PDGF-D–PDGFRβ signaling enhances IL-15–mediated human natural killer cell survival

Shoubao Ma[a,b,1], Tingting Tang[a,1], Xiaojin Wu[c,1], Anthony G. Mansour[a], Ting Lu[a], Jianying Zhang[d], Li-Shu Wang[e], Michael A. Caligiuri[a,b,f,2], and Jianhua Yu[a,b,f,2]

[a]Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, Los Angeles, CA 91010; [b]Hematologic Malignancies Research Institute, City of Hope National Medical Center, Los Angeles, CA 91010; [c]Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou 215123, China; [d]Department of Computational and Quantitative Medicine, City of Hope National Medical Center, Los Angeles, CA 91010; [e]Division of Hematology and Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI 53226; and [f]Comprehensive Cancer Center, City of Hope, Los Angeles, CA 91010

Edited by Marco Colonna, Department of Pathology & Immunology, Washington University in St. Louis School of Medicine, St. Louis, MO; received July 31, 2021; accepted November 24, 2021

The axis of platelet-derived growth factor (PDGF) and PDGF receptor-beta (PDGFRβ) plays prominent roles in cell growth and motility. In addition, PDGF-D enhances human natural killer (NK) cell effector functions when binding to the NKp44 receptor. Here, we report an additional but previously unknown role of PDGF-D, whereby it mediates interleukin-15 (IL-15)–induced human NK cell survival but not effector functions via its binding to PDGFRβ but independent of its binding to NKp44. Resting NK cells express no PDGFRβ and only a low level of PDGF-D, but both are significantly up-regulated by IL-15, via the nuclear factor κB signaling pathway, to promote cell survival in an autocrine manner. Both ectopic and IL-15–induced expression of PDGFRβ improves NK cell survival in response to treatment with PDGF-D. Our results suggest that the PDGF-D–PDGFRβ signaling pathway is a mechanism by which IL-15 selectively regulates the survival of human NK cells without modulating their effector functions.

IL-15 | NK cells | PDGF-D | cell survival | PDGFRβ

Natural killer (NK) cells—a distinct lymphocyte subset in the circulation—play critical roles in antiviral and antitumor immunity (1). One advantage of NK cells is that they recognize "nonself" cells without being activated by specific antigens, allowing a more rapid response than with T cells. This broad cytotoxicity and rapid killing make NK cells ideal for cancer immunotherapy (2). Of note, chimeric-antigen-receptor (CAR)-NK cells have several therapeutic advantages over CAR-T cells (2–4). However, NK cells' shorter lifespan may limit their clinical efficacy. A better understanding of the mechanisms that regulate NK cell survival might therefore improve their clinical application for cancer immunotherapy.

Platelet-derived growth factor (PDGF) is one of the main growth factors that regulate cell growth and division (5). The PDGF family consists of PDGF-A, PDGF-B, PDGF-C, and PDGF-D (5). These ligands bind to two tyrosine kinase receptors, PDGFRα and PDGFRβ (5). Upon activation by PDGFs, PDGF receptors dimerize and undergo autophosphorylation on tyrosine residues in the intracellular domain, which mediates the binding of cofactors and subsequently activates signal transduction, including Ras/Raf/MEK/Erk mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase/protein kinase B (P13K/AKT) (5). PDGFs play prominent roles in cell differentiation, cell growth, cell transformation, and cell migration (5). A recent study showed that PDGF-D is a ligand of NKp44, one of the natural cytotoxicity receptors expressed by activated human NK cells (6). PDGF-D binding to NKp44 prompted NK cells to secrete interferon (IFN)-γ and tumor necrosis factor (TNF)-α, arresting the growth of tumor cells (6). However, little is known about the role of PDGFR signaling in NK cell immunity.

In this study, we report a previously unknown role of PDGF-D: regulation of interleukin 15 (IL-15)–mediated cell survival—not effector functions in human NK cells—that is dependent on PDGFRβ but independent of NKp44. Our findings suggest that the PDGF-D–PDGFRβ signaling pathway is a mechanism by which IL-15 selectively regulates the survival of human NK cells but not their effector functions.

Results

NK Cells Express High Levels of PDGFRβ following IL-15 Stimulation. Previous studies suggested that NK cells and NK leukemia cells might express PDGF receptors (7, 8). However, the expression patterns have not been clearly described. Here, we enriched NK cells from peripheral blood mononuclear cells (PBMCs) of healthy donors (HDs) and examined PDGFRα and PDGFRβ expression by flow cytometry. Unexpectedly, PDGFRα and PDGFRβ could not be detected in resting primary NK cells from HDs (Fig. 1 *A*–*C* and *SI Appendix*, Fig. S1*A*). We then evaluated their levels after stimulation with different cytokines (IL-2, IL-12, IL-15, and IL-18) or their combinations for 24 h. PDGFRα could not be induced by those cytokines or even their combinations (*SI Appendix*, Fig. S1*A*). In contrast, IL-15 induced robust expression of PDGFRβ (Fig. 1 *A*–*C*), and the effect was dose- and time-dependent (Fig. 1 *D* and *E*). IL-12,

Significance

Natural killer (NK) cells belong to a critical innate arm of host immunity against viral infection and malignancies. However, limited expansion and persistence of NK cells in vivo remain major challenges for NK cell-based therapy. Here, we show that platelet-derived growth factor (PDGF)-D–PDGF receptor-beta signaling—a potent stimulator of cell growth and motility—activates NK cells in an autocrine manner and contributes to interleukin-15–mediated NK cell survival but not effector functions, the latter of which were previously shown to depend on the binding of PDGF-D to the NKp44 receptor. Therefore, selectively introducing PDGF signaling into NK cells should benefit NK cell expansion and persistence and/or enhance effector function in NK cell-based immunotherapies.

Author contributions: S.M., M.A.C., and J.Y. designed research; S.M., T.T., and X.W. performed research; A.G.M. and T.L. contributed new reagents/analytic tools; S.M., J.Z., and J.Y. analyzed data; and S.M., L.W., M.A.C., and J.Y. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

[1]S.M., T.T., and X.W. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: mcaligiuri@coh.org or jiayu@coh.org.

This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2114134119/-/DCSupplemental.

Published January 18, 2022.

