal niche at E15.5 and E18.5, concomitant with a down-regulation of N-cad and an up-regulation of E-cadherin (E-cad) in hepatic cells. Moreover, EPCR$^+$ long-term (LT)-HSCs were enriched in the N-cad$^+$Lin$^-$Sca-1$^+$c-Kit$^+$ (LSK) fraction in E12.5 FL, but not in E15.5 or E18.5 FL. In a long-term reconstitution (LTR) activity assay, higher engraftment associated with N-cad$^+$ LSK cells versus N-cad$^-$ LSK cells in E12.5 FL when transplanted into lethally irradiated recipient mice. However, the higher engraftment of N-cad$^+$ LSK cells decreased subsequently in E15.5 and E18.5 FL. It is possible that N-cad expression conferred higher LTR activity to HSCs by facilitating interactions with the perisinusoidal niche, especially at E12.5. The down-regulation of N-cad during FL hematopoiesis may help us better understand the regulation and mobility of HSCs before migration into BM.

1. Introduction

Hematopoietic stem cells (HSCs) are maintained in a unique microenvironment known as the stem cell niche. They self-renew and differentiate into all blood lineages upon exit from the niche. HSC niches in adult bone marrow (BM) are classically identified as osteoblastic and vascular niches, and they support HSC maintenance, proliferation, and differentiation [1,2]. N-cadherin (N-cad) is a key molecule expressed by HSCs and osteoblasts in BM, despite controversies over its role in HSCs [3,4]. For example, it was reported that LT-HSCs were attached to spindle-shaped N-cad$^+$ osteoblastic (SNO) cells [5]. Tie2/Angiopoietin-1 signaling has also been found to play a key role in the maintenance of HSCs through N-cad adhesion with osteoblasts [6]. Nevertheless, subsequent studies began to question the expression of N-cad by primitive HSCs, with some investigators proposing no expression at all [7,8]. Additionally, a recent study did not identify a phenotype in HSCs derived from N-cad knockout mice [9], whereas knockdown of N-cad by shRNA in hematopoietic stem/progenitor cells (HSPCs) accelerated cell division *in vitro* and inhibited long-term engraftment into BM [10]. These controversies appear to have been caused by the specificity of anti-N-cad antibodies (Abs), which may not have been high enough for fluorescence-activated cell sorting (FACS) [11]. To address this issue, our laboratory produced specific anti-N-cad Abs suitable for FACS and immunohistochemistry [12,13]. By using these Abs, a more recent study has revealed that Flamingo and Frizzled 8 were found between N-cad$^+$ osteoblasts and HSCs, where they maintained quiescent long-term HSCs (LT-HSCs) through a noncanonical Wnt pathway [13]. Nonetheless, there are also additional reports showing that a conditional knockout of N-cad in osteoblasts did not affect HSC function [14,15]. However, it is worth noting that N-cad was not deleted from HSCs in this model. Thus, the possibility that other cadherins compensated for the loss in N-cad, such as R-cadherin in stromal cells, cannot be completely ruled out.

Fetal liver (FL) supports HSC expansion and differentiation during hematopoiesis before their migration into BM [16,17]. FL is the primary hematopoietic organ for the expansion and differentiation of HSCs at E11.5–E16.5. After this stage, HSCs begin to migrate into BM to prepare for adult hematopoiesis. Although many attempts have been made to expand HSCs obtained from cord blood, adult BM or mobilized peripheral blood, the main limitation of the *ex vivo* expansion is the loss of self-renewal and multipotency

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ex vivo. Understanding the regulation of FL HSCs during hematopoiesis will be instrumental for investigating an optimal condition of *ex vivo* HSC expansion for clinical practice, which mimics the *in vivo* niche microenvironment [16].

Immunohistochemistry showed N-cad expression in FL cells [18,19] and quantitative PCR detected N-cad expression in FL Lin$^-$Sca-1$^+$c-Kit$^+$ (LSK) CD41$^-$CD48$^-$CD150$^+$ cells [20]. However, the expression and role of N-cad in FL HSCs have not been completely investigated. Based on our previous findings in adult BM, we herein investigate N-cad expression in HSCs by FACS and analyze the characteristics of N-cad$^+$ HSCs in FL. We found N-cad to be down-regulated dramatically in LSK cells during FL hematopoiesis, coinciding with a decrease in endothelial protein C receptor (EPCR) in N-cad$^+$ LSK cells. In immunohistochemical analyses, the detachment of N-cad$^+$ HSCs from Lyve-1$^+$ sinusoidal endothelial cells (ECs) was observed in E15.5 and E18.5 FL. Additionally, a higher long-term reconstitution (LTR) activity was found to associate with HSCs, especially at E12.5, and this was likely facilitated by N-cad.

