Ephrin-B2-expressing natural killer cells induce angiogenesis

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ABSTRACT

Background: Therapeutic angiogenesis aims to induce new blood vessel growth in ischemic tissues; however, previous clinical trials have had limited success. Studies of uterine angiogenesis revealed a specialized subset of natural killer (NK) cells, called uterine NK (uNK) cells, which have unique proangiogenic abilities.

Methods: We show that uNK cells in mice express ephrin-B2, a regulator of angiogenesis, to induce tubule formation in an ex vivo coculture tubule formation assay. We next induced the expression of ephrin-B2 by splenic NK (sNK) cells harvested from male mice.

Results: We showed that induced NK (iNK) cells can also instruct endothelial cells to form tubules using ephrin-B2.

Conclusions: We concluded that Ephrin-B2 is a marker of proangiogenic uNK cells and that a proangiogenic phenotype characterized by ephrin-B2 can be induced in sNK cells to induce therapeutic angiogenesis. (JVS—Vascular Science 2022;3:336-44.)

Clinical significance: Peripheral arterial disease threatens puts another 2 million limbs at risk for amputation annually; however, the clinical trials aimed at inducing angiogenesis within ischemic limbs have yielded disappointing results. New molecular therapies should be developed to address this. Like chimeric antigen receptor T cell therapy for melanoma, engineering a patient’s own cells into a population of proangiogenic cells represents a novel strategy to induce angiogenesis. Previous studies have shown that induction of a proangiogenic phenotype in natural killer cells was able to improve perfusion in the placentas of mice. Dissecting the molecular mechanisms behind these phenomena will elucidate the specific molecular targets required, and streamline the cell engineering protocol. We show that induction on natural killer cells of ephrin-B2, a proangiogenic cell membrane receptor that controls angiogenesis throughout life, induces a proangiogenic phenotype in these cells, which is the first step to a cell-based therapy for critical ischemia.

Keywords: Therapeutic angiogenesis; Ephrin-B2; NK cell; Hypoxia

Therapeutic angiogenesis aims at inducing the growth of new blood vessels within ischemic tissues, and represents a promising alternative to surgical revascularization for peripheral arterial disease (PAD). The vascular endothelial growth factor (VEGF), hypoxia inducible factor 1-alpha, and fibroblast growth factor, have been studied as potential therapies delivered as recombinant proteins or in viral or nonviral gene vectors. Additionally, the TACT, OPTIPEC, PROVASA, and JUVENTAS trials have studied the efficacy of intra-arterial or intramuscular delivery of bone marrow mononuclear cells. Despite promising preclinical studies, these studies unfortunately showed limited improvement in morbidity and mortality.

Some populations of leukocytes induce angiogenesis in tumors and other tissues, and presumably could do so in PAD. Uterine resident natural killer cells (uNK) cells represent their own lineage of NK cells that function to promote angiogenesis in the uterus during both the secretory phase of menstruation as well as during pregnancy, with seemingly unlimited proangiogenic potential throughout the reproductive life of a woman. After interaction with cells of embryonic origin, uNK cells secrete proangiogenic cytokines. During pregnancy, the uterine spiral arteries (SpA) undergo vascular remodeling characterized by loss of vascular smooth muscle cells and dilation of the vessels. Failure of vascular remodeling is linked to gestational hypertension and preeclampsia, which increase the risk of heart failure four-fold, and coronary artery disease, stroke, and death two-fold. Cerdeira et al showed that NK cells from peripheral blood could be induced to express proangiogenic growth factors. These induced decidua-like NK (iNK) cells were shown to improved placental perfusion.

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and decrease uterine artery resistance index in a mouse model of gestational hypertension. Given their incredible ability to induce angiogenesis, further study of idNK cells should be done to determine their potential outside the uterus as a therapy for PAD.