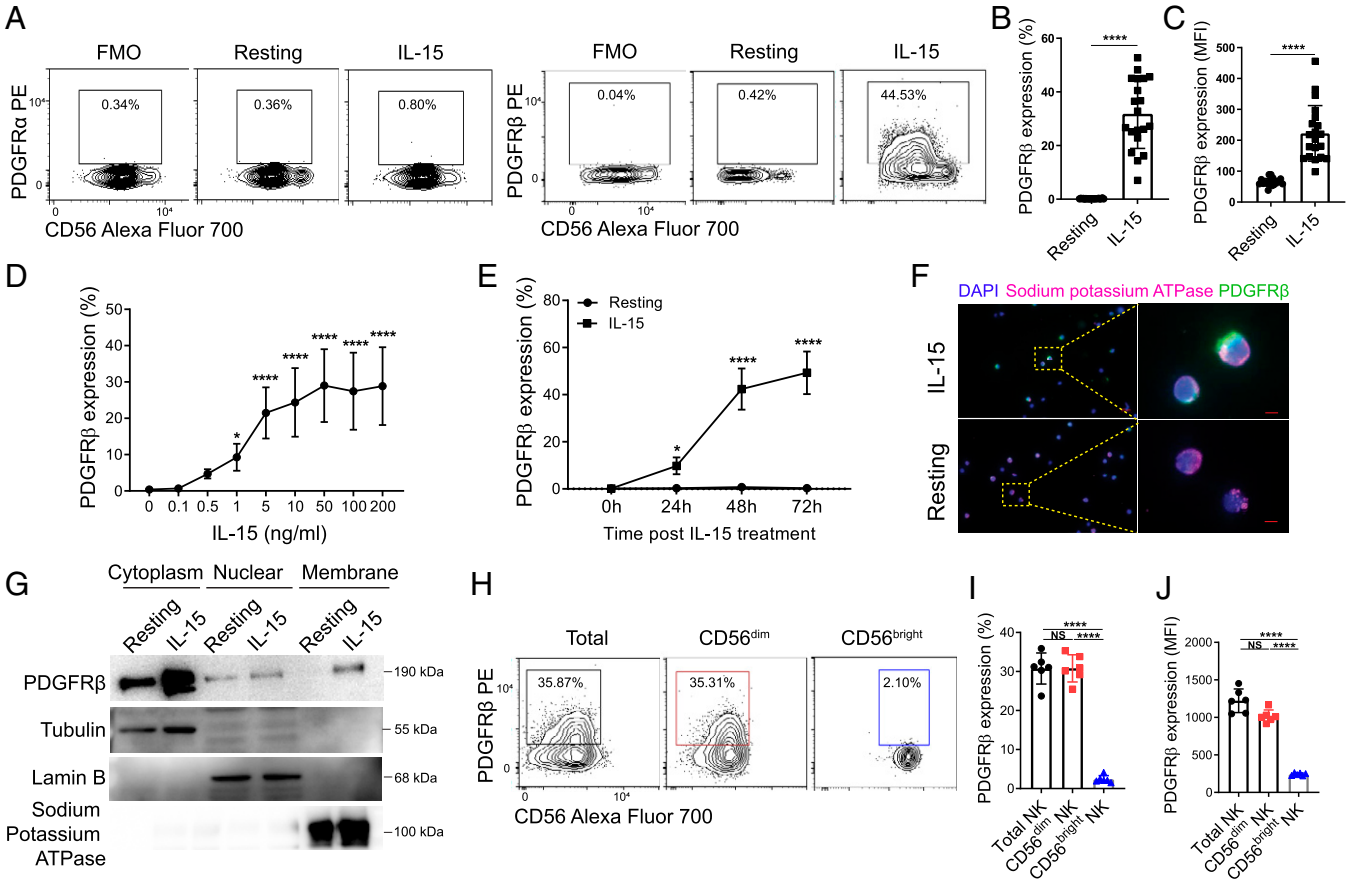

Fig. 1. IL-15 induces PDGFRβ expression in human NK cells. (*A–C*) NK cells were purified from PBMCs of healthy donors and stimulated with IL-15 (10 ng/mL) for 24 h. Expression levels of PDGFRα and PDGFRβ were examined by flow cytometry. Data shown are representative dot plots (*A*), percentages (*B*), and mean fluorescence intensity (MFI) of PDGFRβ in NK cells (*C*) (n = 20). Resting NK cells were used as the control. (*D* and *E*) NK cells were treated with various doses of IL-15 for 24 h (*D*) or with the same dose of IL-15 (10 ng/mL) at the indicated times (*E*). Data shown are the percentages of PDGFRβ$^+$ NK cells among total NK cells (n = 5). (*F*) Immunofluorescence analysis of PDGFRβ expression on resting and IL-15–treated NK cells. Sodium-potassium ATPase was used as a cell membrane marker. (Scale bar, 20 μm.) (*G*) Expression of PDGFRβ in the cytoplasmic, nuclear, and cell membrane fractions of NK cells determined by immunoblotting. (*H–J*) CD56^{dim} and CD56^{bright} NK cells were sorted from PBMCs of healthy donors and treated with IL-15 (10 ng/mL) for 24 h. Expression levels of PDGFRβ were then determined by flow cytometry. Data shown are representative dot plots (*H*), percentages (*I*), and MFI (*J*) of PDGFRβ on NK cells (n = 6). Data represent three independent experiments. Data shown are means ± SD. NS, not significant. $^*P < 0.05$ and $^{****}P < 0.0001$.

IL-18, or the two combined was ineffective (*SI Appendix*, Fig. S1*B*). IL-2, which shares the cognate receptors IL-2Rβ and IL-2Rγc with IL-15, slightly but not significantly induced PDGFRβ expression (*SI Appendix*, Fig. S1*B*) after 24-h culture at the concentration of 10 ng/mL. We further evaluated whether IL-2 could induce PDGFRβ expression at longer time points or higher doses. The results showed that the high doses of IL-2 (200 ng/mL) could only induce weak levels of PDGFRβ (*SI Appendix*, Fig. S1*C*). A longer (up to 72 h) stimulation at the low dose (10 ng/mL) was ineffective (*SI Appendix*, Fig. S1*D*). Our data on IL-2 are consistent with those from a prior study (6). Immunofluorescence confirmed that PDGFRβ was expressed on the membrane after IL-15 stimulation (Fig. 1*F*). Immunoblotting showed that PDGFRβ was enriched in the cytoplasm and translocated to the cell membrane following stimulation by IL-15 (Fig. 1*G*).

NK cells in peripheral blood are typically divided into CD56^{dim} and CD56^{bright} populations (9, 10). Interestingly, we found that IL-15 induced PDGFRβ expression only in CD56^{dim} NK cells and not CD56^{bright} NK cells (Fig. 1 *H–J*), suggesting that PDGFRβ signaling affects only the former. To explore whether our findings in human NK cells could be reproduced in mouse NK cells, we purified splenic NK cells from C57BL/6 mice. However, the murine NK cells did not express PDGFRβ in the resting state or when stimulated with IL-2, IL-12, IL-15, or IL-12 plus IL-15 (*SI Appendix*, Fig. S1*E*). We also could not detect any PDGFRβ after long-term IL-15 stimulation in our IL-15 transgenic mice (11, 12) (*SI Appendix*, Fig. S1*F*). Thus, IL-15 induced PDGFRβ expression only in human NK cells. Taken together, our findings indicate that human NK cells express high levels of PDGFRβ after they are primed with IL-15.

IL-15 Induces PDGFRβ Expression through PI3K/AKT Signaling. To explore the mechanism by which IL-15 induces PDGFRβ expression in NK cells, we first measured messenger RNA (mRNA) levels of the *PDGFRB* gene. They increased significantly after IL-15 treatment (Fig. 2 *A* and *B*), indicating that IL-15 induces *PDGFRB* expression at the transcriptional level. When we inhibited gene transcription with actinomycin D (ActD), IL-15 no longer induced PDGFRβ expression (Fig. 2*C*). In addition, blocking de novo protein synthesis with cycloheximide completely inhibited the PDGFRβ expression induced by IL-15 (Fig. 2*D*).

IL-15 signaling is mediated by at least three downstream signaling pathways in NK cells: MAPK/extracellular-signal-regulated kinase (ERK), PI3K/AKT, and signal transducer and activator of transcription 3/5 (STAT3/5) (*SI Appendix*, Fig. S2*A*)

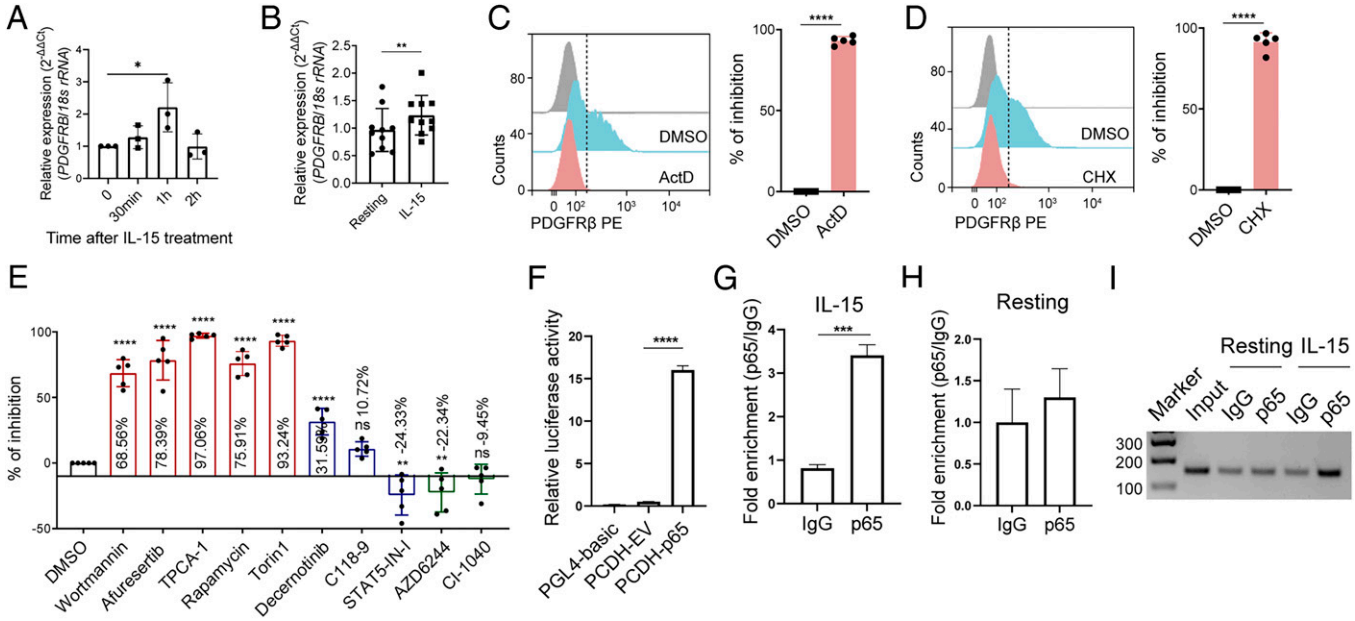

Fig. 2. IL-15–induced PDGFRβ expression is mediated by PI3K/AKT signaling. (*A* and *B*) Primary NK cells were treated with IL-15 (10 ng/mL) for the indicated times. mRNA levels of *PDGFRB* at different time points (*A*) ($n = 3$) or at 1 h (*B*) ($n = 10$) were examined by qPCR. (*C* and *D*) Primary NK cells were pretreated with ActD (5 μg/mL) (*C*) or cycloheximide (CHX, 20 μg/mL) (*D*) for 1 h, washed twice with RPMI-1640, and then treated with IL-15 (10 ng/mL) for 24 h. Dimethyl sulfoxide (DMSO) was used as a control. Expression levels of PDGFRβ were examined by flow cytometry ($n = 5$). Data shown are representative histograms and percentage of inhibition with the following equation: % inhibition = 100 × [1 − (DMSO-inhibitor)/DMSO]. (*E*) Primary NK cells were pretreated with wortmannin (1 μM), afuresertib (10 μM), TPCA-1 (1 μM), rapamycin (10 μM), torin1 (10 μM), decernotinib (10 μM), C118-9 (10 μM), STAT5-IN-1 (10 μM), AZD6244 (10 μM), or CI-1040 (10 μM) for 1 h, washed twice with RPMI 1640, and then treated with IL-15 (10 ng/mL) for 24 h. DMSO was used as control. Data shown are percent of inhibition ($n = 5$). The mean value of the inhibitory rate is shown. (*F*) Luciferase reporter assay shows that p65 activates *PDGFRB* gene transcription. (*G–I*) Binding of p65 to the *PDGFRB* promoter in IL-15–treated NK cells (*G*) or resting NK cells (*H*) as determined by ChIP-qPCR or PCR (*I*) ($n = 3$). Data represent three independent experiments. Data shown are means ± SD. NS, not significant. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