2. Materials and methods

2.1. Animals

Ly5.1 and Ly5.2 adult female C57/BL6 mice, pregnant or non-pregnant, were purchased from Sankyo Labo Service Corporation. To obtain FL, embryos were dissected from sacrificed pregnant mice at E12.5, E15.5, and E18.5. Femurs and tibias were used for BM analysis. Animals were handled according to the Guidelines for the Care and Use of Laboratory Animals of Keio University School of Medicine, with the approval of the institutional review board of Keio University.

2.2. Antibodies

Anti-N-cad monoclonal antibodies (Abs, AbD13077 and AbD13081) were produced as previously described [12] and subsequently used for flow cytometry and cell sorting experiments. The following monoclonal Abs were also used for flow cytometry and cell sorting: anti-c-Kit (2B8, BD Biosciences), anti-Sca-1 (E13-161.7, BD Biosciences), anti-CD45.1 (A20, BD Biosciences), anti-CD45.2 (104, BD Biosciences), anti-CD4 (L3T4, BD Biosciences), anti-CD8 (53-6.72, BD Biosciences), anti-B220 (RA3-6B2, BD Biosciences), anti-TER-119 (TER-119, eBioscience), anti-Gr-1 (RB6-8C5, BD Biosciences), anti-Mac-1 (M1/70, BD Biosciences), anti-CD34 (RAM34, eBioscience), anti-CD41 (MWReg30, BD Biosciences), anti-CD48 (HM48-1, BioLegend), anti-CD150 (TC15-12F12.2, BioLegend), and anti-EPCR (RCR-16, BioLegend). The following Abs were used for immunohistochemistry: biotinylated anti-mouse N-cad (AbD13077), goat anti-mouse EPCR (AF2749, R&D Systems), goat anti-mouse c-Kit (AF1356, R&D Systems), rabbit anti-mouse lymphatic vessel endothelial receptor 1 (LYVE-1) (103-PA50AG, ReliaTech), and goat anti-mouse E-cad (AF748, R&D Systems).

2.3. FL cell isolation and sorting

FL mononuclear cells were isolated from E12.5, E15.5, and E18.5 wild type mice. Immunostaining for flow cytometry was performed as previously described [21]. Stained cells were analyzed and sorted using a FACSVantage DiVa flow cytometer (BD Biosciences) and a FACSAria cell sorter (BD Biosciences).

2.4. Long-term competitive BM reconstitution assay

N-cad$^+$ LSK or N-cad$^-$ LSK cells derived from Ly5.1 E12.5, E15.5, or E18.5 mouse FL were sorted and transplanted (~500 cells/recipient) intravenously into lethally irradiated Ly5.2 recipient mice, together with Ly5.2 BM-derived unfractionated mononuclear competitor cells (~2×10^5 cells/recipient). Thereafter, peripheral blood (PB) cells were screened monthly for evidence of Ly5.1 and Ly5.2 chimera using flow cytometry. HSC capacity of transplanted cells in engrafted BM was analyzed for the presence of the LSK fraction 4 months after transplantation. Bone marrow mononuclear cells (BMMNCs, ~5×10^6 cells) were transplanted into lethally irradiated mice 4 months after the first BM transplantation (BMT), and the frequency of donor-derived cells in PB was analyzed monthly.

2.5. Immunofluorescence staining

FL was fixed in 4% paraformaldehyde [wt/vol] in phosphate buffer solution (PBS) overnight at 4 °C. After washing with PBS, FL was