Ephrin-B2 is a transmembrane receptor tyrosine kinase that controls the development and maintenance of arterial identity by blood vessels throughout life, and thus is considered a master regulator of angiogenesis. It is perhaps not surprising that it is also involved in uterine angiogenesis, because this is a time of rapid and widespread vascular remodeling and angiogenesis within the uterus. During remodeling, SpA switch from an arterial to a venous phenotype. Vascular phenotype is controlled by the receptor-ligand pair ephrin-B2 and EphB4. In vein grafts, ephrin-B2 stimulation promotes patency by stimulating EphB4 to maintain a venous phenotype. Although ephrin ligands and their receptors, the eph family, have previously been identified in lymphocytes and other cells of the immune system, peripheral NK cells have not been characterized as ephrin- or Eph-expressing cells. uNK cells, however, were shown to express ephrin-B2 by immunohistochemistry, and it was speculated that ephrin-B2 promoted uNK cell trafficking to arteries instead of veins. However, the function of ephrin-B2 on uNK cells has not been more closely studied. We hypothesized that uNK cells use ephrin-B2 to induce angiogenesis, and that idNK cells would also use ephrin-B2 to orchestrate angiogenesis.

**METHODS**

**Study design.** uNK cells from virgin mice were harvested and the function of ephrin-B2 explored and first analyzed by flow cytometry and immunofluorescence to confirm previous reports. Flow cytometric and immunofluorescence data were confirmed by qualitative reverse transcriptase polymerase chain reaction. Interaction between uNK cells and endothelial cells (ECs) was shown in an ex vivo coculture tubule formation assay. Unless otherwise indicated, data points represent individual experimental replicates, comprising either unique mice or unique pools of mice.

**Statistical analysis.** To compare the mean fluorescence intensity or percentage of NK cells positive for ephrin-B2, unpaired t tests were performed. For analysis of the tubule formation assay the tubule lengths were first standardized to the positive control (VEGF). This was done to ensure consistency in analysis between technical replicates. The groups were first analyzed by one-way analysis of variance, then by post hoc t tests.

**Mice.** C57BL/6 mice aged 6 to 8 weeks were housed and experiments performed in accordance with the Institutional Animal Care and Use Committee of Rosalind Franklin University of Medicine and Science, under protocol B21-07, in North Chicago, IL. C57BL/6 mice were humanely killed via CO2 asphyxiation and the uterus and spleen were harvested.

Isolation of NK cells from mice. To enrich splenic NK cells, spleens were crushed and passed through a 100 μm strainer into 2% fetal bovine serum (FBS) in phosphate-buffered saline (PBS). For uNK cells, the uterus was minced and digested using 50 μg/mL Liberase (Roche, Basel, Switzerland), crushed, and passed through a 100-μm strainer. NK cells were isolated from the resulting single cell suspensions using the EasySep Mouse NK Cell Isolation Kit from Stemcell Technologies (Vancouver, BC, Canada), according to the manufacturer’s instructions.

**Immunofluorescence**. NK cells were fixed on slides in 4% formaldehyde. Primary antibodies and concentrations for immunofluorescence included 1:100 rabbit anti-Ephrin-B2 (Abcam ab131536; Cambridge, UK), 1:1000 goat anti-Rabbit IgG Alexa Fluor Plus 594 (ThermoFisher Scientific, A-32740; Waltham, MA), and 1:1000 donkey anti-Mouse IgG Alexa Fluor Plus 488 (ThermoFisher Scientific, A-21202). Slides were covered with ProLong Diamond Antifade Mountant with DAPI (Invitrogen, p36962; Waltham, MA). Slides were imaged with a Nikon Eclipse 80i fluorescence microscope.

**Antibodies and flow cytometry.** Cells were stained for viability with LIVE/DEAD Fixable Violet Dead Cell Stain Kit, for 405 nm excitation (Invitrogen, Cat. L34955). Antibodies and dilutions used for flow cytometry are as follows: 1:20 NK1.1-FITC (Biolegend, Cat. 108706; San Diego, CA), 1:20 CD11b-PerCP/Cy5.5 (Biolegend, Cat. 301328), 1:20 NKp46-PE/Cy7 (Biolegend Cat. 137618), 1:20 CD45-BV605 (Biolegend Cat. 501340), and 1:25 Ephrin-B2-PE (Santa Cruz Biotechnology, sc-398735; Santa Cruz, CA). Cells were stained and washed in PBS according to the manufacturer’s instructions (Invitrogen). Fc receptors were blocked by incubating cells in 500 μL of 2.5 μg/mL TruStain FcXTM PLUS (anti-mouse CD16/32) (Biolegend) in PBS with 2% FBS for 10 minutes at 4°C. Cells were fixed in BD Biosciences (East Rutherford, NJ) Cytofix/cytoperm solution according to manufacturer’s instructions. Cells were analyzed with the BD FACSLyric flow cytometer.