(13). To determine which of these pathways regulates IL-15–mediated PDGFRβ induction, we pretreated NK cells with inhibitors that target specific pathway components and then stimulated the cells with IL-15 (*SI Appendix*, Fig. S2*A*). The PI3K inhibitor wortmannin and the pan-AKT kinase inhibitor afuresertib inhibited PDGFRβ expression by ∼70% (Fig. 2*E* and *SI Appendix*, Fig. S2*B*). Activated AKT activates downstream molecular proteins such as nuclear factor κB (NF-κB) and mTOR (13). We found that TPCA-1, an inhibitor of IkappaB kinase (an upstream component of NF-κB signaling), completely prevented IL-15 from stimulating PDGFRβ expression (Fig. 2*E* and *SI Appendix*, Fig. S2*B*). Treating NK cells with mTOR inhibitors, such as rapamycin or torin1, also dramatically and significantly blocked PDGFRβ expression by IL-15 (Fig. 2*E* and *SI Appendix*, Fig. S2*B*). In contrast, blocking Janus kinase 3 (JAK3) (decernotinib) signaling reduced PDGFRβ expression by approximately one-third (Fig. 2*E* and *SI Appendix*, Fig. S2*B*); treatment with STAT signaling inhibitors (e.g., the STAT3 inhibitor C118-9 or the STAT5 inhibitor STAT5-IN-1) or MAPK signaling inhibitors such as the MEK1 inhibitor AZD6244 or the MEK1/2 inhibitor CI-1040 produced either little inhibition or even promoted IL-15–induced PDGFRβ expression (Fig. 2*E* and *SI Appendix*, Fig. S2*B*). These data suggest that IL-15 likely induces PDGFRβ expression through the PI3K/AKT pathway. To strengthen our hypothesis, we showed that p65, a subunit of the NF-κB complex that is downstream of the PI3K/AKT pathway, has a binding site in the promoter region of *PDGFRB* (*SI Appendix*, Fig. S2*C*). Moreover, a luciferase reporter assay showed that p65 directly activated *PDGFRB* gene transcription (Fig. 2*F*). Chromatin immunoprecipitation (ChIP)-qPCR showed that IL-15–stimulated NK cells—but not resting NK cells—had significantly higher levels of p65 associated with the *PDGFRB* promoter than the normal immunoglobulin G (IgG) control (Fig. 2 *G–I*), indicating that p65 binds directly to the *PDGFRB* gene promoter in IL-15–stimulated NK cells. Of note, we found that IL-2 induced a level of phosphor (p)-p65 significantly lower than IL-15 at all the time points that we tested except for the 5-min early time point, when IL-2 induced a slightly higher level of p-p65 than IL-15 (*SI Appendix*, Fig. S2 *D* and *E*). This suggests that IL-2 is unable to strongly and durably induce the high levels of p-p65 that NK cells seem to require to up-regulate PDGFRβ. Collectively, our data demonstrate that PDGFRβ expression in human NK cells is regulated by PI3K/AKT/NF-κB, which is downstream of IL-15 signaling.

Transcriptional programs are regulated by chromatin accessibility, which can be indicated by transposase recognition and histone 3 lysine 27 acetylation (H3K27ac) (14, 15). High accessibility of chromatin to active gene promoters positively correlates with gene transcription (14, 16). To explore whether the accessibility of chromatin to the *PDGFRB* locus differs between $CD56^{dim}$ and $CD56^{bright}$ populations, we analyzed online datasets containing Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq) and H3K27ac ChIP-seq, both of which were performed in $CD56^{bright}$ and $CD56^{dim}$ NK cells (17). We found that both the ATAC-seq signal and enrichment of the H3K27ac modification in the promoter region of *PDGFRB* were higher in $CD56^{dim}$ NK cells than in $CD56^{bight}$ NK cells (*SI Appendix*, Fig. S3*A*). These results suggest that *PDGFRB* has stronger promoter activity in $CD56^{dim}$ NK cells than in $CD56^{bright}$ NK cells, which may explain the differential regulation of PDGFRβ expression in the two NK cell populations (Fig. 1 *H–J*).

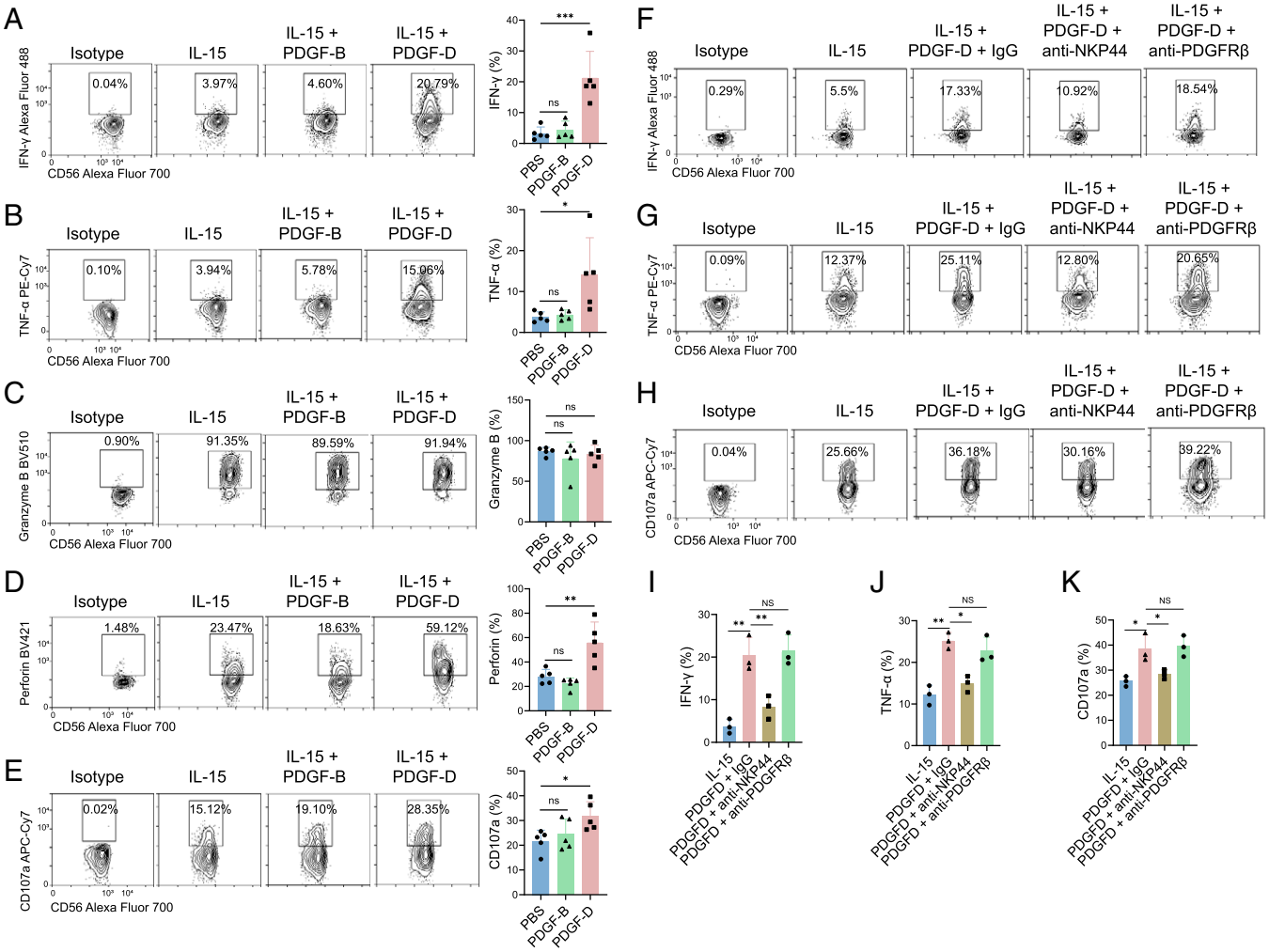

Fig. 3. PDGF-D enhances NK cell effector functions through NKp44 but not PDGFRβ. (*A–E*) Primary NK cells were treated with IL-15 (10 ng/mL) in the presence of PDGF-B (50 ng/mL) or PDGF-D (50 ng/mL) for 48 h. Expression levels of IFN-γ, TNF-α, granzyme B, perforin, and CD107a were examined by flow cytometry (n = 5). (*F–K*) Primary NK cells were treated with IL-15 (10 ng/mL) plus PDGF-D (50 ng/mL) in the presence of anti-NKp44 (10 μg/mL) or anti-PDGFRβ (10 μg/mL) for 48 h. Expression levels of IFN-γ, TNF-α, and CD107a were examined by flow cytometry (n = 3). Data represent three independent experiments. Data shown are means ± SD. NS, not significant. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

PDGFRβ Does Not Affect NK Cell Effector Functions. When we investigated the function of PDGFRβ in NK cells, we found that PDGFRβ$^+$ and PDGFRβ$^-$ NK cells had comparable IFN-γ, TNF-α, granzyme B, and perforin expression (*SI Appendix*, Fig. S4 *A–D*). The two populations also had similar CD107a expression levels and NK cell cytotoxicity against K562 cells (*SI Appendix*, Fig. S4 *E* and *F*), indicating that the presence of PDGFRβ is not essential for NK cell effector functions. We also ectopically expressed PDGFRβ on primary NK cells, using a lentivirus that overexpressed PDGFRβ (*SI Appendix*, Fig. S4 *G* and *H*). The transduced cells showed similar expression levels of CD107a or IFN-γ compared to NK cells transduced with empty vector, in the stimulation of K562 cells or IL-12 plus IL-18, respectively (*SI Appendix*, Fig. S4 *I* and *J*). We also compared functional receptors on the surfaces of these two populations. PDGFRβ$^+$ and PDGFRβ$^-$ NK cells expressed similar levels of activation receptors (such as CD25, CD69, NKG2D, NKp30, and NKp44) and inhibitory receptors (such as NKG2A and KLRG1) (*SI Appendix*, Fig. S4*K*).