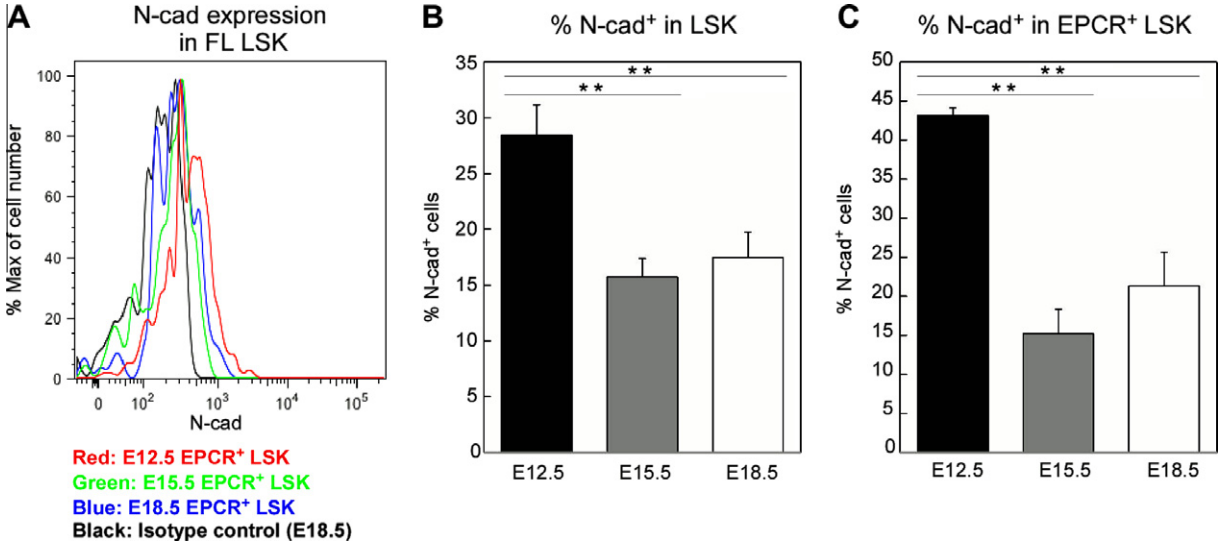

Fig. 1. N-cad expression in LSK and EPCR$^+$ LSK cells by flow cytometric analysis. (A) The flow cytometric pattern of N-cad in E12.5 FL EPCR$^+$ LSK (red), E15.5 FL EPCR$^+$ LSK (green), and E18.5 FL EPCR$^+$ LSK (blue) cells using the N-cad Ab produced by our laboratory is shown. E18.5 EPCR$^+$ LSK cells using an isotype Ab (black) served as the control. Comparison of N-cad$^+$ cells in E12.5, E15.5, and E18.5 mouse FL LSK (B) and EPCR$^+$ LSK (C) cells. Data represent means ± SD ($n = 4$, $^{**}P < 0.01$). N-cad expression was highest in E12.5 FL LSK and E12.5 FL EPCR$^+$ LSK cells.

infused with increasing concentrations of sucrose (10% [wt/vol], 20% [wt/vol] and 30% [wt/vol]), embedded in OCT compound (Sakura Finetek Japan), and frozen at −30 °C. Frozen FL was sectioned at a thickness of 10 μm at −25 °C, and cross-sections were transferred onto a silanized glass slide (Dako). Sections were washed with PBS and incubated with Protein Block Serum-Free Ready-to-use (PBSFR) blocking solution (Dako) to inhibit non-specific staining, followed by incubation with PBSFR-diluted primary Abs overnight at 4 °C. After washing with PBS, sections were stained with PBSFR-diluted corresponding secondary Abs for 90 min at room temperature: Alexa Fluor® 488-conjugated donkey anti-goat IgG (Invitrogen); Cy3-conjugated streptavidin (Invitrogen); Alexa Fluor® 647-conjugated donkey anti-goat IgG (Invitrogen); or DyLight™ 649-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories). For CD45 and Ter119 immunostaining, fluorescein isothiocyanate (FITC)-conjugated anti-CD45 (30-F11) or FITC-conjugated anti-TER-119 (TER-119) were used directly. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen). As the control, isotype control anti-N-cad Ab by our laboratory was used for primary staining, followed by secondary Abs.

2.6. Statistical analysis

Results are shown as the mean ± SD. Student's *t*-test was used to determine significant differences. $P < 0.05$ was taken as statistically significant.

3. Results

3.1. High expression of N-cad in E12.5 FL HSCs decreases in E15.5 and E18.5 FL

To investigate the N-cad expression level in FL HSCs, we performed flow cytometric analysis with N-cad AbD13077. E12.5, E15.5 and E18.5 mouse FL cells were each gated against LSK and EPCR$^+$ LSK fractions. A comparison of flow cytometric patterns corresponding to E12.5, E15.5 and E18.5 FL EPCR$^+$ LSK cells clearly demonstrated the highest expression of N-cad in E12.5 FL (Fig. 1A). Specifically, the percentage of N-cad$^+$ cells in the LSK fraction was highest in E12.5 FL (28.4 ± 2.7%), but this percentage decreased in E15.5 (15.7 ± 1.7%) and E18.5 (17.5 ± 2.3%) FL (Fig. 1B). Although the percentage of N-cad$^+$ cells in a more primitive EPCR$^+$