**Cell lines and tubule formation assay.** Primary mouse ECs derived from mouse uterine microvasculature were ordered from Cell Biologics Inc (Chicago, IL). Cells were passaged in complete mouse EC media with growth factors (Cell Biologics — M1168) at 37°C and 5% CO2. Cells were used for coculture tubule formation assays between passages two and six. For cocultures, 1:1 ratio of ECs to NK cells were incubated overnight on Matrigel (Corning, Corning, NY). ECs were stained with 2 μg/mL calcein AM before imaging (ThermoFisher Scientific).
Fig 1. Immunomagnetic negative selection enriches NK cells from single cell suspensions of spleen and uterus. (A) The spleens of nonpregnant female mice were mechanically disrupted and passed through a 100-µm filter to generate a single cell suspension. Suspensions were analyzed by flow cytometry. Natural killer (NK) cells were identified as single, live, CD45^+CD11b^low/NK1.1^ cells. Myeloid cells were identified as single, live, CD45^+CD11b^hi cells. B and T cells were collectively identified as single, live, CD45^+CD11b^low/NK1.1^ cells. (B) Splenic single cell suspensions were enriched for NK cells by immunomagnetic negative selection and analyzed by flow cytometry. Whole myeloid enriched myeloid, whole B/T enriched B/T, whole NK enriched NK.
For positive control, ECs were stimulated with 50 ng/mL recombinant VEGF-120 (R&D Systems, 494-VE-005; Minneapolis, MN). For ephrin-B2 blocking experiments, NK cells were incubated at room temperature for 30 minutes with 100 μg/mL of rabbit pAb anti-mouse ephrin-B2 (Abcam, ab131536), washed, and then used for coculture experiments. TNYL-RAW-miniPeg and scrambled peptide control were custom ordered from Alan Scientific and added to cocultures at a final concentration of 5 μmol/L.

**Induction of angiogenic phenotype in sNK cells.** sNK cells were enriched from male mice spleens by immunomagnetic negative selection. Cells were incubated in RPMI (Gibco, Grand Isle, NY) with 10% FBS (Sigma-Aldrich, St Louis, MO), 1 U/mL penicillin/streptomycin (Gibco), and supplemented with 10 ng/mL recombinant mouse IL-15 (R&D Systems), 2 ng/mL recombinant mouse transforming growth factor-β (R&D Systems), 1 μmol/L 5-aza-2′-deoxycytidine (Sigma-Aldrich).

**VEGF enzyme-linked immunosorbent assay.** The supernatants from coculture tubule formation assays were collected the next day and analyzed by enzyme-linked immunosorbent assay kit (R&D Systems). The total VEGF concentration was determined using the DuoSet Mouse VEGF enzyme-linked immunosorbent assay. The mean fluorescence intensity of ephrin-b2 in uNK cells was significantly higher than that of sNK cells (Fig 2, C) (sNK, 20.7 ± 44.3; uNK, 356.3 ± 159.6; P < .0001). To confirm that uNK cells expressed ephrin-b2, we enriched single cell suspensions of spleen or uterus for NK cells using immunomagnetic negative selection (Fig 1, E, F). uNK cells express significantly more Efnb2 mRNA relative to Gapdh than sNK cells (Fig 2, D) (sNK, 0.4 ± 0.9; uNK cells, 11.4 ± 13.9; P = .02).