When PDGFRβ binds to its two ligands, PDGF-B or PDGF-D, it induces downstream signaling, including Ras/Raf/MAPK and PI3K/AKT, resulting in cell growth (5). We therefore treated IL-15–primed NK cells with PDGF-B or PDGF-D and then evaluated NK cell function. PDGF-D—but not PDGF-B—induced the expression of IFN-γ, TNF-α, perforin, and CD107a but not granzyme B (Fig. 3 *A–E*). A prior study reported that PDGF-D can interact with NKp44 to stimulate the secretion of IFN-γ and TNF-α from NK cells (6). To determine whether that stimulation was dependent on NKp44 or PDGFRβ, we added NKp44- or PDGFRβ-neutralizing antibodies to the culture system. Blocking NKp44—but not PDGFRβ— significantly abrogated the increased expression of IFN-γ, TNF-α, and CD107a (Fig. 3 *F–K*). These data indicate that PDGF-D−PDGFRβ signaling does not affect NK cell activation or effector functions.

PDGFRβ Signaling Contributes to IL-15–Mediated NK Cell Survival. IL-15 is a key cytokine for NK cell proliferation and survival through prosurvival Bcl-2 family proteins, such as BCL-2, BCL-XL, and MCL-1 (11, 18–21). We therefore determined whether PDGFRβ on NK cells regulates IL-15–mediated cell proliferation or survival. PDGFRβ$^+$ NK cells grew faster than PDGFRβ$^-$ NK cells in vitro (Fig. 4*A*). PDGFRβ$^+$ NK cells also showed significantly higher Ki67 expression compared with PDGFRβ$^-$ NK cells (Fig. 4 *B* and *C*). In addition, PDGFRβ$^+$ NK cells had fewer annexin V$^+$ apoptotic cells than PDGFRβ$^-$ NK cells (Fig. 4 *D* and *E*), indicating that PDGFRβ signaling

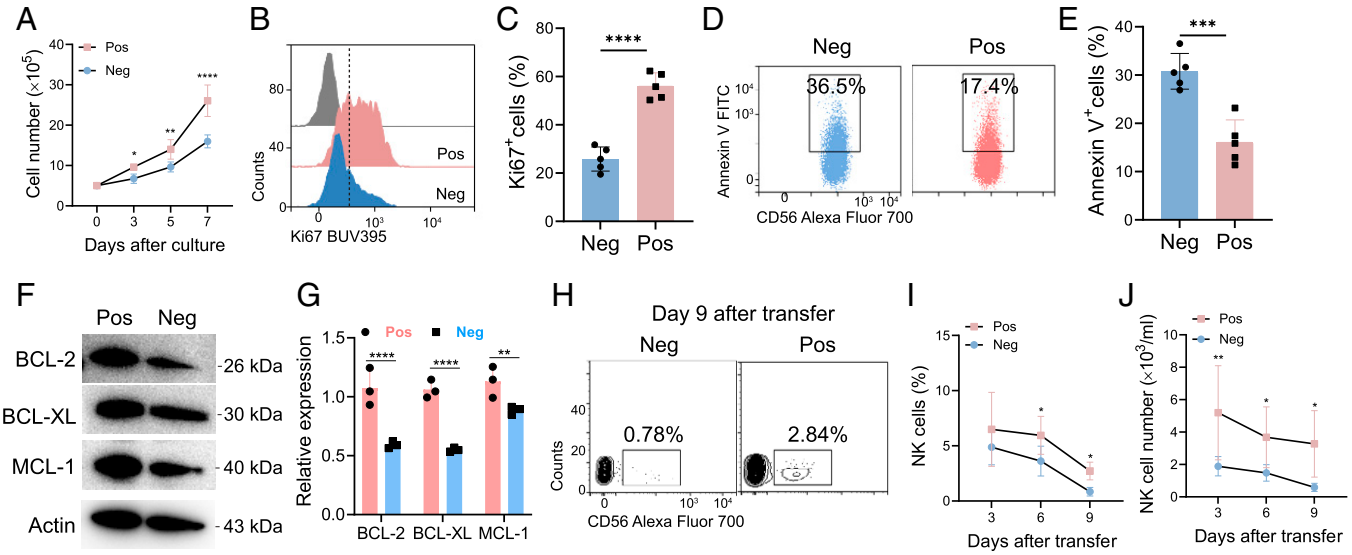

Fig. 4. PDGFRβ promotes IL-15–mediated NK cell survival in vitro and in vivo. (*A*) 5×10^5 sorted PDGFRβ$^+$ (Pos) and PDGFRβ$^-$ (Neg) NK cells were cultured in vitro for 7 d in the presence of IL-15 (10 ng/mL). The cells were counted via trypan blue exclusion assay on days 3, 5, and 7 ($n = 5$). (*B* and *C*) On day 7, Ki67 levels in PDGFRβ$^+$ and PDGFRβ$^-$ NK cells were determined by flow cytometry ($n = 5$). (*D* and *E*) Annexin V levels in PDGFRβ$^+$ and PDGFRβ$^-$ NK cells on day 7 were determined by flow cytometry ($n = 5$). (*F* and *G*) Immunoblotting showing protein levels of BCL-2, BCL-XL, and MCL-1 in PDGFRβ+ (Pos) and PDGFRβ− (Neg) NK cells purified by fluorescence-activated cell sorting (FACS) (n = 3). (*H–J*) 1×10^7 sorted PDGFRβ$^+$ or PDGFRβ$^-$ IL-15-transduced NK cells were injected into NOD/SCID/IL-2rg (NSG) mice. Blood samples were collected for analysis at the indicated time after adoptive transfer. Data shown are representative dot plots on day 9 after adoptive transfer (*H*), percentages (*I*), and absolute numbers (*J*) of PDGFRβ$^+$ and PDGFRβ$^-$ NK cells on days 3, 6, and 9 after adoptive transfer ($n = 5$). Data represent three independent experiments. Data shown are means ± SD. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

enhances NK cell survival. Immunoblotting further demonstrated that PDGFRβ$^+$ NK cells had significantly higher levels of BCL-2, BCL-XL, and MCL-1 compared to PDGFRβ$^-$ NK cells (Fig. 4 *F* and *G*). However, we found equivalent levels of the IL-15 receptor (CD122) in PDGFRβ$^+$ and PDGFRβ$^-$ NK cells (*SI Appendix*, Fig. S5 *A* and *B*), indicating that these two populations may have similar responsiveness to IL-15. To address whether PDGFRβ also promotes NK cell persistence in vivo, we stably transduced NK cells with soluble IL-15 (*SI Appendix*, Fig. S6*A*). PDGFRβ$^+$ and PDGFRβ$^-$ NK cells were transduced with similar efficiency (*SI Appendix*, Fig. S6*B*). The cells were then sorted, and equal numbers were injected into NOD/SCID/IL-2rg (NSG) mice (*SI Appendix*, Fig. S6*A*). We found that PDGFRβ$^+$ NK cells were persisting 9 d after injection, while PDGFRβ$^-$ NK cells could not (Fig. 4*H*). The percentage and the absolute number of PDGFRβ$^+$ NK cells were also significantly higher than those of PDGFRβ$^-$ NK cells 3 and 6 d after injection (Fig. 4 *I* and *J*). Collectively, these results demonstrate that PDGFRβ contributes to IL-15–mediated NK cell survival in vitro and in vivo.

IL-15 Maintains NK Cell Survival in Part through a PDGF-D−PDGFRβ Autocrine Pathway. Theoretically, PDGFRβ needs to bind to its ligand to maintain NK cell survival, and our data showed that PDGFRβ$^+$ NK cells grow better than PDGFRβ$^-$ NK cells. We therefore hypothesized that NK cells might express ligands that recognize. Using BioGPS (https://biogps.org) to screen for genes that encode ligands of PDGFR, we found that human NK cells express high mRNA levels of *PDGFD* but not *PDGFA*, *PDGFB*, or *PDGFC* (Fig. 5*A*). We confirmed this finding using qPCR, finding 20-fold-higher levels of mRNAs for *PDGFD* in NK cells than for the other family members (Fig. 5*B*). Compared to T cells and B cells, only NK cells expressed high levels of *PDGFD* (Fig. 5*B*). In addition, we found that IL-15 stimulation significantly increased mRNA and protein levels of *PDGFD* in NK cells, as determined by qPCR (Fig. 5*C*), flow cytometry (Fig. 5 *D* and *E*), immunoblotting (Fig. 5*F*), and enzyme-linked immunosorbent assay (Fig. 5*G*). Interestingly, we also detected a binding site for p65 in the promoter region of *PDGFD* (*SI Appendix*, Fig. S2*C*), and a luciferase reporter assay showed that p65 directly activated *PDGFD* gene transcription (Fig. 5*H*). Moreover, ChIP-qPCR revealed that p65 was significantly enriched compared with a normal IgG control in IL-15–stimulated NK cells but not in resting NK cells (Fig. 5 *I* and *J*). Taken together, our data demonstrate that NK cells express PDGF-D in an autocrine manner and that PDGF-D can be up-regulated by p65 downstream of IL-15 signaling.