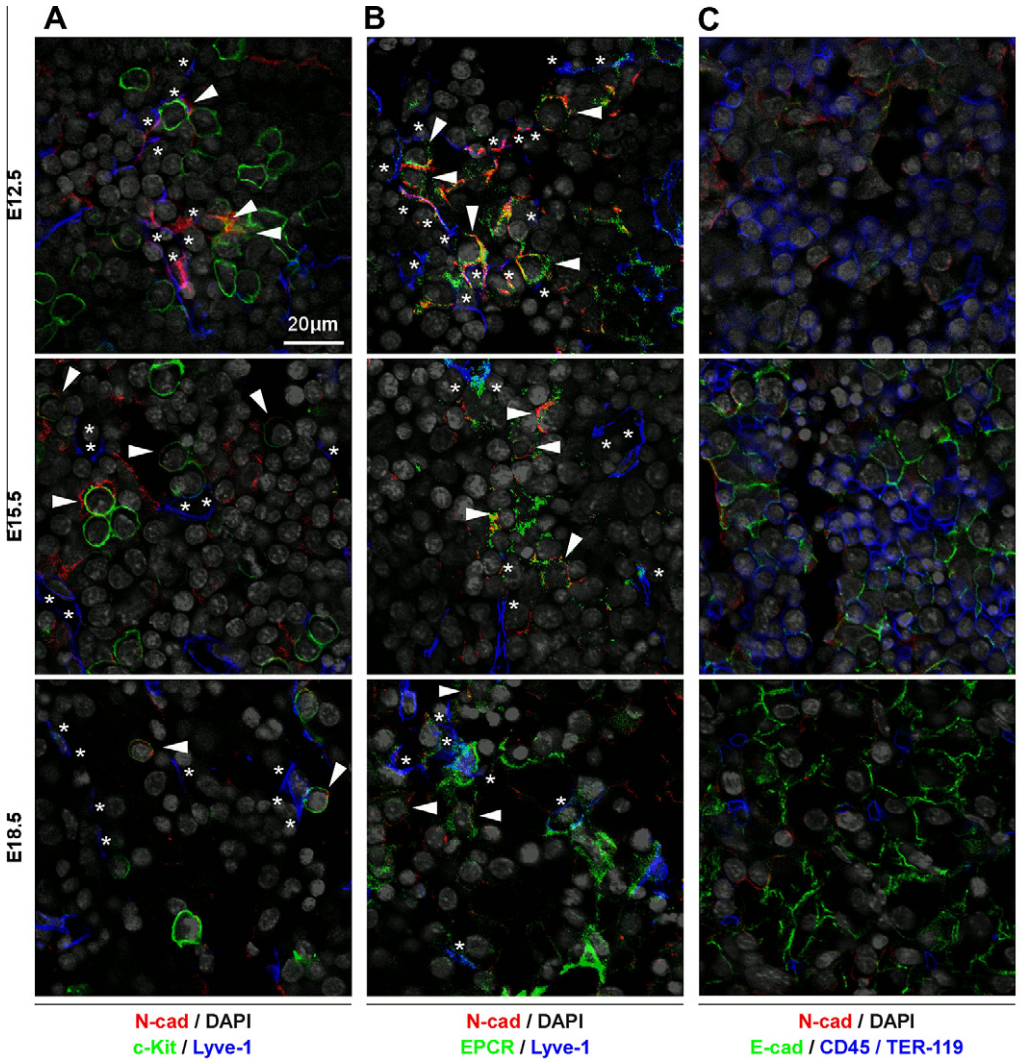

Fig. 2. Immunofluorescence staining of N-cad in E12.5, E15.5, and E18.5 mouse FL. (A) N-cad (red) and c-Kit (green) positive HSCs (arrowheads) were observed in mouse FL from E12.5 to E18.5, along with Lyve-1 immunoreactivity in sinusoidal ECs (blue, asterisks). (B) A small percentage of cells stained for EPCR (green) and N-cad (red) (arrowheads). Likewise, ECs were positive for Lyve-1 (blue, asterisks). (C) N-cad (red) and E-cad (green) localization is shown in E12.5, E15.5, and E18.5 FL. Blood cells were immunostained with CD45 and TER-119 (blue) Abs. All magnifications are ×1200.

LSK fraction was even higher (43.2 ± 1.0%), it too decreased in E15.5 (15.2 ± 3.1%) and E18.5 (21.3 ± 4.3%) FL (Fig. 1C). These data illustrate that N-cad expression is highest in E12.5 FL HSCs and that this expression associated with EPCR$^+$ LT-HSCs.

3.2. N-cad$^+$ HSCs co-localize with Lyve-1$^+$ endothelial cells in E12.5 mouse FL but gradually detach in E15.5 and E18.5

To investigate the localization of N-cad in HSCs, frozen FL sections were immunostained using anti-N-cad AbD13077. As a marker of immature HSCs, N-cad was co-stained with c-Kit and EPCR. Interestingly, we found a large number of N-cad$^+$c-Kit$^+$ and N-cad$^+$EPCR$^+$ HSCs in E12.5 FL residing along or adjacent to Lyve-1$^+$ ECs, some of which were also N-cad$^+$ (Fig. 2A and B). Consistent with FACS data, however, the expression of N-cad decreased in E15.5 and E18.5 so that N-cad$^+$ cells were hardly seen at later stages of development, especially in E18.5 FL. Although some N-cad$^+$c-Kit$^+$ and N-cad$^+$EPCR$^+$ HSCs could still be found to co-localize with Lyve-1$^+$ sinusoidal ECs in E15.5 and E18.5 FL, others were detached from Lyve-1$^+$ ECs. Also, fewer N-cad$^+$Lyve-1$^+$ ECs were detected at later stages, indicating less interaction with N-cad$^+$ HSCs. These findings suggest that HSCs have begun to migrate into BM.