**RESULTS**

**Ephrin-B2 expression distinguishes uNK cells from peripheral NK cells.** To confirm expression of ephrin-B2, NK cells were harvested from the uterus (uNK) or spleen (sNK) of nonpregnant mice and analyzed by flow cytometry (Fig 1, A-D). NK cells were identified as single, live, CD45+CD11b−/lowNK1.1+ cells. Myeloid cells were identified as CD45−CD11b+ cells, and the remaining non-NK lymphoid population including B and T lymphocytes was collectively identified as CD45+CD11b−/lowNK1.1− cells. We detected strong ephrin-B2 expression by NK cells in the uterus (Fig 2, A). Myeloid cells, sNK cells, and uNK cells all expressed some ephrin-B2; however, uNK cells were the most positive population (Fig 2, B) (spleen B/T cells, 8.4% ± 1.9%; uterine B/T cells, 5.5% ± 1.3%; P = .20; spleen myeloid cells, 41.5% ± 9.0%; uterine myeloid cells, 46.2% ± 5.1%; P = .88; spleen NK, 21.2% ± 4.4%; uNK, 71.5% ± 14.6%; P < .0001). Male sNK cells had the lowest expression of ephrin-B2, and significantly fewer sNK cells were positive for ephrin-B2 in males than in females (Fig 2, B) (male sNK, 4.0% ± 2.4%). For female mice, the mean fluorescence intensity of ephrin-b2 in uNK cells was significantly higher than that of sNK cells (sNK, 20.7 ± 44.3; uNK, 356.3 ± 159.6; P < .0001). Male sNK cells had the lowest expression of ephrin-B2, and significantly fewer sNK cells were positive for ephrin-B2 in males than in females (Fig 2, B) (male sNK, 4.0% ± 2.4%). For female mice, the mean fluorescence intensity of ephrin-b2 in uNK cells was significantly higher than that of sNK cells (Fig 2, C) (sNK, 20.7 ± 44.3; uNK, 356.3 ± 159.6; P < .0001). To confirm that uNK cells expressed ephrin-b2, we enriched single cell suspensions of spleen or uterus for NK cells using immunomagnetic negative selection (Fig 1, E, F). uNK cells express significantly more Efnb2 mRNA relative to Gapdh than sNK cells (Fig 2, D) (sNK, 0.4 ± 0.9; uNK cells, 11.4 ± 13.9; P = .02).

**uNK cells use ephrin-B2 to induce EC tubule formation.** We hypothesized that uNK cells induce angiogenesis using ephrin-B2. NK cells were preincubated with a blocking antibody (Ab) to ephrin-B2 or an isotype control followed by an overnight coculture with suspended ECs on Matrigel matrix. When incubated with uNK cells, ECs formed tubes that were similar in length to the VEGF positive control (Fig 3, A). Blocking ephrin-B2 on uNK cells resulted in significantly less tubule formation by the ECs (uNK + Iso, 113% ± 26%; uNK + Block, 27% ± 20%; P < .01), sNK cells induced little tubule formation in the presence of either isotype control or blocking Ab, although there was a significant decrease in tubule length in the presence of the blocking Ab (sNK + Iso, 29.2 ± 10.3%; sNK + Block, 10.0 ± 3.1%; P = .02). We asked whether this effect was due to the blocking Ab interfering directly with the ECs. We observed no difference in tubule formation when ECs were stimulated with VEGF plus the isotype control or VEGF plus the blocking Ab (110.8 ± 12.4% vs 80.0 ± 24.0% correspondingly; P = .5). We concluded that uNK cells are strong inducers of angiogenesis and that this effect is mediated by ephrin-B2.

**uNK cell ephrin-B2 interacts with ECs via EphB4.** To further characterize the role of ephrin-B2 on uNK cell-mediated angiogenesis, we asked whether uNK cell ephrin-B2 was interacting with one of its receptors, EphB4, on ECs. TNYL-RAW is a peptide that specifically blocks the interaction of ephrin-B2 with EphB4, but not...
with other EphB receptors, and this is significant because the ephrin-B2-EphB4 binding interaction is known to regulate angiogenesis and EC migration.17,21,22 Compared with a scrambled control peptide (scram), when uNK cells and ECs were cocultured in the presence of TNYL-RAW, endothelial tubule formation was compromised (Fig 4, A). When standardized to the positive control, significantly shorter tubules formed in the presence of TNYL-RAW (Fig 4, B; scram, 104.3% ± 37.2% of positive control; TNYL, 43.6% ± 25.6% of positive control; P = .004). Importantly, we found that TNYL-RAW had no effect on tubules formed in response to VEGF (Fig 4, B). Because ephrin-B2 is known to regulate processing of the VEGF receptor by ECs, and because this would affect tubule formation, we measured VEGF in the coculture supernatant.23 There was no significant difference in VEGF concentration in the supernatants (Fig 3, C) (uNK ± scram, 46.0 ± 16.4 pg/mL; uNK ± TNYL-RAW, 54.21 ± 16.4