Finding that NK cells express PDGF-D led us to hypothesize that IL-15 may maintain NK cell survival through a PDGF-D–PDGFRβ autocrine pathway. PDGF-D treatment of NK cells promoted cell expansion in the presence of IL-15 (Fig. 6*A*). In contrast, PDGF-D–blocking antibody inhibited NK cell expansion (Fig. 6*B*). Also, PDGF-D enhanced the expansion of primary NK cells transduced with PDGFRβ (Fig. 6*C*). In addition, when we treated NK cells with a PDGFRβ-neutralizing antibody to block PDGF-D−PDGFRβ signaling, NK cell expansion triggered by PDGF-D decreased significantly (Fig. 6*D*). However, neutralizing NKp44 did not affect PDGF-D–mediated NK cell growth (Fig. 6*E*). An immunoblot showed that PDGF-D treatment increased the expression levels of BCL-2, BCL-XL, and MCL-1, in an NKp44-independent but PDGFRβ-dependent manner (Fig. 6 *F–I*). Further studies showed that PDGF-D inhibited apoptosis of PDGFRβ$^+$ NK cells—but not PDGFRβ$^-$ NK cells—in vitro and in vivo (Fig. 6 *J* and *L*). PDGF-D did not affect NK cell proliferation as shown by similar levels of Ki67 in vitro and in vivo (Fig. 6 *K* and *M*), suggesting that PDGF-D promotes IL-15–mediated NK cell survival but not proliferation, though PDGFRβ$^+$ NK cells are more proliferative than PDGFRβ$^-$ NK cells (Figs. 4 *B* and *C* and 6*K*). Thus, our findings reveal a mechanism by which IL-15 promotes NK cell survival: IL-15 induces NK cells to express both PDGF-D and PDGFRβ; then, engagement of PDGFRβ by PDGF-D promotes NK cell survival (*SI Appendix*, Fig. S7).

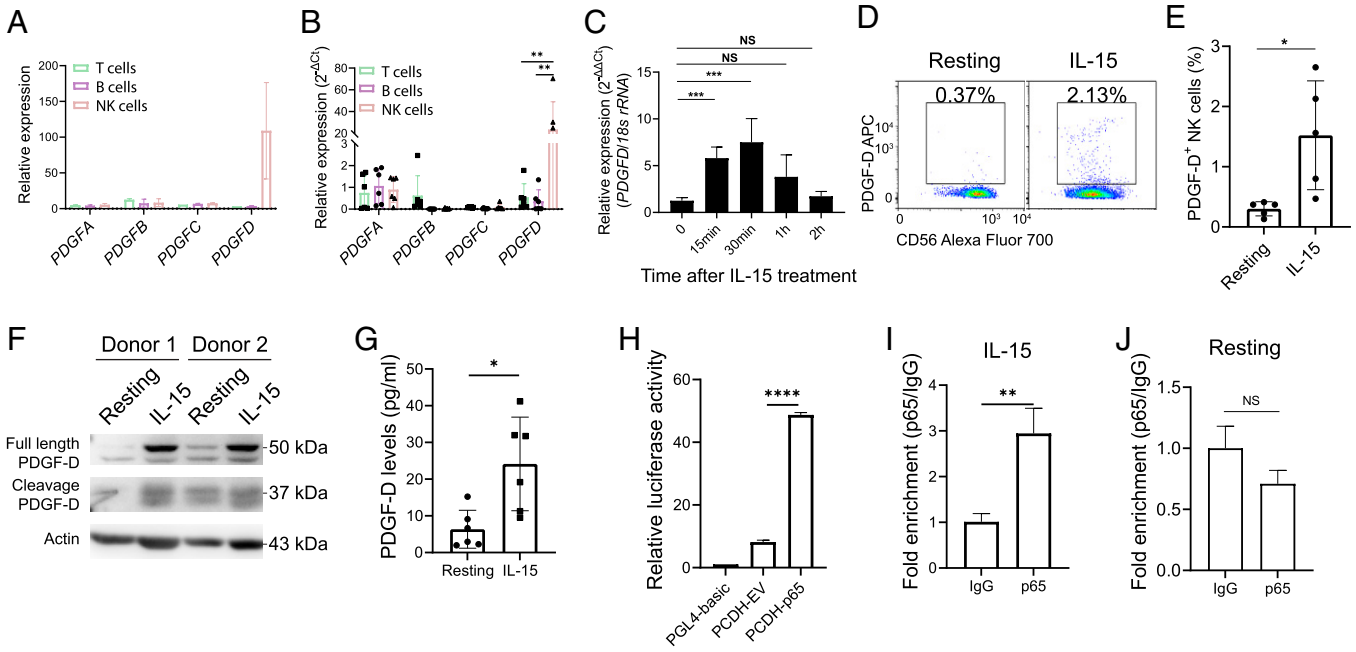

Fig. 5. IL-15 induces PDGF-D expression in an autocrine manner. (*A*) mRNA levels of *PDGFA*, *PDGFB*, *PDGFC*, and *PDGFD* in T cells, B cells, and NK cells were analyzed using the BioGPS online tool. (*B*) mRNA levels of *PDGFA*, *PDGFB*, *PDGFC*, and *PDGFD* in T cells, B cells, and NK cells were examined by qPCR ($n = 10$). (*C*) Primary NK cells were treated with IL-15 (10 ng/mL) for the indicated times. mRNA levels of *PDGFD* were examined by qPCR ($n = 3$). (*D* and *E*) Representative dot plots and percentages of PDGF-D levels in NK cells after IL-15 (50 ng/mL) treatment for 24 h. Resting NK cells were used as control ($n = 5$). (*F*) Immunoblotting shows the full length and cleavage of PDGF-D in resting and IL-15-treated NK cells. (*G*) ELISA shows PDGF-D levels in supernatants of NK cell cultures ($n = 6$). (*H*) Luciferase reporter assay shows that p65 activates *PDGFD* gene transcription. (*I* and *J*) Binding of p65 to the *PDGFD* promoter in IL-15–treated (*I*) or resting NK cells (*J*) as determined by ChIP-qPCR ($n = 3$). Data represent three independent experiments. Data shown are means ± SD. NS, not significant, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

Discussion

In this study, we showed that PDGF-D−PDGFRβ signaling—a potent stimulator of cell growth and motility—activates an autocrine pathway that contributes to IL-15–mediated survival of human NK cells. Our findings therefore expand our understanding of the mechanism by which IL-15 signaling regulates NK cell immunity. Moreover, introducing PDGF-D−PDGFRβ signaling into NK cells might help enhance their survival and improve NK cell-based immunotherapy.

NK cells in humans and mice share many features, including expression of the transcription factors T-bet and Eomes and activation-induced production of IFN-γ, TNF-α, granzyme B, and perforin. In humans, NK cells are typically defined as $CD3^-CD56^+$ cells, and they can be further divided into $CD3^-CD56^{dim}$ and $CD3^-CD56^{bright}$ populations (1, 22). In mice, NK cells are defined as $CD3^-NK1.1^+$ cells that typically express NKp46, CD49b, CD11b, and CD27 but not CD127 (23). In this study, we found that IL-15 could not induce PDGFRβ expression in mouse NK cells, indicating that PDGF-D−PDGFRβ signaling does not contribute to IL-15–mediated cell proliferation and survival in that system. We also found that only $CD56^{dim}$ NK cells expressed PDGFRβ after IL-15 stimulation. $CD56^{dim}$ NK cells, which account for more than 90% of peripheral NK cells, are cytolytic, whereas the $CD56^{bright}$ subset is immunoregulatory, mainly through cytokine production (22). Because $CD56^{bright}$ cells are immature precursors of mature $CD56^{dim}$ NK cells (9, 22, 24), our findings indicate that only mature NK cells can express PDGFRβ. This is in agreement with a previous report showing that both $CD56^{bright}$ and $CD56^{dim}$ NK cells robustly express IL-15Rβ/γ, while these two NK cell subsets may have separate IL-15–mediated signaling pathways that activate different transcriptional regulatory networks (25). Gene expression levels should also be controlled by chromatin accessibility (14). Our analysis of publicly available databases indeed indicated that, in the promoter region of the *PDGFRB* locus, $CD56^{dim}$ NK cells are more accessible to transposons with higher levels of the H3K27Ac histone modification than are $CD56^{bright}$ NK cells.

PDGF-D−PDGFRβ signaling has important functions in the regulation of cell growth and survival. PDGF-D has been recognized as a ligand of NKp44, one of the natural cytotoxicity receptors expressed by activated NK cells (6). PDGF-D prompted NK cells to secrete IFN-γ and TNF-α, arresting the growth of tumor cells (6). We confirmed that PDGF-D induced the production of IFN-γ, TNF-α, and granzyme B in IL-15–activated NK cells and that induction was dependent on NKp44 but not on PDGFRβ. In addition, we provided direct evidence that NK cells can express PDGFRβ and interact with PDGF-D to promote their survival in the presence of IL-15. Therefore, our findings, together with the prior report, suggest that PDGF-D not only enhances effector function through NKp44 but also promotes cell survival through PDGFRβ in human NK cells. In cancer, PDGF-D is abundant and is known to stimulate tumor growth and angiogenesis through PDGFRβ (6, 26–28). Therefore, future development of PDGF-D or PDGFRβ inhibitors to target tumor cells for cancer therapy should be pursued with caution, as inhibiting PDGF signaling might impair host antitumor responses by NK cells. On the other hand, selectively introducing PDGF signaling into NK cells might benefit NK cell expansion, persistence, and enhancement of effector function during NK cell-based immunotherapy.