Thereafter, we examined the expression of E-cadherin (E-cad), a marker of hepatoblasts and hepatocytes, together with N-cad expression in FL. Interestingly, E-cad expression in hepatic cells increased drastically in E15.5 and E18.5 FL, with its level being lowest in E12.5 FL (Fig. 2C). In general, N-cad expression was down-regulated, while E-cad expression in hepatic cells was up-regulated during development. This change in cadherin expression may be related to the migration of HSCs from the FL perisinusoidal niche into BM.

3.3. EPCR$^+$ LT-HSCs are enriched in N-cad$^+$ LSK cells in E12.5 FL, but not in E15.5 or E18.5 FL

EPCR$^+$ LSK cells are relatively quiescent, which indicates that they are immature stem cells with a high hematopoietic ability [21]. To investigate the expression of EPCR, a LT-HSC marker, in N-cad$^+$ LSK cells and N-cad$^-$ LSK cells, we analyzed flow cytometric patterns corresponding to E12.5, E15.5, and E18.5 FL (Fig. 3A–C). In E12.5 FL, EPCR expression was significantly higher in the N-cad$^+$ LSK fraction (32.9 ± 2.9%), while it decreased in the N-cad^{low} LSK (19.4 ± 1.5%) and N-cad$^-$ LSK (14.4 ± 3.0%) fractions (Fig. 3D). On the other hand, no significant difference in the percentage of EPCR$^+$ cells was detected in E15.5 and E18.5 FL LSK fractions, despite a similar tendency of EPCR expression with the average of that in N-cad$^+$ HSCs being the highest (Fig. 3E and F). These results suggest that N-cad expression in the LSK fraction enriches EPCR$^+$ LT-HSCs in the E12.5 FL LSK fraction, whereas N-cad expression only does not in in E15.5 or E18.5 FL.

3.4. The increase of N-cad$^+$ LSK cells in LTR activity versus N-cad$^-$ LSK cells in E12.5 FL decreases in E15.5 and E18.5 FL

To examine the self-renewal function of N-cad$^+$ LSK cells, we compared the BM LTR activity of E12.5 FL, E15.5, and E18.5