Fig 2. Uterine natural killer (uNK) cells, but not splenic NK (sNK) cells, express ephrin-B2 in nonpregnant female mice. (A) (Left) Representative histograms of ephrin-B2 or isotype staining in B/T cells, myeloid cells, and NK cells, from the uterus (above, red) or spleen (below, blue) of nonpregnant female mice. Isotype control is depicted for each population in gray. Right: immunofluorescent staining for ephrin-B2 in NK cells enriched from uterus or spleen by immunomagnetic negative selection. (B) Percentage of cells that are ephrin-B2-positive by flow cytometry in splenic or uterine populations without immunomagnetic negative selection. (C) Mean fluorescent intensity of ephrin-B2 in splenic or uNK cells (t test). (D) The expression of Ephrin-B2 (Efnb2) was confirmed by reverse transcriptase polymerase chain reaction analysis. Ct values were standardized to Gapdh expression. Data represent mean ± standard deviation. ns, not significant.
pg/mL; \( P = .41 \). We concluded that ephrin-B2 expressed by uNK cells induces tubule formation by interacting with ECs via the receptor EphB4.

**sNK cells are induced to express ephrin-B2 and become proangiogenic.** Because male sNK cells were the lowest ephrin-b2-expressing cells, we asked whether ephrin-B2 expression is inducible in male sNK cells, we harvested NK cells from the spleens of male mice. We induced a proangiogenic phenotype in NK cells following the protocol developed by Cerdeira et al.\(^{14}\) Briefly, freshly isolated NK cells incubated for 1 week in the presence of transforming growth factor-\( \beta \) and the demethylating agent, 5-aza-2'-deoxycytidine in 1% oxygen (induced NK [iNK] cells) or at 21% oxygen (control). Compared with freshly harvested sNK cells and control NK cells, iNK cells had a modest but significant increase in ephrin-B2 expression by flow cytometry (Fig 5, A) (fresh, 4.0% ± 2.1%; control, 4.6% ± 2.0%; induced, 8.2% ± 2.8%; control vs induced \( P = .0259 \)). The expression of \( Efnb2 \) by iNK cells was confirmed by reverse transcriptase polymerase chain reaction and, although there was no difference in the expression of \( Efnb2 \) between fresh and iNK cells, there was a significant increase of \( Efnb2 \) by iNK cells compared with control NK cells (Fig 5, B) (fresh, 11.6 ± 3.9; control, 0.2 ± 0.5; induced, 14.5 ± 12.6; control vs induced \( P = .0108 \)). To determine whether iNK cells can induce tubule formation by ECs via
ephrin-B2-EphB4 engagement, we cocultured the control or iNK cells with ECs on Matrigel overnight in the presence of TNYL-RAW or a scram (Fig 5, C). Tubules formed by iNK cells were significantly longer than those formed by control NK cells (Fig 5, D). We concluded that induction of ephrin-B2 allows NK cells to induce angiogenesis.

DISCUSSION

We show that NK cells expressing ephrin-B2 induce angiogenesis by interacting with ECs via the ephrin-B2 receptor, EphB4. In female mice, uNK cells, and to a limited extent sNK cells, can induce tubule formation by ECs, and ephrin-B2 expression by the NK cells was the mediator of tubule formation. We also showed that the NK cell-mediated induction of tubule formation depends on the interaction of ephrin-B2 with its receptor, EphB4, by using the peptide TYNL-RAW. This finding suggests that NK cells are not just promoting angiogenesis, but may also control vessel phenotype by modulating ephrin-B2-EphB4 signaling. Further study is needed to determine if and to what extent the ECs are upregulating EphB4 in response to uNK cells.