Large granular lymphocyte (LGL) leukemia is a lymphoproliferative disease characterized by a clonal expansion of cytotoxic T or NK cells. Aggressive T cell and NK cell LGL leukemia is resistant to therapy, producing a poor prognosis (29). It is recognized that IL-15 and PDGF play crucial roles in LGL leukemia expansion by promoting NK cell or leukemic LGL survival (8, 30). LGL leukemia cells promote their survival by

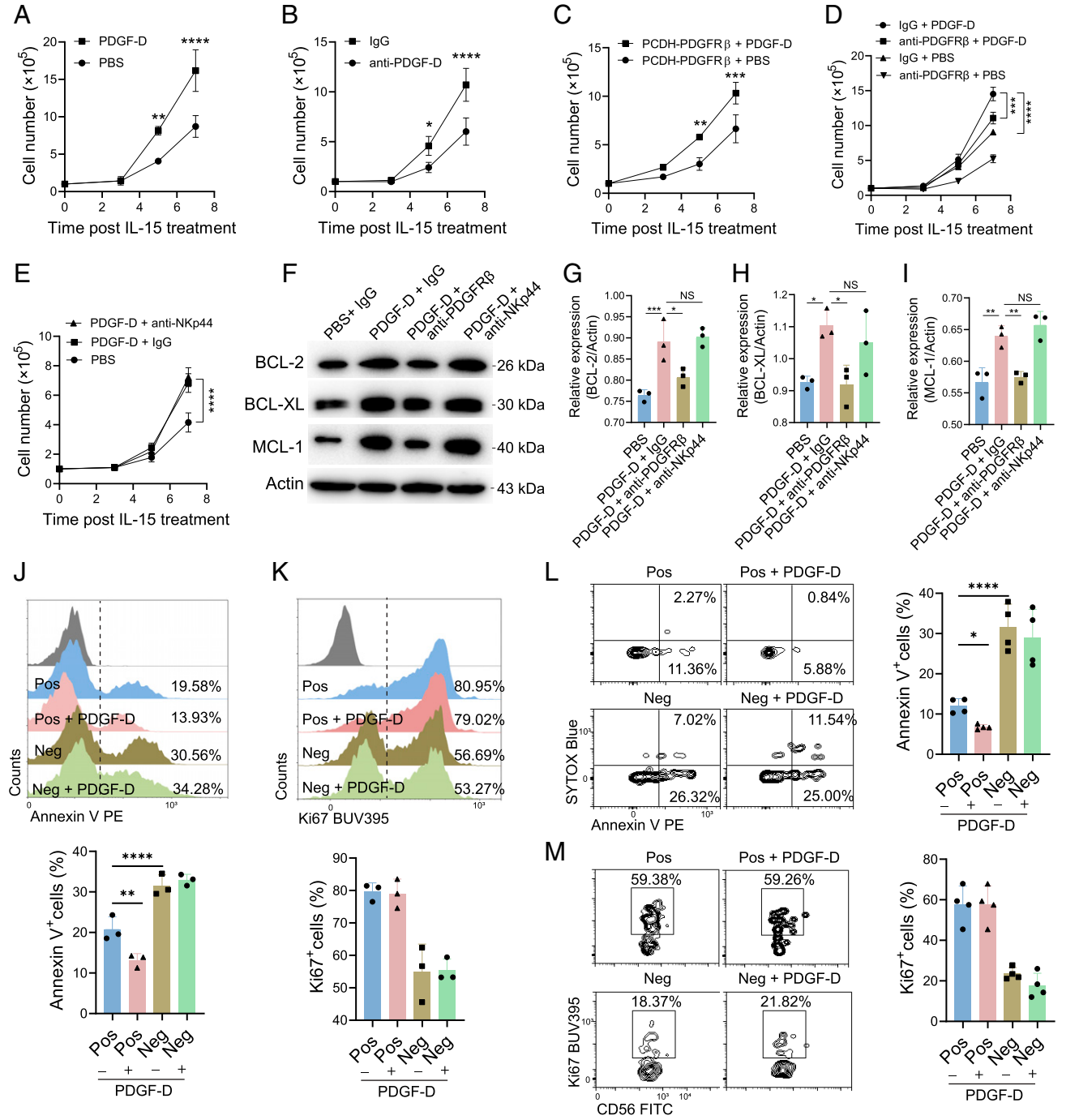

Fig. 6. IL-15 maintains NK cell survival through a PDGF-D–PDGFRβ autocrine pathway. (*A*) 1×10^5 primary NK cells were cultured in vitro for 7 d in the presence of IL-15 (10 ng/mL) and PDGF-D (50 ng/mL). Cells were counted by trypan exclusion assay on days 3, 5, and 7 ($n = 3$). (*B*) 1×10^5 primary NK cells were cultured in vitro for 7 d in the presence of IL-15 (10 ng/mL) and anti-PDGF-D (10 μg/mL) or control IgG (10 μg/mL). Cells were counted by trypan exclusion assay on days 3, 5, and 7 ($n = 3$). (*C*) 1×10^5 PDGFRβ-transduced NK cells were cultured in vitro for 7 d in the presence of IL-2 (10 ng/mL) and PDGF-D (50 ng/mL). The cells were counted by trypan exclusion assay on days 3, 5, and 7 ($n = 3$). (*D*) 1×10^5 primary NK cells were cultured in vitro for 7 d in the presence of IL-15 (10 ng/mL) and PDGF-D (50 ng/mL) as well as anti-PDGFRβ (10 μg/mL). Cells were counted by trypan exclusion assay on days 3, 5, and 7 ($n = 3$). (*E*) 1×10^5 primary NK cells were cultured in vitro for 7 d in the presence of IL-15 (10 ng/mL) and PDGF-D (50 ng/mL) as well as anti-NKp44 (10 μg/mL). Cells were counted by trypan exclusion assay on days 3, 5, and 7 ($n = 3$). (*F–I*) Primary NK cells were cultured in the presence of IL-15 (10 ng/mL) and PDGF-D (50 ng/mL) as well as anti-NKp44 (10 μg/mL) or anti-PDGFRβ (10 μg/mL) for 48 h. The cultured NK cells were then harvested for immunoblotting to determine protein levels of BCL-2, BCL-XL, and MCL-1 ($n = 3$). (*J* and *K*) 1×10^6 sorted PDGFRβ$^+$ (Pos) and PDGFRβ$^-$ (Neg) NK cells pre-treated with IL-15 (10 ng/mL) for 24 h were cultured in the presence of PDGF-D (50 ng/mL) for 48 h without IL-15. Cell apoptosis and proliferation were analyzed by annexin V staining (*J*) and Ki67 staining (*K*), respectively. Data shown are representative histograms and summary data ($n = 3$). (*L* and *M*) 5×10^6 sorted PDGFRβ$^+$ (Pos) and PDGFRβ$^-$ (Neg) NK cells overexpressing IL-15 were injected into NOD/SCID/IL-2rg (NSG) mice. The mice were injected intravenously with PDGF-D (1 μg per mouse) or phosphate-buffered saline daily for 3 d. After they were killed, adoptively transferred NK cells (CD45$^+$CD56$^+$) in their peripheral blood were identified by flow cytometry. The cells were stained for annexin V (*L*) and Ki67 (*M*). Data shown are representative histograms and summary data ($n = 4$). Data represent three independent experiments. Data shown are means ± SD. NS, not significant, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

expressing high levels of PDGFRβ and PDGF-B to activate an autocrine regulatory pathway (8). Therefore, PDGFRβ signaling not only contributes to normal NK cell survival but also is a key survival factor in LGL leukemia. However, the factors that prompt LGLs to express PDGF remain to be discovered (8). Our findings indicate that IL-15 signaling is a key initiation factor that drives PDGF-D and PDGFRβ expression in normal NK cells. We reported earlier that IL-15 plays a central role in the genesis of LGL leukemia and that overexpression of IL-15 as a single growth factor can initiate the leukemic transformation of LGLs (12, 31, 32). We therefore hypothesize that IL-15–PDGF signaling may play a causal role in the pathogenesis of LGL leukemia and that targeting IL-15–PDGF signaling might be a potential therapy for the disease.

In conclusion, we report a previously unknown role for PDGF-D−PDGFRβ signaling: positive regulation of IL-15–mediated cell survival of human NK cells without an effect on NK cell effector functions. Thus, our findings advance the mechanistic understanding of IL-15 signaling in NK cell immunity and point to the introduction of PDGF signaling into NK cells as a promising strategy for advancing NK cell-based therapies against tumors with the precaution of leukemogenesis potentially driven by the signaling.

Materials and Methods

Isolation of Primary Human NK Cells. Peripheral blood samples from de-identified healthy donors were obtained from the Michael Amini Transfusion Medicine Center of City of Hope National Medical Center under institutional review board-approved protocols. NK cells were enriched using the RosetteSep Human NK Cell Enrichment Mixture (STEMCELL Technologies) and Ficoll-Paque (GE Healthcare). The purity of primary NK cells ($CD3^-CD56^+$) was confirmed with flow cytometry. $CD56^{bright}$ and $CD56^{dim}$ NK cells were sorted with a FACSAria Fusion Flow Cytometer (BD Biosciences).