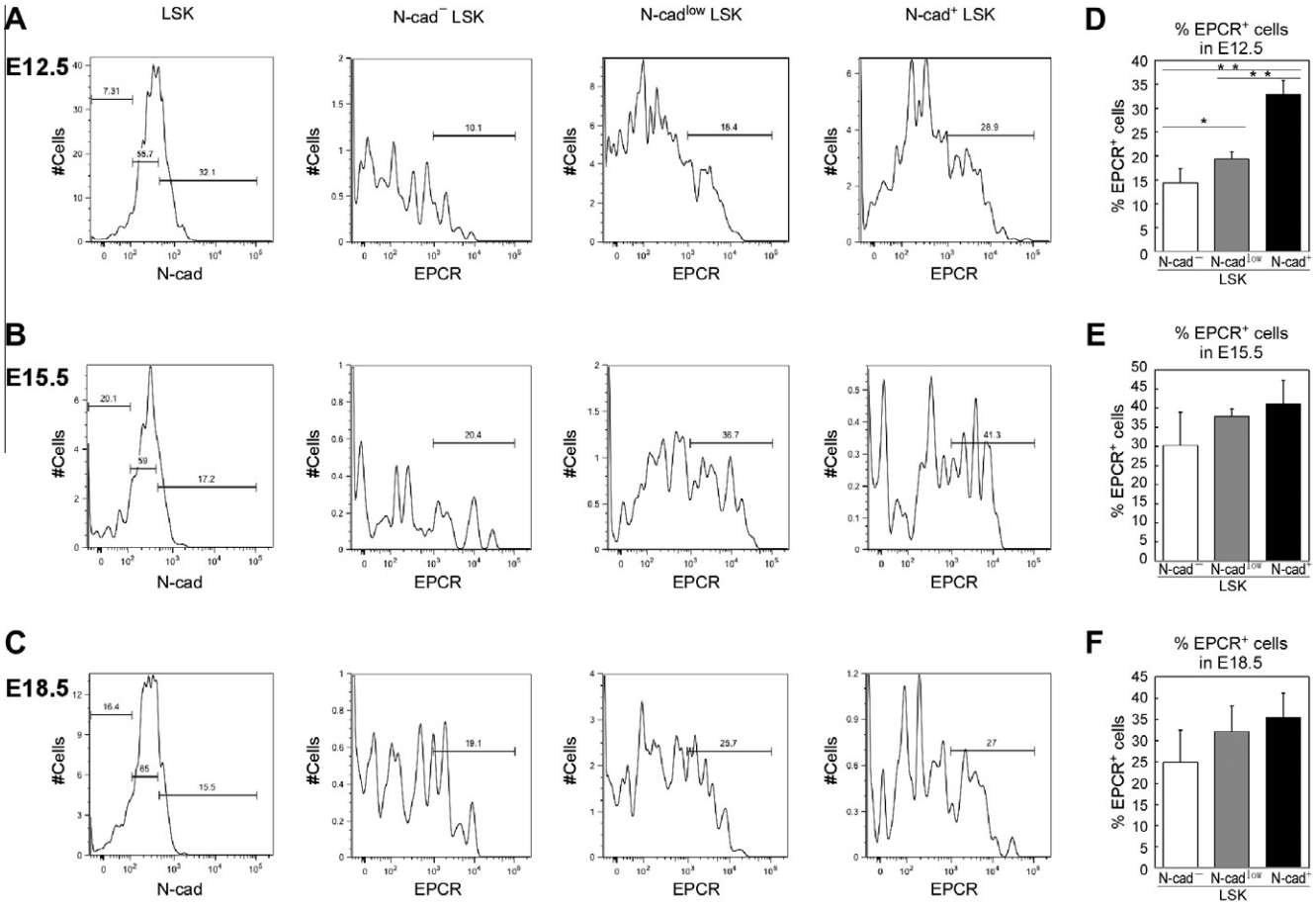

Fig. 3. Gating of EPCR in N-cad$^+$ LSK, N-cad^{low} LSK, and N-cad$^-$ LSK cells by flow cytometric analysis. (A–C) To assess EPCR expression, E12.5, E15.5, or E18.5 mouse FL cells were gated against the LSK fraction, followed by their separation into N-cad$^-$, N-cad^{low}, and N-cad$^+$ fractions. (D–F) The percentage of EPCR$^+$ cells in N-cad$^+$, N-cad^{low}, and N-cad$^-$ fractions in E12.5 (D), E15.5 (E), and E18.5 (F) FL is shown. Data represent means ± SD ($n = 4$, $^*P < 0.05$, $^{**}P < 0.01$).