Ephrin-B2 or its receptor, EphB4, have shown promise as therapies to induce angiogenesis in ischemic limbs and in ischemic cardiovascular disease. Because male sNK cells had the lowest expression of ephrin-B2, and because the burden of cardiovascular disease is higher in males, we sought to determine whether ephrin-B2 expression could be induced and showed that they gain the ability to promote angiogenesis in a tubule formation assay. Our results support those of Cerdeira et al and Cavalli et al, which show that human INK cells have the ability to be reinjected into mice where they improve vascular remodeling and placental perfusion. We go one step further by identifying a cell-cell interaction-based
mechanism by which iNK cells induce angiogenesis that is reliant on ephrin-B2. Although RNA expression of Efnb2 by iNK cells does not seem to be higher than in fresh sNK cells, it is worth noting that these cells incubated under hypoxic conditions for 1 week and did not lose Efnb2 expression like control cells did. Cavalli et al.\textsuperscript{15} show that induced cells upregulate cell stress-related pathways, which can affect a number of housekeeping genes, although we found no difference in Gapdh expression. Our results also show that their induction protocol can be used in mouse models, making it applicable to in vivo angiogenesis assays. We believe that iNK cells expressing ephrin-B2 are excellent candidates for further study as a strategy for therapeutic angiogenesis, and that, like those injected into mice by Cavalli et al.\textsuperscript{15}, iNK cells can also function in human tissues to improve tissue perfusion outside the uterus. The application of iNK cells to mouse models of peripheral ischemia would determine the functionality of these cells in tissues outside the uterus.

Although our data uncover a promising molecular mechanism to induce angiogenesis in engineered cells, limitations to our model require additional studies before transitioning our model into in vivo studies. Namely, detailed analysis of interactions outside of ephrin-b2 between NK cells and ECs in our model would identify additional or alternative pathways leading to the induction of angiogenesis. Although our methods required EC starvation and the tubule formation assays were conducted in growth factor-free media, we cannot
rule out the secretion of growth factors by the NK cells or the ECs in response to their interactions. However, we found no difference in the supernatant of VEGF between TYNL-RAW and scrambled control wells, which strengthens our conclusion that ephrin-b2 is the primary mechanism regulating NK cell-mediated EC tubule formation. Our study design used the length of tubules formed by ECs as the measure for angiogenic capacity of NK cells; this measure is useful, but does not fully represent the complex process of angiogenesis. Further studies should be done detailing the phenotype of ECs after incubation in the presence of NK cells, and whether the induction of ephrin-B2 on NK cells affects EC gene expression, as well as other measures of angiogenesis such as EC migration assays.

CONCLUSIONS

uNK cells use ephrin-B2 to induce angiogenesis. Inducing ephrin-B2 on peripheral NK cells results in a proangiogenic cell population. Ephrin-B2-expressing induced NK cells are good candidates for further study as a strategy for therapeutic angiogenesis.