Antibodies, Cytokines, and Inhibitors. Fluorochrome-conjugated mouse anti-human antibodies against CD3 (UCHT1), CD56 (B159), PDGFRα (αR1), PDGFRβ (28D4), IFN-γ (4S. B3), TNF-α (MAb11), CD107a (H4A3), granzyme B (GB11), perforin (δG9), CD25 (M-A251), CD69 (FN50), NKG2D (1D11), NKp30 (p30-15), NKp44 (p44-8), NKG2A (131411), and Ki67 (B56) and isotype controls were purchased from BD Biosciences. Anti-human KLRG1 (13F12F2) was purchased from eBioscience. APC-conjugated human PDGF-D antibody was purchased from Assaypro LLC. Anti-mouse CD3 (17A2), NK1.1 (PK136), and PDGFRβ (APB5) were purchased from BioLegend. PDGF receptor β (28E1) rabbit mAb (#3169), Phospho-NF-κB p65 (Ser536) (93H1) rabbit mAb (#3033), NF-κB p65 (D14E12) rabbit mAb (#8242), Bcl-2 (124) mouse mAb (#15071), Bcl-xL (54H6) rabbit mAb (#2764), Mcl-1 (D2W9E) rabbit mAb (#94296), β-tubulin (9F3) rabbit mAb (#2128), and lamin B1 (D9V6H) rabbit mAb (#13435) were purchased from Cell Signaling Technology. Alexa Fluor 488-conjugated anti-PDGF receptor beta (sc-19995 AF488) was purchased from Santa Cruz. Recombinant anti-SCDGFB/PDGF-D antibody (ab181845), recombinant anti-IL2 receptor beta/p75 antibody (ab271040), and recombinant anti-sodium-potassium ATPase antibody (ab76020) were purchased from Abcam. Beta-actin monoclonal antibody (66009-1-Ig) was purchased from Proteintech. Recombinant human IL-2 (200-02), IL-12 p70 (200-12), and IL-15 (200-15); recombinant murine IL-2 (402-ML-020), IL-12 (419-ML-010/CF), and IL-15 (447-ML-010/CF); and recombinant human IL-18 (B001-5), PDGF-BB (220-BB-010), and PDGF-DD (1159-SB-025) were purchased from R&D Systems. Purified anti-human CD336 (NKp44) antibody (325104), purified mouse IgG1, and κ isotype control antibody (40140150) were purchased from BioLegend. Human PDGFR beta antibody (AF385) was purchased from R&D Systems. Wortmannin, afuresertib, TPCA-1, decernotinib, AZD6244, CI-1040, and cycloheximide were purchased from Selleck Chemicals. Rapamycin, Torin1, and STAT5-IN-1 were purchased from MedChemExpress. Actinomycin D (catalog no. A9415) was purchased from Sigma-Aldrich. Brefeldin A was purchased from BioLegend.

NK Cell Culture, Transduction, and Treatment. NK cells were cultured in RPMI 1640 with 10% heat-inactivated fetal bovine serum (FBS) (Gibco) at 37 °C in a 5% CO_2 humidified incubator. For NK cell transduction, PDGFRB complementary DNA (cDNA) open reading frame clone (catalog no. HG10514-G) was purchased from Sino Biological and cloned into pCDH-CMV-MCS-EF1-copGFP lentivirus vector (System Biosciences). To produce lentivirus, we transfected the lentiviral transfer vector DNA, together with psPAX2 packaging (Addgene) and pMD2.G envelope plasmid DNA (Addgene), into HEK293T cells, using a polyethyenimine transfection protocol (Polysciences). Concentrated lentivirus was added to primary NK cells cultured in RPMI medium 1640 supplemented with 10% FBS and 10 μg/mL polybrene with 2,000 × *g* centrifugation for 2 h. Cells were cultured for an additional 48 h. For cytokine treatment, NK cells were treated with IL-2, IL-12, IL-15, IL-18, or a combination for the indicated time. The cells were then collected for flow cytometry analysis. For the inhibition assay, NK cells were pretreated with the indicated inhibitors for 1 h, washed twice with RPMI 1640, and then treated with IL-15 for 24 h.

Mouse NK Cell Isolation and Treatment. Mouse NK cells were isolated from the spleen of C57BL/6J or IL-15 transgenic mice, using the EasySep Mouse NK Cell Isolation Kit (STEMCELL Technologies) as previously described (11). Cells were treated with IL-2, IL-12, IL-15, or IL-12 plus IL-15 for 24 h and then collected for flow cytometry.

Flow Cytometry. Cells were stained with the indicated cell-surface markers and/or fixed/permeabilized using a Fixation/Permeabilization Kit (eBioscience). For intracellular staining of IFN-γ and PDGF-D, 5 μg/mL Brefeldin A (BioLegend) was added for 4 h before cell harvest. Intracellular staining of Ki67 was performed by fixing and permeabilizing with the Foxp3/Transcription Factor Staining Kit (eBioscience). For CD107a staining, CD107a antibody was added into the culture for 4 h in the presence of Brefeldin A. Annexin V staining was performed using an FITC Annexin V Apoptosis Detection Kit according to the manufacturer's protocol (BD Biosciences). Flow cytometry analysis was performed on BD LSRFortessa X-20 (BD Biosciences). Data were analyzed using NovoExpress software (Agilent Technologies).

Immunofluorescence Assay. Resting NK cells and IL-15–treated NK cells were fixed with 4% formaldehyde, blocked with 5% bovine serum albumin, and then stained with Alexa Fluor 488-conjugated anti-PDGF receptor beta and anti-sodium- potassium ATPase antibody overnight at 4 °C. The cells were washed and incubated with Alexa Fluor 647-conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch) at room temperature for 1 h. Cells were rinsed three times in 1× phosphate-buffered saline and one drop of the Diamond Antifade Mountant with DAPI (Thermo Scientific) was then applied. Cover slide-mounted specimens were visualized, and images were acquired using a Zeiss microscope.

Quantitative Real-Time RT-PCR (qPCR) and Immunoblotting. RNA was isolated using an RNeasy Mini Kit (Qiagen) and reversely transcribed to cDNA with a PrimeScript RT Reagent Kit with gDNA Eraser (Takara Bio) according to the manufacturer's protocol. mRNA expression levels were analyzed using SYBR Green qPCR Master Mix and a QuantStudio 12K Flex Real-Time PCR System (both from Thermo Fisher Scientific). Primer sequences used are as follows: *PDGFRB* forward, 5′- TGATGCCGAGGAACTATTCATCT-3′; *PDGFRB* reverse, 5′- TTTCTTCTCGTGCAGTGTCAC-3′; *PDGFD* forward, 5′- TTGTACCGAAGAGATGAGACCA -3′; *PDGFD* reverse, 5′- GCTGTATCCGTGTATTCTCCTGA -3′; *18S rRNA* forward, 5′- TGTGCCGCTAGAGGTGAAATT -3′; and *18S rRNA reverse*, 5′- TGGCAAATGCTTTCGCTTT -3′. Relative amplification values were normalized to the amplification of *18S rRNA*. Immunoblotting was performed according to standard procedures, as previously described (11). Cellular fractionation separation was performed using NE-PER Nuclear and Cytoplasmic Extraction Reagents and the Mem-PER Plus Membrane Protein Extraction Kit (Thermo Scientific).

Enzyme-Linked Immunosorbent Assay (ELISA). Primary NK cells (5×10^6 per well) were plated in a six-well plate in RPMI 1640 supplemented with 10% FBS. The cells were treated with recombinant human IL-15 (50 ng/mL) for 24 h, and supernatants were collected and frozen at −80 °C for later use. The PDGF-D concentration in the supernatant was measured using a Human PDGF-DD Quantikine ELISA Kit (DDD00; R&D Systems).

Luciferase Reporter Assay. The promoter regions of *PDGFRB* and *PDGFD* were amplified and cloned into the pGL4-basic luciferase reporter vector (Promega). HEK293T cells purchased from ATCC were cotransfected with the pGL4-*PDGFRB* or pGL4-*PDGFD* reporter plasmid as well as with a p65-overexpression plasmid or empty vector. A pRL-TK Renilla reporter plasmid (Promega) was added to normalize transfection efficiency. The cells were harvested for lysis 24 h after transfection, and luciferase activity was quantified fluorimetrically with Dual-Luciferase Reporter Assay System (Promega). A p65 overexpression plasmid was used as previously described (33). Primer sequences for cloning the *PDGFRB* and *PDGFD* promoters were as follows: *PDGFRB* forward, 5′- CGGGGTACCCAAAGACCTGGCCAGGCCCCCTCT-3′; *PDGFRB* reverse, 5′-CCGCTCGAGCTGGCAGCCTCAGGAGCTCACACCA-3′; *PDGFD* forward,

5′- CCGCTCGAGCAAAGAGATTAGGAACTTTATTTCT-3′; and *PDGFD* reverse, 5′- CCCAAGCTTTGACGGGACAAACAACAGGTTGA -3′.

ChIP Assay. Primary NK cells were treated with IL-15 for 1 h. The cells were cross-linked in 1% formaldehyde and quenched with glycine buffer. ChIP assays were carried out using a Pierce Magnetic ChIP Kit (catalog no. 26157; Thermo Scientific) according to the manufacturer's instructions. Digested chromatin was incubated overnight with a p65 ChIP-grade antibody (#8242; Cell Signaling Technology) or IgG control antibody (#3900; Cell Signaling Technology). The enriched chromatin was analyzed by qPCR using the following primers: *PDGFRB* forward, 5′-AAATGATCTCCCTGGGTGCCA-3′; *PDGFRB* reverse, 5′-CGCGTGCGTCTGTTTTCAA-3′; *PDGFD* forward, 5′- TCCTTAGTGTCTCTCCC AGGG-3′; and *PDGFD* reverse, 5′- AAATTTAGGTTTGTGGGCCATG-3′.