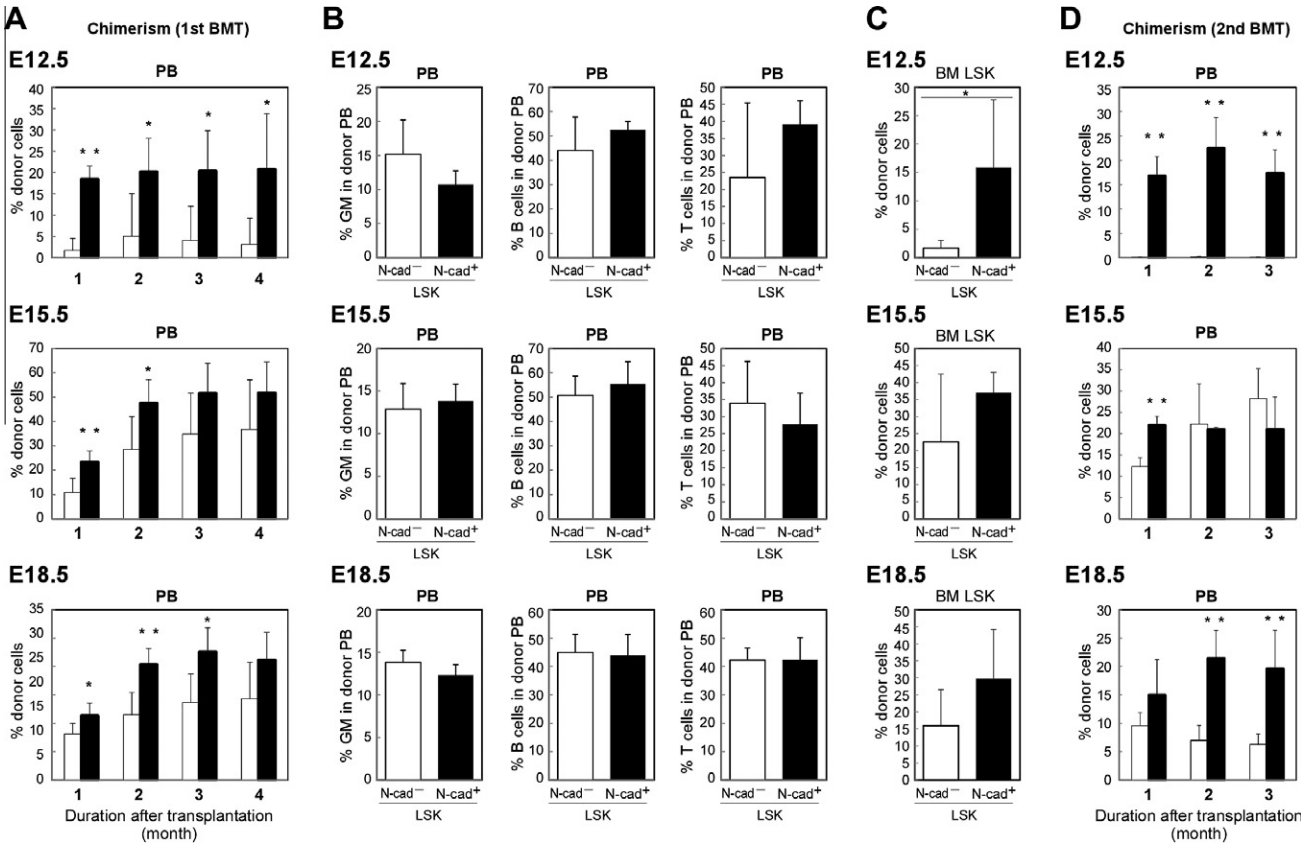

Fig. 4. LTR activity in N-cad$^+$ LSK and N-cad$^-$ LSK cells from E12.5, E15.5, and E18.5 FL. (A) Approximately 500 N-cad$^+$ LSK (black bars) or N-cad$^-$ LSK cells (white bars) isolated from E12.5, E15.5, and E18.5 Ly5.1 mice were transplanted into lethally irradiated Ly5.2 recipient mice. The chimeras of donor-derived cells in PB were analyzed 1–4 months after the first BMT ($n = 4$, $^*P < 0.05$, $^{**}P < 0.01$). (B) The frequency of macrophages and granulocytes (GM), B or T cells in donor-derived PB cells 4 months after the first BMT in recipient mice where E12.5, E15.5, or E18.5 FL LSK cells were transplanted. Data represent the means ± SD ($n = 4$, $P > 0.05$). (C) Engraftment of donor LSK cells into BM. Data represent the means ± SD ($n = 4$, $^*P < 0.05$). (D) The frequency of donor-derived cells in PB after the second BMT. Approximately 5×10^6 BMMNCs were transplanted into lethally irradiated second recipient mice 4 months after the first BMT. Data represent the means ± SD ($n = 4$, $^{**}P < 0.01$).

N-cad$^+$ LSK versus N-cad$^-$ LSK cells. We isolated N-cad$^+$ and N-cad$^-$ LSK cells from E12.5, E15.5, and E18.5 FL using anti-N-cad Ab (AbD13081), which were then transplanted into lethally irradiated recipient mice. The chimerism of Ly5.1 E12.5 FL donor-derived cells in peripheral blood (PB) was significantly higher in N-cad$^+$ LSK cells versus N-cad$^-$ LSK cells 1 month after the first BMT and it lasted until 4 months after BMT. Although E15.5 and E18.5 FL N-cad$^+$ LSK cells also exhibited higher LTR activity than N-cad$^-$ LSK cells 1 month after BMT, this elevation in LTR activity decreased gradually (Fig. 4A). On the other hand, analysis of PB 4 months after the first BMT for macrophages and granulocytes (GM), B or T cells revealed that no differentiative preference in PB was seen between N-cad$^+$ and N-cad$^-$ cells among E12.5, E15.5, or E18.5 FL (Fig. 4B). Next, we investigated the engraftment of donor cells in BM 4 months after BMT. E12.5 FL N-cad$^+$ LSK cells showed a significantly higher percentage of donor-derived cells in BM LSK cells than N-cad$^-$ cells. However, no difference in chimerism in BM LSK cells was detected in BMT in E15.5 and E18.5 FL-derived cells (Fig. 4C).

To further investigate the LTR activity of N-cad$^+$ and N-cad$^-$ HSCs, we performed serial BMT. As shown in Fig. 4D, donor-derived cells isolated from the first recipient mice where E12.5 N-cad$^+$ LSK cells were transplanted showed significantly higher reconstitution in secondary recipient mice versus those from E12.5 N-cad$^-$ LSK transplanted mice. Higher reconstitution of cells isolated from the first recipient mice that received E15.5 N-cad$^+$ LSK cells versus those from E15.5 N-cad$^-$ LSK transplanted mice was only seen 1 month after the second BMT. Lastly, donor-derived cells isolated from the first recipient mice that received E18.5 N-cad$^+$ LSK cells showed significantly higher reconstitution 2 and 3 months after, but not 1 month after the second BMT. Interestingly, although N-cad$^+$ LSK cells derived from E15.5 or E18.5 FL did not show significant elevation of reconstitution compared to N-cad$^-$ LSK cells 4 months after the first BMT, they exhibited higher reconstitution in the second BMT.