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REFERENCES
1. Annex BH, Cooke JP. New Directions in Therapeutic Angiogenesis and Arteriogenesis in Periphreral Arterial Disease. Circ Res 2021;128:1944-57.
2. Iyer SR, Annex BH. Therapeutic Angiogenesis for Periphreral Artery Disease. JACC Basic Transl Sci 2017;2:503-12.
3. Giacca M, Zacchigna S. VEGF gene therapy: therapeutic angiogenesis in the clinic and beyond. Gene Ther 2012;19:622-9.
4. Duong Van Huyen J-P, Smadja DM, Bruneval P, Gaussem P, Dal-Cortivo L, Julia P, et al. Bone marrow-derived mononuclear cell therapy induces distal angiogenesis after local injection in critical leg ischemia. Mod Pathol Off J U S Can Acad Pathol Inc 2008;21:837-46.
5. Walter DH, Krankenberg H, Balzer JO, Kalka C, Baumgartner I, Schluter M, et al. PROVASA Investigators. Intraarterial administration of bone marrow mononuclear cells in patients with critical limb ischemia: a randomized-start, placebo-controlled pilot trial (PROVASA). Circ Cardiovasc Interv 2011;4:26-37.
6. Teraa M, Sprengers RW, Schutgens REG, Slaper-Cortenbach IC, van der Graaf Y, Algra A, et al. Effect of Repeated Intra-Arterial Infusion of Bone Marrow Mononuclear Cells in Patients With No-Option Limb Ischemia. Circulation 2015;131:851-60.
7. Matoba S, Tsutsumi T, Murohara T, Inami T, Katsuda Y, Ito M, et al. TACT Follow-up Study Investigators. Long-term clinical outcome after intramuscular implantation of bone marrow mononuclear cells (Therapeutic Angiogenesis by Cell Transplantation [TACT] trial) in patients with chronic limb ischemia. Am Heart J 2008;156:1010-8.
8. Sojka DK, Yang L, Yokoyama WM. Uterine natural killer cells: To protect and to nurture. Birth Defects Res 2018;101:531-8.
9. Lash GE, Schiessl B, Kirkley M, Innes BA, Cooper A, Searle RF, et al. Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. J Leukoc Biol 2006;80:572-80.
10. Filipovic I, Chiassone L, Vaccaro P, Hamilton RS, Ingegnere T, Doisne J-M, et al. Molecular definition of group I innate lymphoid cells in the mouse uterus. Nat Commun 2018;9:4492.
11. Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. Hypertension 2013;62:1046-54.
12. Wu P, Hathihotowu R, Kwok CS, Babu A, Kotronias RA, Rushton C, et al. Preeclampsia and Future Cardiovascular Health. Circ Cardiovasc Qual Outcomes 2017;10:e003497.
13. Brosens IA, Robertson WB, Dixon HC. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu 1972;1:177-91.
14. Cerdeira AS, Rajakumar A, Royle CM, Lo A, Hussain Z, Thadhani RI, et al. Conversion of peripheral blood NK cells to a decidual NK-like phenotype by a cocktail of defined factors. J Immunol 2013;190:3939-48.
15. Cavalli RC, Cerdeira AS, Pernicone E, Korkes HA, Burke SD, Rajakumar A, et al. Induced Human Decidual NK-Like Cells Improve Utero-Placental Perfusion in Mice. PLoS One 2016;11:e0164353.
16. Zhang J, Dong H, Wang B, Zhu S, Croy BA. Dynamic changes occur in patterns of endometrial EFNB2/EphB4 expression during the period of spiral arterial modification in mice. Biol Reprod 2008;79:450-8.
17. Wolf K, Hu H, Isaji T, Dardik A. Molecular identity of arteries, veins, and lymphatics. J Vasc Surg 2019;69:253-62.
18. Jadowsic CC, Feigel A, Yang C, Feinstein AJ, Kim ST, Collins MJ, et al. Reduced adult endothelial cell EphB4 function promotes venous remodeling. Am J Physiol Cell Physiol 2013;304:C627-35.
19. Muto A, Yi T, Harrison KD, Dávalos A, Fancher TT, Ziegler KR, et al. Eph-B4 prevents venous adaptive remodeling in the adult arterial environment. J Exp Med 2021;208:561-75.
20. Darling TK, Lamb TJ. Emerging Roles for Eph Receptors and Ephrin Ligands in Immunity. Front Immunol 2019;10.
21. Noberini R, Mitra S, Salvucci O, Valancia F, Duggineni S, Prigozhina N, et al. Induced Human Decidual NK-Like Cells Improve Utero-Placental Perfusion in Mice. PLoS One 2011;6:e28681.
22. Koolpe M, Burgess R, Dail M, Pasquale EB. EphB receptor-binding peptides identified by phage display enable design of an antagonist with ephrin-like affinity. J Biol Chem 2005;280:17301-11.
23. Wang Y, Nakayama M, Pitulescu ME, Schmidt TS, Bochenek ML, Sakakibara A, et al. Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis. Nature 2010;465:483-6.
24. Broqueres-You D, Leré-Déan C, Merkulova-Rainon T, Mansounga CS, Allanic D, Hainaud P, et al. Ephrin-B2—Activated Peripheral Blood Mononuclear Cells From Diabetic Patients Restore Diabetes-Induced Impairment of Postischemic Neovascularization. Diabetes 2012;61:2621-32.
25. Yang D, Jin C, Ma H, Huang M, Shi G-P, Wang J, et al. EphrinB2/EphB4 pathway in postnatal angiogenesis: a potential therapeutic target for ischemic cardiovascular disease. Angiogenesis 2016;19:297-309.

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