^{51}Cr Release Cytotoxicity Assay. NK cell cytotoxicity against K562 cells was evaluated by standard ^{51}Cr release assays as previously described (11). PDGFRβ^{+} and PDGFRβ^{-} NK cells were sorted and cocultured with ^{51}Cr-labeled K562 cells in a 96-well V-bottom plate at ratios of 5:1, 2.5:1, and 1.25:1 for 4 h at 37 °C in a 5% CO_2 incubator. Supernatant harvested from each well was transferred into a 96-well Luma plate and analyzed using a MicroBeta Scintillation Counter (Wallac; PerkinElmer). Percentages of killing were calculated using the following equation: % specific lysis = 100× ((test ^{51}Cr release) − (spontaneous ^{51}Cr release))/((maximal ^{51}Cr release) − (spontaneous ^{51}Cr release)).

NK Cell Transduction and Adoptive Transfer. The full-length human IL-15 sequence was cloned into a pCIR retrovirus vector. To produce retrovirus, GP2-293 cells were transfected with that vector, using Lipofectamine 3000 reagent (Thermo Fisher). NK cell transduction was performed using RetroNectin (Takara Bio)-coated plates with 2,000 × *g* centrifugation for 2 h, as described previously (3). The infected cells were washed and cultured with rhIL-2 (1,000 IU/mL) for 48 h. Transduced NK cells were expanded for 7 d, using irradiated (25 Gy) autologous PBMCs as described previously (3). Then, 1×10^7 sorted PDGFRβ^{+} or PDGFRβ^{-} NK cells were injected into NOD/SCID/IL-2rg (NSG) mice (The Jackson Laboratory), followed by detection of survival of PDGFRβ^{+} or PDGFRβ^{-} NK cells in peripheral blood by flow cytometry on days 3, 6, and 9 after adoptive transfer. To investigate the effect of PDGF-D in this setting, 5×10^6 sorted PDGFRβ^{+} or PDGFRβ^{-} NK cells were intravenously coinjected with PDGF-D (1 μg per mouse) or phosphate-buffered saline on day 0 and PDGF-D were administered similarly on both day 1 and 2. On day 3, mice were killed and peripheral blood samples were collected to analyze survival of PDGFRβ^{+} or PDGFRβ^{-} NK cells.

Statistical Analysis. Unpaired Student's *t* tests (two-tailed) were performed to compare two independent groups. One-way ANOVA was performed to compare three or more independent groups. Linear mixed models were performed to compare matched groups by accounting for the variance–covariance structure due to repeated measures (over time) from the same subjects. Two-way ANOVA or linear mixed models were applied to two-factor analyses. Data analyses were performed by software Prism and SAS9.4. *P* values were adjusted for multiple comparisons using Holm-Sidak's procedure. A P value <0.05 was considered statistically significant.

Data Availability. All study data are included in the article and/or *SI Appendix*.

ACKNOWLEDGMENTS. This work was supported by grants from the NIH (NS106170, AI129582, CA247550, CA163205, CA223400, CA068458, CA265095, and CA210087), the US Department of Agriculture (2020-07696), the Leukemia & Lymphoma Society (1364-19), and the Breast Cancer Alliance (2021 Exceptional Project Award).

1. A. G. Freud, B. L. Mundy-Bosse, J. Yu, M. A. Caligiuri, The broad spectrum of human natural killer cell diversity. *Immunity* **47**, 820–833 (2017).
2. A. Yilmaz, H. Cui, M. A. Caligiuri, J. Yu, Chimeric antigen receptor-engineered natural killer cells for cancer immunotherapy. *J. Hematol. Oncol.* **13**, 168 (2020).
3. R. Ma *et al.*, An oncolytic virus expressing IL15/IL15Rα combined with off-the-shelf EGFR-CAR NK cells targets glioblastoma. *Cancer Res.* **81**, 3635–3648 (2021).
4. E. Liu *et al.*, Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N. Engl. J. Med.* **382**, 545–553 (2020).
5. J. Andrae, R. Gallini, C. Betsholtz, Role of platelet-derived growth factors in physiology and medicine. *Genes Dev.* **22**, 1276–1312 (2008).
6. A. D. Barrow *et al.*, Natural killer cells control tumor growth by sensing a growth factor. *Cell* **172**, 534–548.e19 (2018).
7. G. M. Gersuk, J. M. Holloway, W. C. Chang, P. K. Pattengale, Inhibition of human natural killer cell activity by platelet-derived growth factor. *Nat. Immun. Cell Growth Regul.* **5**, 283–293 (1986).
8. J. Yang *et al.*, Platelet-derived growth factor mediates survival of leukemic large granular lymphocytes via an autocrine regulatory pathway. *Blood* **115**, 51–60 (2010).
9. J. Yu *et al.*, CD94 surface density identifies a functional intermediary between the CD56bright and CD56dim human NK-cell subsets. *Blood* **115**, 274–281 (2010).
10. M. A. Cooper *et al.*, Human natural killer cells: A unique innate immunoregulatory role for the CD56(bright) subset. *Blood* **97**, 3146–3151 (2001).
11. S. Ma *et al.*, The RNA m6A reader YTHDF2 controls NK cell antitumor and antiviral immunity. *J. Exp. Med.* **218**, e20210279 (2021).
12. J. Yu *et al.*, NKp46 identifies an NKT cell subset susceptible to leukemic transformation in mouse and human. *J. Clin. Invest.* **121**, 1456–1470 (2011).
13. A. Mishra, L. Sullivan, M. A. Caligiuri, Molecular pathways: Interleukin-15 signaling in health and in cancer. *Clin. Cancer Res.* **20**, 2044–2050 (2014).
14. S. L. Klemm, Z. Shipony, W. J. Greenleaf, Chromatin accessibility and the regulatory epigenome. *Nat. Rev. Genet.* **20**, 207–220 (2019).
15. K. Karmodiya, A. R. Krebs, M. Oulad-Abdelghani, H. Kimura, L. Tora, H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells. *BMC Genomics* **13**, 424 (2012).
16. T. Kouzarides, Chromatin modifications and their function. *Cell* **128**, 693–705 (2007).
17. P. L. Collins *et al.*, Gene regulatory programs conferring phenotypic identities to human NK cells. *Cell* **176**, 348–360.e12 (2019).
18. Y. Wang *et al.*, The IL-15-AKT-XBP1s signaling pathway contributes to effector functions and survival in human NK cells. *Nat. Immunol.* **20**, 10–17 (2019).
19. W. E. Carson *et al.*, Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J. Exp. Med.* **180**, 1395–1403 (1994).
20. N. D. Huntington *et al.*, Interleukin 15-mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1. *Nat. Immunol.* **8**, 856–863 (2007).
21. W. E. Carson *et al.*, A potential role for interleukin-15 in the regulation of human natural killer cell survival. *J. Clin. Invest.* **99**, 937–943 (1997).
22. A. G. Freud, J. Yu, M. A. Caligiuri, Human natural killer cell development in secondary lymphoid tissues. *Semin. Immunol.* **26**, 132–137 (2014).
23. J. Yu, A. G. Freud, M. A. Caligiuri, Location and cellular stages of natural killer cell development. *Trends Immunol.* **34**, 573–582 (2013).
24. M. Luetke-Eversloh, M. Killig, C. Romagnani, Signatures of human NK cell development and terminal differentiation. *Front. Immunol.* **4**, 499 (2013).
25. J. A. Wagner *et al.*, CD56bright NK cells exhibit potent antitumor responses following IL-15 priming. *J. Clin. Invest.* **127**, 4042–4058 (2017).
26. J. Liu *et al.*, PDGF-D improves drug delivery and efficacy via vascular normalization, but promotes lymphatic metastasis by activating CXCR4 in breast cancer. *Clin. Cancer Res.* **17**, 3638–3648 (2011).
27. L. Xu, R. Tong, D. M. Cochran, R. K. Jain, Blocking platelet-derived growth factor-D/platelet-derived growth factor receptor beta signaling inhibits human renal cell carcinoma progression in an orthotopic mouse model. *Cancer Res.* **65**, 5711–5719 (2005).
28. W. J. LaRochelle *et al.*, Platelet-derived growth factor D: Tumorigenicity in mice and dysregulated expression in human cancer. *Cancer Res.* **62**, 2468–2473 (2002).
29. A. Moignet, T. Lamy, Latest advances in the diagnosis and treatment of large granular lymphocytic leukemia. *Am. Soc. Clin. Oncol. Educ. Book* **38**, 616–625 (2018).
30. R. Zhang *et al.*, Network model of survival signaling in large granular lymphocyte leukemia. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 16308–16313 (2008).
31. A. Mishra *et al.*, Aberrant overexpression of IL-15 initiates large granular lymphocyte leukemia through chromosomal instability and DNA hypermethylation. *Cancer Cell* **22**, 645–655 (2012).
32. T. A. Fehniger *et al.*, Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. *J. Exp. Med.* **193**, 219–231 (2001).
33. W. Dong *et al.*, The mechanism of anti-PD-L1 antibody efficacy against PD-L1-negative tumors identifies NK cells expressing PD-L1 as a cytolytic effector. *Cancer Discov.* **9**, 1422–1437 (2019).