In summary, the elevation of N-cad$^+$ LSK cells in LTR activity versus N-cad$^-$ LSK cells both in PB and BM was striking in E12.5 FL, whereas this increase in LTR activity was decreased in E15.5 and E18.5 FL. These data suggest that high N-cad expression in E12.5 FL LSK cells may contribute to higher LTR activity, but that it is down-regulated during the development of fetal hematopoiesis.

4. Discussion

In this study, we produced anti-N-cad antibodies and investigated the role of N-cad in FL hematopoiesis [12]. We demonstrated that the N-cad expression level in HSCs changed dramatically during embryogenesis from E12.5 to E18.5. N-cad$^+$ HSCs at E12.5 also expressed a high level of EPCR, which indicates that N-cad and EPCR can be useful markers for immature HSCs at E12.5. Interestingly, a decrease in EPCR expression was noted in N-cad$^+$ HSCs concomitant with a decline in N-cad expression in FL HSCs, suggesting that HSC characteristics may have changed during FL development. Moreover, there was no significant difference in EPCR expression in

N-cad$^+$ HSCs versus N-cad$^-$ HSCs at E15.5 and E18.5. It is possible that the contribution of N-cad to the primitiveness of HSCs at these later stages may not be as great as that at E12.5. Consistent with FACS data, higher LTR activity was observed in N-cad$^+$ HSCs versus N-cad$^-$ HSCs at E12.5, but this activity decreased at E15.5 and E18.5. Taken together, these data suggest that N-cad$^+$ HSC function may change drastically during FL hematopoiesis. It is also worth noting that the engraftment of N-cad$^+$ HSCs at E15.5 and E18.5 was significantly better than the engraftment of N-cad$^-$ HSCs 1 month after BMT. This seems to confirm the important role of N-cad in fetal HSCs residing in the BM niche as well as in adult HSCs.

Although much remains to be known about the niche and the regulation of HSCs in FL, our group has already demonstrated the importance of the perisinusoidal niche for HSCs by using EPCR as a LT-HSC marker [21]. In this particular study, we clearly detected the existence of N-cad$^+$c-Kit$^+$ cells and N-cad$^+$EPCR$^+$ HSCs, both localizing adjacent to Lyve-1$^+$ sinusoidal ECs. Some Lyve-1$^+$ sinusoidal ECs were also positive for N-cad when our anti-N-cad Ab was used for immunofluorescence staining. The attachment of FL HSCs to the perisinusoidal niche, especially at E12.5, resembled cell adhesion between adult HSCs and the BM niche. Taken together with FACS and LTR activity data, we conclude that N-cad is an important component of the HSC niche in FL, thereby enabling HSCs to reside in the perisinusoidal region by reinforcing the interaction between ECs and the extracellular matrix, especially at E12.5. During FL development, E-cad expression was dramatically up-regulated in hepatoblasts and hepatocytes, and the number of E-cad$^+$ hepatoblasts and hepatocytes increased. E-cad$^+$ hepatocytes consequently occupied a major portion of FL, while the percentage of HSCs decreased significantly. We also detected a higher level of N-cad$^+$ HSCs which do not reside along the Lyve-1$^+$ sinusoidal ECs at E15.5 and especially at E18.5, but fewer at E12.5. It indicates that N-cad$^+$ HSCs in the perisinusoidal niche may gradually exit during FL development. On the other hand, FACS data showed a decrease in N-cad expression in HSCs from E12.5 to E18.5. Thus, we believe that the hematopoietic ability of N-cad$^+$ HSCs change during hematopoiesis. At a later stage of embryogenesis, such as during E18.5, HSCs are believed to migrate into BM, where adult hematopoiesis is known to take place.

In conclusion, we speculate that N-cad$^+$ HSCs are maintained in the perisinusoidal niche via N-cad-based adhesion and that a down-regulation in N-cad results in their exit out of the perisinusoidal niche and migration into BM. This functional shift in N-cad expression may explain why N-cad$^+$ HSCs do not show a great advantage in LTR activity over N-cad$^-$ HSCs at later stages of hematopoiesis. The dramatic down-regulation of N-cad during hematopoiesis may help us better understand the regulation and mobility of FL HSCs before migration into BM.

Acknowledgments

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